Lipid Peroxidation as Determined by Plasma Isoprostanes Is Related to Disease Severity in Mild Asthma

Lisa G. Wood[a], Dominic A. Fitzgerald[b,1], Peter G. Gibson[c], David M. Cooper[b], and Manohar L. Garg[a,*]

[a]Discipline of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, University of Newcastle, Newcastle, New South Wales, 2308, Australia, [b]Department of Paediatrics, John Hunter Children's Hospital, Rankin Park, New South Wales, 2305, Australia, and [c]Airway Research Centre, Department of Respiratory Medicine, John Hunter Hospital, Rankin Park, New South Wales, 2305, Australia

ABSTRACT: Oxidative stress is believed to play an important role in the pathophysiology of asthma. Recently discovered F2-isoprostanes, of which 8-iso-$PGF_{2\alpha}$ is the most well-known isomer, have emerged as the most reliable marker of *in vivo* oxidative stress. The aim of this study was to examine 8-iso-$PGF_{2\alpha}$ as a biomarker of oxidative stress in mild asthma in relation to endogenous and dietary antioxidant protection. Total (free and esterified) plasma 8-iso-$PGF_{2\alpha}$, plasma dietary antioxidants (vitamins E and C, β-carotene, Zn, and Se), and erythrocyte antioxidant enzyme activities (glutathione peroxidase and superoxide dismutase) were measured in 15 mild asthmatics and 15 age- and sex-matched controls. Total plasma 8-iso-$PGF_{2\alpha}$ levels [median (quartile 1 – quartile 3)] were significantly increased in the asthmatics [213 pg/mL (122–455) vs. 139 pg/mL (109–174), P = 0.042]. The 8-iso-$PGF_{2\alpha}$ levels were found to be associated with clinical asthma severity (P = 0.044) and inhaled corticosteroid use (P = 0.027) in asthmatics. No differences were observed in the plasma dietary antioxidant vitamins. The asthmatics had significantly lower plasma levels of Zn (P = 0.027) and Se (P = 0.006). Plasma Se correlated negatively with 8-iso-$PGF_{2\alpha}$ (r = –0.725, P = 0.002). No differences between the groups were observed for glutathione peroxidase or superoxide dismutase, however, superoxide dismutase activity was negatively associated with asthma severity (P = 0.042). In conclusion, oxidative stress is increased in mild asthmatics, as reflected by increased plasma levels of 8-iso-$PGF_{2\alpha}$ and a deficiency in plasma Zn and Se. The isoprostane 8-iso-$PGF_{2\alpha}$ may provide a useful tool in intervention studies aimed at improving clinical status in asthma.

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Oxidative stress is believed to play an important role in the pathophysiology of asthma (1,2), which is characterized by many factors such as bronchial hyperresponsiveness, increased vascular permeability with edema of airway walls, mucus hypersecretion with small airway plugging, and infiltration by inflammatory cells (1). Inflammatory cells that are sequested and activated in asthmatic airways include mast cells, macrophages, eosinophils, neutrophils, lymphocytes, and platelets (1). These cells release a variety of mediators that are involved in the inflammatory response, including a range of toxic reactive oxygen species (ROS), such as the superoxide, hydrogen peroxide, and hydroxyl radicals (1,2). These ROS can have many detrimental effects on airway function such as peroxidation of membrane lipids, leading to epithelial cell disruption and/or death; DNA damage; alteration in important biomolecules such as surface receptor proteins and enzymes; enhanced release of arachidonic acid from membranes causing smooth muscle contraction; impaired β-adrenergic responsiveness; increased airway reactivity and secretions; and increased vascular permeability (1). Many of these effects will contribute to the variable and reversible airway narrowing that is characteristic of asthma, suggesting that ROS may contribute to the pathophysiology of the disease by several different mechanisms.

Evidence for the occurrence of oxidative stress in asthma includes: increased exhaled hydrogen peroxide and nitric oxide (3,4), increased thiobarbituric acid-reactive substances (TBARS) (2), decreased Trolox equivalent antioxidant capacity (TEAC) (2), altered status of antioxidant enzymes [glutathione peroxidase (GSHPx), superoxide dismutase (SOD), and catalase] (4–8) and their cofactors (Se and Zn) (5,6,9), decreased vitamin E (4,10), decreased vitamin C (10), increased generation of ROS from inflammatory cells *in vitro* (11), and decreased levels of lipoperoxidation substrates (12). Although measurements such as the *in vitro* TBARS test, breath alkane levels, hydroperoxides, conjugated dienes, and others are satisfactory in many circumstances, they have met criticism by a number of investigators, particularly when examining oxidative stress *in vivo* (13,14). These tests have limited specificity and/or sensitivity for oxidative stress or

[1]Present address: Department of Respiratory Medicine, New Children's Hospital, Parramatta, New South Wales, 2124, Australia.

