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Ascorbic acid supplementation attenuates exercise-induced bronchoconstriction in patients with asthma ☆

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KEYWORDS
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Summary
Background: Previous research has shown that diet can modify the bronchoconstrictor response to exercise in asthmatic subjects.
Objective: Determine the effect of ascorbic acid supplementation on pulmonary function and several urinary markers of airway inflammation in asthmatic subjects with exercise-induced bronchoconstriction (EIB).
Methods: Eight asthmatic subjects with documented EIB participated in a randomized, placebo controlled double-blind crossover trial. Subjects entered the study on their usual diet and were placed on either 2 weeks of ascorbic acid supplementation (1500 mg/day) or placebo, followed by a 1-week washout period, before crossing over to the alternative diet. Pre- and post-exercise pulmonary function, asthma symptom scores, fraction of exhaled nitric oxide (F_ENO), and urinary leukotriene (LT) C_4–E_4 and 9α, 11β-prostagladin (PG)F_2] were assessed at the beginning of the trial (usual diet) and at the end of each treatment period.
Results: The ascorbic acid diet significantly reduced ($p<0.05$) the maximum fall in post-exercise FEV_1 ($-6.4\pm2.4\%$) compared to usual ($-14.3\pm1.6\%$) and placebo diet ($-12.9\pm2.4\%$). Asthma symptoms scores significantly improved ($p<0.05$) on the ascorbic acid diet compared to the placebo and usual diet. Post-exercise F_ENO, LTC_4–E_4 and 9α, 11β-PGF_2 concentration was significantly lower ($p<0.05$) on the ascorbic acid diet compared to the placebo and usual diet.

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Conclusion: Ascorbic acid supplementation provides a protective effect against exercise-induced airway narrowing in asthmatic subjects.

Introduction

Exercise is a powerful trigger of asthma symptoms, and up to 90% of asthmatics experience exercise-induced bronchoconstriction (EIB) a condition in which there is a transient narrowing of the airways during and/or following exercise.[1] EIB is not an isolated disorder or specific disease, but rather part of the spectrum of asthmatic disease where exercise is one of many stimuli that may induce airflow limitation.[2]

The trigger for EIB most likely involves drying and cooling of the intrathoracic airway as the result of an increased ventilatory demand during exercise.[2] It has been suggested that dry air hyperpnea initiates a transient dehydration, which causes an increase in the osmolarity of the airway surface fluid,[3] thereby activating epithelial cells, histamine and proinflammatory eicosanoids (prostaglandins and leukotrienes) and subsequent bronchoconstriction.[4] It has also been suggested that rapid rewarming of the airways following exercise may lead to vascular hyperemia and airway edema,[5] which may contribute further to the airway narrowing.

At present the treatment of EIB almost exclusively involves the use of pharmacotherapy to treat this condition. However, there is mounting evidence that dietary modification and/or supplementation has potential to modify the EIB response in asthmatic individuals.[6] Dietary antioxidants and their influence on the severity of EIB are of particular interest because oxidative damage has been implicated in asthma.[7] Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been shown to induce proinflammatory cell activation, increase mucus secretion, and increase hyperresponsiveness, all of which lead to epithelial damage and airway inflammation.[7] Thus, antioxidant supplementation may be instrumental in reducing EIB severity by inhibiting this cascade of proinflammatory events as a result of neutralizing the effects of excess ROS/RNS.

Ascorbic acid (vitamin C) is the most extensively investigated antioxidant for effects on asthma and has been shown in numerous case–control and cross-sectional studies to be associated with a reduced risk of asthma.[8] Interventional studies investigating the role of either a single dose or extended antioxidant supplementation on EIB have generally shown a protective effect.[9–15]

However, no studies to date have attempted to investigate a number of biological markers of airway inflammation associated with EIB on a diet supplemented with ascorbic acid. Therefore, the main aim of this study was to determine the effects of 2 weeks of ascorbic acid supplementation on pulmonary function and several urinary markers of airway inflammation in asthmatic subjects with EIB. We hypothesized that ascorbic acid supplementation would decrease markers of airway inflammation and reduce the bronchoconstrictor response to exercise.

Methods

Subjects

Eight subjects (2 males, 6 females, age 24.5 ± 4.8 yr, $\dot{V}O_{2\,max}$ 48.7 ± 10.9 ml/kg/min, height 176.8 ± 13.5 cm) with physician-diagnosed asthma were recruited from a population of university students and the local community, and all indicated that they were recreationally active. All subjects had clinically treated mild-to-moderate persistent asthma with an FEV_1 greater than 70% of predicted (Table 1), and documented EIB (usual diet) as indicated by a drop of greater than 10% in post-exercise FEV_1 compared with pre-exercise values.[16]

All subjects had a history of chest tightness, shortness of breath and intermittent wheezing following exercise. As per previous studies, subjects were asked to discontinue taking leukotriene receptor antagonists [(LTRAs; $N = 3$ montelukast (Singulair®)] 12 h prior to testing,[10,13,14] and 4 days prior to testing to abstain from taking combined inhaled corticosteroids (ICS) and long-acting β_2-agonists [$N = 2$, fluticasone propionate (Flovent®) and salmeterol (Advair®)] or combined long-acting β_2-agonists and LTRAs [$N = 3$ salmeterol (Advair®) and montelukast (Singulair®)].[9] In addition, all subjects were asked to refrain from taking caffeine, and to avoid physical activity 12 and 24 h, respectively, before exercise testing. Subjects were asked to abstain from taking antioxidant supplements other than those given during the course of the study. Each subject completed a health questionnaire and gave written informed consent prior to enrollment in the study. All testing procedures and informed consent were approved by the

Table 1 Pre-exercise (baseline) pulmonary function.

	Diet		
	Usual	Ascorbic acid	Placebo
FVC (l