*To whom correspondance should be addressed at Discipline of Nutrition and Dietetics, Faculty of Medicine & Health Sciences, University of Newcastle, Callaghan, NSW, 2308, Australia.
E-mail: ndmg@medicine.newcastle.edu.au

Abbreviations: BHT, butylated hydroxytoluene; EIA, enzyme immunoassay; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; GC–MS, gas chromatography–mass spectrometry; GSHPx, glutathione peroxidase; Hb, hemoglobin; HPLC, high-performance liquid chromatography; ICP–MS, inductively coupled plasma–mass spectrometry; 8-iso-$PGF_{2\alpha}$, isomer of F2-isoprostane; MDA, malondialdehyde; ROS, reactive oxygen species; SEM, standard error of the mean; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substance; TEAC, Trolox equivalent antioxidant capacity; TxB_2, tritium-labeled thromboxane.

may be unreliable when applying the techniques to human subjects (14).

Isoprostanes, a recently discovered marker of oxidative stress, can be measured in plasma, urine, or other biological fluids (15–18), and overcome many of the methodological problems surrounding other markers. They are prostaglandin-like compounds produced *in vivo via* the cyclooxygenase-independent free radical-catalyzed oxidation of arachidonic acid (16). The most well-known isomer is 8-iso-$PGF_{2\alpha}$. Isoprostanes are now accepted to be the most accurate and reliable marker of oxidative stress, because they are structurally stable, are produced *in vivo*, and are present in relatively high concentrations (16). Indeed, as a marker of oxidative stress, the 8-iso-$PGF_{2\alpha}$ determination of carbon tetrachloride-induced lipid peroxidation is believed to be 20 times more sensitive than measurement of TBARS (17). Furthermore, isoprostane levels have been shown to increase in experimental models of injury, and can be suppressed using antioxidants (16).

The aim of this study was to examine oxidative stress in mild asthma using plasma 8-iso-$PGF_{2\alpha}$ as a biomarker and to examine antioxidant defenses in asthmatics, by studying both endogenous (erythrocyte GSHPx and SOD) and dietary (plasma vitamins E and C, β-carotene, Zn, and Se) antioxidant protection.

MATERIALS AND METHODS

Subject recruitment. Fifteen asthmatic subjects and 15 age- and gender-matched healthy controls were recruited for the study. All asthmatics studied had attended asthma clinics at the John Hunter Hospital and undergone pulmonary function testing in our laboratory. The diagnosis of asthma was made by a respiratory physician based upon a history of episodic respiratory symptoms, a doctor's prior diagnosis of asthma, the use of inhaled asthma therapy (β_2 agonists, corticosteroids, cromolyn, long-acting β_2 agonists), and all patients demonstrated a >12% improvement in their forced expiratory volume in 1 s (FEV1) in response to bronchodilator in our pulmonary function laboratory (19,20). All patients were clinically stable from an asthma viewpoint. Specifically, none had required oral corticosteroids for 3 mon prior to participation in this study. None had altered their preventive medications in the 4 wk prior to enrollment. The exclusion criteria were (i) age less than 5 yr (unable to perform reproducible spirometry), (ii) vitamin supplements taken in the last 4 wk, (iii) presence of other diseases known to be associated with elevated oxidative stress (cancer, diabetes, arthritis, or cystic fibrosis). Informed written consent was obtained from the subjects and/or their guardians. Ethics approval was obtained from the Hunter Area Health Service and the University of Newcastle Human Research Ethics Committees.

Subject characteristics. Each subject was assessed by a respiratory physician, who administered a questionnaire to assess current symptoms and treatment, past severity, and prior hospitalization. Subsequently, each subject's asthma severity was classfied as infrequent episodic, frequent episodic, or persistent, using standard criteria (19) (Table 1). All subjects were clinically stable when assessed. Routine pulmonary function tests were performed in all subjects using a spirometer (Medgraphics 1085D Breeze™ cardiorespiratory diagnostic software 1991, St. Paul, MN) with established normal values (21). FEV1 and forced vital capacity (FVC) were recorded and compared to predicted values. Height was measured with a Holtain, Crymych, Dyfed stadiometer. Weight was recorded using GEC/Avery digital scales (model number 824/890). Blood was collected in EDTA-coated tubes for full blood counts, performed using a Coulter Gen-S analyzer.

Vitamins and minerals. Blood samples were collected in EDTA-coated tubes and then centrifuged at 3,000 rpm at 4°C for 10 min. Plasma was collected and frozen at –70°C within half an hour of blood collection. Plasma levels of vitamins A and E and β-carotene were separated on a reversed-phase high-performance liquid chromatography (HPLC) column and measured using a variable wavelength ultraviolet (UV)-visible detector. Samples were thawed, mixed with ethanol to precipitate proteins, and vortexed; then hexane was added. After vortexing again, samples were centrifuged and the hexane phase removed and injected into an HPLC column [lab-packed Whatman ODS 3 (5 µm) 300 × 3.5 mm i.d.], with a flow rate of 1 mL/min, run time of 20 min, at ambient temperature. At 0.01 min, vitamin A was measured at 310 nm; at 5.5 min, vitamin E was measured at 280 nm; and at 9.0 min, β-carotene was measured at 450 nm. Plasma vitamin C was separated on a reversed-phase HPLC column and measured using an electrochemical detector. Samples were mixed with trichloracetic acid to precipitate proteins, vortexed, and centrifuged; and the supernatant was injected into an HPLC column [lab-packed Whatman ODS 3 (5 µm) 150 × 3.5 mm i.d.], with a flow rate of 1 mL/min, run time of 15 min, at ambient temperature. Measurements were made with an amperometric electrochemical detector with potential +0.6 V vs. Ag/AgCl reference electrode. Plasma levels of zinc, selenium, and copper were analyzed by inductively coupled plasma–mass spectrometry (ICP–MS). Samples were diluted in an ammonium EDTA-based diluent in a quantitative appli-

TABLE 1
Classification of Asthma

	Infrequent episodic	Frequent episodic	Persistent
Frequency of asthma exacerbation	< Every 6 wk	At least every 4–6 wk	Symptoms on most days
Use of bronchodilators	Not needed between exacerbations	< 3 Times weekly	Most days
Interval preventative therapy	Not required	May be required	Always required

cation. Platinum and rhodium were used as internal standards in the diluent. Calibration was by additions calibration in a pooled plasma base.

Total (free and esterified) 8-iso-$PGF_{2\alpha}$ assays. Blood samples were collected in EDTA-coated tubes, containing reduced glutathione (Sigma Chemical Company, St. Louis, MO) as an antioxidant. The samples were centrifuged at 3,000 rpm at 4°C for 10 min. The plasma fraction was removed and stored at −70°C in tubes precoated with butylated hydroxytoluene (BHT) (Sigma) for 8-iso-$PGF_{2\alpha}$ analysis. To an aliquot of plasma, a known amount of tritium-labeled thromboxane B_2 (TxB_2) (Amersham, Arlington Heights, IL) was added, to allow determination of recovery rate after purification procedure. Ethanol was added, the sample was chilled at 4°C, then centrifuged at 1,500 × *g* for 10 min to remove the precipitated proteins. The supernatant was decanted, an equal volume of 15% KOH was added and the resultant solution incubated at 40°C for 1 h, to cleave any esterified isoprostane. The sample was diluted with H_2O, then the pH was lowered with HCl to below 4.0. The sample was passed through a Sep-Pak C-18 reversed-phase cartridge (Waters, Milford, MA) which had been activated by rinsing with methanol, then H_2O. After passing the sample through, the cartridge was rinsed again with H_2O, then hexane. Finally, the 8-iso-$PGF_{2\alpha}$ was eluted with ethyl acetate containing 1% methanol. This solvent was evaporated using N_2, and the sample reconstituted with assay buffer. Purified sample was added to aqueous biodegradable counting scintillant (Amersham), and counted using a liquid scintillation counter, to determine recovery rates. A quantity of the remaining portion was analyzed with an 8-isoprostane enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Absorbance values were measured by using a plate reader and a wavelength of 405 nm, and the raw data were corrected for recovery. The assay was validated by adding a series of known amounts of pure 8-iso-$PGF_{2\alpha}$ standard to equal volumes of purified plasma. The concentration of total 8-iso-$PGF_{2\alpha}$ in these samples was determined by using enzyme immunoassay (EIA). A high correlation (0.99) was obtained between the known amounts of pure 8-iso-$PGF_{2\alpha}$ added and the concentration determined by EIA. The antiserum used in this assay has a 100% cross-reactivity with 8-iso-$PGF_{2\alpha}$; 0.2% each with $PGF_{2\alpha}$, $PGF_{3\alpha}$, PGE_1, and PGE_2; and 0.1% with 6-keto-$PGF_{1\alpha}$. The detection limit of the assay is 4 pg/mL. This kit has been used to measure 8-iso-$PGF_{2\alpha}$ concentration in human plasma, bronchoalveolar lavage, and other fluids (15,18,22).

GSHPx enzyme assay. Whole blood was collected into EDTA-coated tubes and centrifuged at 8,500 × *g* at 4°C for 10 min. Plasma was discarded, and cells were washed with 10 vol of ice-cold buffer (50 mM Tris-HCl, pH 7.5, containing 5 mM EDTA and 1 mM dithiothreitol). Samples were centrifuged again at 8,500 × *g* at 4°C for 10 min, and supernatant was discarded. Cells were then lysed by adding exactly 4 vol of ice-cold deionized water. After centrifuging again at 8,500 × *g* at 4°C for 10 min, supernatant was collected and stored at −70°C for analysis. Erythrocyte GSHPx activities were measured using a GPx-340 spectrophotometric assay kit (Bioxytech; OXIS International, Portland, OR), to obtain values in units per mL. The hemoglobin (Hb) concentration of the samples was also measured using Sigma Kit No. 525 for Total Hemoglobin, to allow erythrocyte GSHPx activity to be expressed as units per g of Hb.

SOD enzyme assay. Whole blood was collected into EDTA-coated tubes, and centrifuged at 3,000 rpm at 4°C for 10 min. The erythrocyte pellet was separated and stored at −70°C before analysis. The erythrocyte pellet was thawed and resuspended in 4 vol of ice-cold water and vortexed thoroughly. Ice-cold extraction reagent (ethanol/chloroform, 62.5:37.5 vol/vol) was added to the erythrocyte suspension and vortexed for 30 s. Samples were centrifuged at 3,000 × *g* at 4°C for 10 min. The upper phase was collected and stored at −70°C for analysis. Erythrocyte Zn/Cu-SOD activities were measured using SOD-525 spectrophotometric assay kit (Bioxytech; OXIS International), to obtain values in units per mL. The Hb concentration of the samples was also measured using Sigma Kit No. 525 for Total Hemoglobin, to allow erythrocyte Zn/Cu-SOD activity to be expressed as units per mg of Hb.

Dietary intake. Dietary intake was assessed using the 24-h recall method (23). Analysis of food records was conducted using the Diet/1 Nutrient Calculation Software, which is based on the 1992 Australian food tables and the composition of Australian manufactured foods (24). The mean intakes of energy, protein, fat, carbohydrates, fiber, retinol, β-carotene, vitamin A equivalents, vitamin C, iron, and zinc for each subject group were determined from these.

Statistical analysis. Results were analyzed using a standard computer statistical package (Minitab Inc. Version 12 for Windows 1997, State College, PA). Data were tested for normality using the Anderson-Darling test. Statistical comparisons were performed using the paired Student *t*-test for normally distributed data and the Wilcoxon paired test for nonparametric data. Correlations between variables were studied by linear regression, with calculation of Pearson's correlation coefficient for normal data, and Spearman's rank correlation coefficient for nonparametric data. Subgroup analysis was done using the Kruskal-Wallis test for nonparametric variables and the analysis of variance test for normal variables (25). The mean ± standard error is reported for normal data; for nonparametric data the median (quartile 1 – quartile 3) is reported. Differences were considered significant when $P < 0.05$.

RESULTS

Demographic data are reported for 15 asthmatic subjects (median age 14.0 yr) and 15 age- and sex-matched healthy controls (median age 14.0 yr) (Table 2). There were no significant differences between the mean height or weight of the two groups. Pulmonary function testing revealed no difference in lung function between the groups as indicated by the percentage predicted FEV1, percentage predicted FVC, and

TABLE 2
Characteristics of Healthy Controls and Asthmatics

Variable	Controls (n = 15)	Asthmatics (n = 15)	P value
Sex (M/F)	8/7	8/7	
Age (yr)[a]	14.0 (11.0–29.0)	14.0 (11.0–29.0)	1.000
<18 yr	9	9	
≥ 18 yr	6	6	
Smoking status (Y/N)	0/15	0/15	
Height (cm)[b]	164.0 ± 3.8	165.7 ± 4.7	0.576
Weight (kg)[b]	60.8 ± 4.6	62.1 ± 6.4	0.754
%FEV1[b]	102.7 ± 2.7	99.5 ± 4.3	0.533
%FVC[b]	102.5 ± 3.0	103.6 ± 4.4	0.836
FEV1/FVC (%)[b]	86.1 ± 1.8	82.7 ± 1.8	0.164
White cell count (10^9/L)[a]	5.8 (5.6–6.3)	6.9 (5.0–8.4)	0.167
Neutrophil count (10^9/L)[b]	2.91 ± 0.23	3.21 ± 0.30	0.260
Eosinophil count (10^9/L)[a]	0.20 (0.10–0.23)	0.30 (0.20–0.40)	0.014

[a]Non-parametric data analyzed by using Wilcoxon paired test. Values reported are median (quartile 1 – quartile 3).
[b]Normal data analyzed using Student's paired *t*-test. Values reported are mean ± SEM.

FEV1/FVC (Table 2). Subjects were classified as infrequent episodic, frequent episodic, and persistent asthmatics (Table 3) (19).

As expected, the peripheral blood eosinophil count was significantly higher in the asthmatic group (P = 0.014) than in the controls, indicative of the disease often being atopic in nature (Table 2). Eosinophil count was associated with clinical asthma severity (P = 0.032) and inhaled corticosteroid use (P = 0.003).

Total (free and esterified) plasma 8-iso-$PGF_{2\alpha}$ levels were found to be elevated in the asthmatics compared to the controls (P = 0.042) (Fig. 1). The 8-iso-$PGF_{2\alpha}$ levels were associated with clinical asthma severity (P = 0.044) and inhaled corticosteroid use (P = 0.027) in asthmatics (Figs. 2A and 2B). All subjects had normal lung function (Table 2), and no correlation was observed between total 8-iso-$PGF_{2\alpha}$ levels and lung function. There was no relationship between lung function and disease severity.

Plasma levels of Zn and Se were both significantly lower in the asthmatics (P = 0.027 and 0.006, respectively) (Table 4), with Se levels correlating negatively with total plasma 8-iso-$PGF_{2\alpha}$ levels (r = –0.725, P = 0.002). Erythrocyte SOD activity was lower in the asthmatics, with this difference approaching significance (P = 0.076). SOD activity was negatively associated with clinical severity of asthma (P = 0.042), with the more severely asthmatic subjects having reduced SOD activity. There was, however, no significant difference in the erythrocyte activity levels of GSHPx and no association between GSHPx activity and clinical severity.

Plasma levels of the dietary antioxidants β-carotene and vitamins E and C were similar in the two groups. There was no correlation between total plasma 8-iso-$PGF_{2\alpha}$ and plasma dietary antioxidants. Dietary analysis indicates that there was no significant difference in the nutrient intake of the groups (Table 5).

DISCUSSION

This study demonstrated reduced levels of plasma Zn and Se, increased total plasma 8-iso-$PGF_{2\alpha}$ levels, and a negative cor-

TABLE 3
Clinical Characteristics of Asthmatics

Clinical severity, *n* (%)	Infrequent episodic	7 (47)
	Frequent episodic	4 (27)
	Persistent	4 (27)
Current medications, *n* (%)	Short-acting β_2-agonist	12 (80)
	Long-acting β_2-agonist	1 (7)
	Cromoglycate	2 (13)
	Ipratropium	1 (7)
	Inhaled corticosteroid	5 (33)
	Fluticasone propionate, 125 µg	3 (20)
	Budesidonide, 200–600 µg	2 (13)
Oral corticosteroid use for asthma in past 2 yr, *n* (%)		5 (33)
Duration of asthma, yr[a]		10.0 (8.0–16.0)

[a]Values reported are median (quartile 1 – quartile 3).

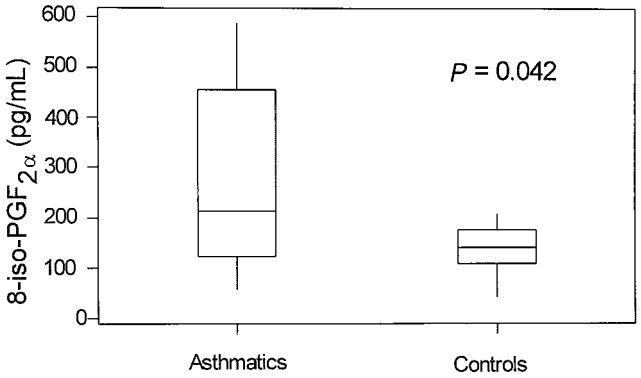

FIG. 1. Total plasma 8-iso-$PGF_{2\alpha}$ levels in 15 asthmatics vs. 15 age- and gender-matched controls. Plot shows medians and interquartile ranges (quartile 1 – quartile 3).

relation between plasma 8-iso-$PGF_{2\alpha}$ and Se levels in mild asthmatics, providing further evidence that oxidative stress is elevated in asthma. Moreover, this study showed a positive association between asthma severity and the level of total plasma 8-iso-$PGF_{2\alpha}$ and a negative association between asthma severity and erythrocyte SOD enzyme activity. By implication, simple dietary antioxidant supplementation may provide adjunctive therapy for asthmatics, especially those more severely affected.

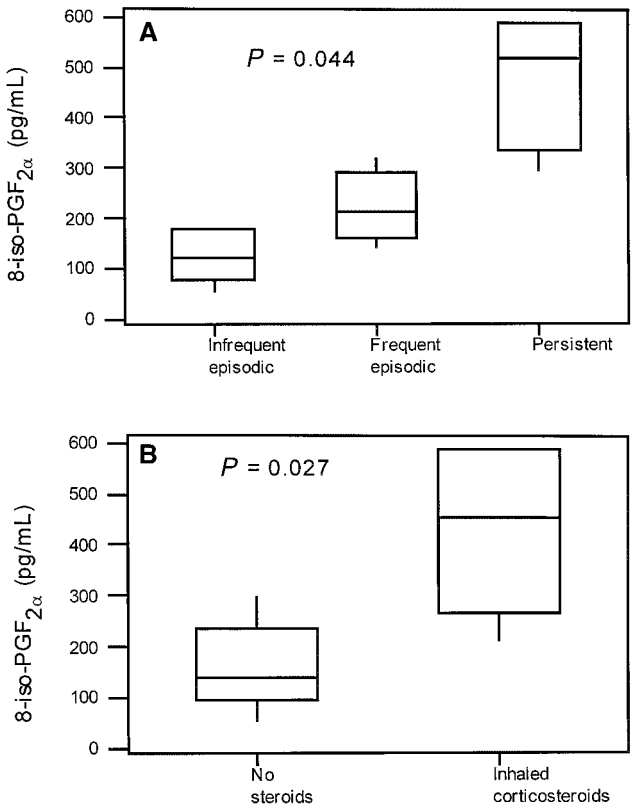

FIG. 2. (A) Total plasma 8-iso-$PGF_{2\alpha}$ levels in asthmatics with infrequent episodic (n = 7), frequent episodic (n = 4), and persistent (n = 4) asthma. An association between 8-iso-$PGF_{2\alpha}$ and asthma severity is observed (P = 0.044). (B) Total plasma 8-iso-$PGF_{2\alpha}$ levels in steroid-naïve asthmatics (n = 10) vs. asthmatics using inhaled corticosteroids (n = 5). An association between 8-iso-$PGF_{2\alpha}$ and steroid use is observed (P = 0.027). Plots show medians and interquartile ranges (quartile 1 – quartile 3).

Inflammation of the airways is believed to be a main contributor to oxidative stress in asthma (1). Inflammatory cells that may release ROS into asthmatic airways include mast cells, macrophages, neutrophils lymphocytes, platelets, and, in particular, eosinophils (1). In our study, the higher eosinophil count in the asthmatics and the association between eosinophil count and asthma severity demonstrate that severity of asthma is related to the degree of inflammation. Furthermore, the positive association demonstrated between eosinophil count and inhaled corticosteroid use indicates that inflammation persists despite steroid use in these patients.

As inflammation is persistent and increases with disease severity in the asthmatics, it is not surprising that we observed an elevation in oxidative stress, as measured by plasma 8-iso-$PGF_{2\alpha}$ levels, and a positive association between 8-iso-$PGF_{2\alpha}$ levels and asthma severity. A similar trend was recently reported by Montuschi *et al.* (18), who reported elevated 8-iso-$PGF_{2\alpha}$ levels in breath condensate of asthmatics, with 8-iso-$PGF_{2\alpha}$ levels increasing as asthma severity increased. The positive association we observed between 8-iso-$PGF_{2\alpha}$ levels and inhaled corticosteroid use also agrees with the work of Montuschi *et al.* (18), who reported higher 8-iso-$PGF_{2\alpha}$ in breath condensate of severe asthmatics using oral steroids, compared to mild asthmatics who were steroid-naive. In contrast, studies using other indices of oxidative stress have reported inhibition of oxidation in response to steroid use (3,26). In our study, it is likely that 8-iso-$PGF_{2\alpha}$ concentrations remained elevated in patients using inhaled corticosteroids because the treatment had not effectively controlled their asthma, resulting in residual inflammation and oxidative stress. Studies examining the relationship between steroid use, residual inflammation, and oxidative stress are warranted.

As isoprostanes have also been observed to be potent vasoconstrictory agents in rat lungs (27), one can speculate about their contribution to the airway narrowing that is characteristic of asthma. Although the situation in humans *in vivo* may be different, the full impact of elevated isoprostane levels on pulmonary function remains to be established, and isoprostanes may prove to have an important biological role as well as being an important *in vivo* marker of oxidative stress.

While there is evidence suggesting 8-iso-$PGF_{2\alpha}$ may also be produced enzymatically by cyclooxygenase activity in some cells and tissues (28), this does not appear to occur in humans *in vivo* (29). Thus, this marker is still believed to be an accurate indicator of oxidative stress (30). The EIA methodology used in this study to measure 8-iso-$PGF_{2\alpha}$ provides an inexpensive, accessible alternative to analysis by gas chromatography–mass spectrometry (GC–MS). The values of total 8-iso-$PGF_{2\alpha}$ we observed in normal plasma using the EIA method are similar to those obtained by Morrow *et al.* using GC–MS (31). As discussed by Morrow and Roberts (16), GC–MS assay is expensive and labor-intensive and uses technology that is not widely available. Thus, the use of spe-

TABLE 4
Biochemical Markers—Plasma Levels of 8-iso-$PGF_{2\alpha}$, Vitamin E, Vitamin C, β-Carotene, Vitamin A, Zn, Se, Cu, and Erythrocyte Levels of GSHPx and SOD Activity

Biochemical marker	Controls	Asthmatics	*P* value
8-Iso-$PGF_{2\alpha}$ (pg/mL)[a]	139 (109 – 174)	213 (122 – 455)	0.042
Vitamin E (μmol/L)[a]	17 (14 – 21)	17 (15 – 20)	0.900
Vitamin C (μmol/L)[b]	57.2 ± 7.4	54.2 ± 7.1	0.562
β-Carotene (μmol/L)[a]	0.4 (0.2 – 0.6)	0.3 (0.2 – 0.6)	0.701
Vitamin A (μmol/L)[a]	1.6 (1.4 – 1.9)	1.6 (1.3 – 1.9)	0.286
Zn (μmol/L)[b]	12.8 ± 0.4	11.5 ± 0.4	0.027
Se (μmol/L)[b]	1.35 ± 0.06	1.16 ± 0.08	0.006
Cu (μmol/L)[a]	14.5 (13.0 – 16.2)	14.1 (12.3 – 17.9)	0.932
GSHPx (U/g Hb)[b]	18.2 ± 1.3	19.8 ± 1.1	0.361
SOD (U/mg Hb)[b]	2.03 ± 0.07	1.85 ± 0.06	0.076

[a]Non-parametric data analyzed by using Wilcoxon paired test. Values reported are median (quartile 1 – quartile 3).
[b]Normal data analyzed using Student's paired *t*-test. Values reported are mean ± SEM. GSHPx, glutathione peroxidase; SOD, superoxide dismutase; Hb, hemoglobin.

cific immunoassays has expanded research in this area, with several studies using the EIA methodology recently being reported (15,18,22).

Our data showed a significant deficiency in plasma Zn levels in asthmatics, supporting results from other researchers (32). Zn plays an important role as an antioxidant, with the probable mechanisms being stabilization of sulfhydryl groups to prevent intramolecular disulfide formation, displacement of bound Cu and Fe to prevent electron transfer, and reduction of free radical production in neutrophils (33). Thus, the consequence of Zn deficiency in the asthmatics is reduced antioxidant protection.

Zn is also a cofactor for the antioxidant enzyme, Cu,Zn-SOD. Located primarily in the cytosol and mitochondria of cells, SOD catalyzes the dismutation of the superoxide anion into oxygen and hydroperoxide, which is then acted upon by GSHPx to form water. The activity and synthesis of this enzyme, however, are not decreased by dietary Zn deficiency (34), as Zn can be replaced at the structural site by other metals. Previous studies of SOD activity in asthmatics are inconsistent, with both decreased activity (7,26) and increased activity (8) being reported. These data are difficult to compare owing to variations in disease severity and differences in the cell types and blood components being measured. In our study, erythrocyte SOD activity was not significantly reduced in the asthmatics. There was, however, a negative association between SOD activity and asthma severity. This suggests that SOD levels are only diminished in the case of severe oxidative stress, when the oxidant burden exceeds the host's ability to upregulate antioxidant enzyme protection.

The asthmatic subjects in our study also had low plasma Se levels, supporting results of other researchers, who have reported decreased levels of Se in whole blood (5,9), plasma/serum (6,9), and erythrocytes (6). Se is an essential component of the GSHPx enzyme, necessary for both its synthesis and activity. Thus, many reports have linked Se deficiency to a decrease in GSHPx activity (5,6). Our data, however, which are also supported by other researchers (9,26), showed no deficiency in erythrocyte GSHPx activity despite low plasma Se levels. This suggests that Se has another func-

TABLE 5
Nutrient Intake of Healthy Controls and Asthmatics

Nutrient/kg body weight	Controls	Asthmatics	*P* value
Energy (kJ)[a]	171.3 ± 12.4	158.6 ± 10.7	0.360
Protein (g)[a]	1.7 ± 0.2	1.3 ± 0.1	0.116
%Protein[a]	16.9 ± 1.0	14.7 ± 0.9	0.119
Fat (g)[a]	1.5 ± 0.2	1.3 ± 0.2	0.415
%Fat[b]	32 (30 – 36)	31 (21 – 41)	0.496
Carbohydrate (g)[a]	5.1 ± 0.4	5.1 ± 0.3	0.948
%Carbohydrate[a]	49.7 ± 2.4	53.9 ± 3.1	0.269
Fiber (g)[a]	0.35 ± 0.03	0.34 ± 0.01	0.981
Vitamin A (μg)[a]	6.8 ± 1.1	7.0 ± 1.1	0.926
β-Carotene (μg)[b]	42.6 (18.1 – 90.6)	16.7 (11.0 – 36.1)	0.164
Vitamin C (mg)[b]	1.7 (1.1 – 3.4)	2.0 (1.3 – 3.3)	0.932
Iron (mg)[a]	0.22 ± 0.02	0.20 ± 0.02	0.442
Zinc (mg)[b]	0.20 (0.10 – 0.28)	0.11 (0.09 – 0.18)	0.201

[a]Normal data analyzed using Student's paired *t*-test. Values reported are mean ± SEM.
[b]Non-parametric data analyzed using Wilcoxon paired test. Values reported are median (quartile 1 – quartile 3).

tion, independent of its association with GSHPx. The negative correlation observed between Se and total plasma 8-iso-$PGF_{2\alpha}$ levels suggests that Se has a role as an antioxidant. In humans, only about 10% of total erythrocyte Se is bound to GSHPx (35). Much of the remaining Se is incorporated into several different selenoproteins, most of which have redox functions, suggesting that an antioxidant role is likely. Selenoprotein P is one such protein, which has been identified as having a protective effect against oxidation (36). In the case of Se deficiency, as observed in our asthmatics, it is possible that while the available Se is used to maintain GSHPx activity, the production and activity of other selenoproteins such as Selenoprotein P have been reduced, thereby reducing overall antioxidant protection. In future studies, measurement of these selenoproteins will be important if the role of Se in asthma is to be better understood.

Plasma concentrations of vitamins C and E and β-carotene in the asthmatics showed no deficiencies compared to the controls. Although some have found similar results (37), others have shown a decrease in plasma levels of vitamin C (38) and erythrocyte vitamin E (4). This inconsistency may be due to differences in the dietary intake and/or asthma severity of the subjects in each study. In our study, patients had relatively mild disease (no subjects required oral steroids). Also, the dietary analysis indicates that the nutrient intake of the asthmatics in our study was similar to the control group. Thus, while the presence of ROS was apparently elevated in the asthmatics, the utilization of these antioxidant vitamins was not increased. This suggests that the mechanisms described above, involving the minerals Se and Zn, may be the first line of antioxidant defense in asthma. Alternatively, another recent report (10) suggested that peripheral blood markers may be less sensitive than direct lung measurements. Thus, plasma antioxidant levels may be a poor reflection of local antioxidant defenses in the lung and while plasma antioxidant vitamin levels are normal, deficiencies may be occurring in the lung lining fluid. This highlights the importance of directly measuring the antioxidant defenses in the lung in future studies.

In conclusion, this study indicates that oxidative stress is elevated and contributes to the clinical severity of asthma, with a deficiency in plasma Se and Zn being usual. As these minerals have been shown to play an important role in antioxidant defense, it would be sensible to supplement asthmatic patients with these nutrients, in order to minimize the effects of oxidant stress. A previous report of Se supplementation improving antioxidant defenses and clinical symptoms in asthmatics (39) is encouraging and suggests that further investigation of oxidative stress during Se and Zn supplementation is warranted. The discovery of 8-iso-$PGF_{2\alpha}$ as the best indicator of oxidative stress *in vivo* allows examination of the effects of future antioxidant interventions in asthmatics.

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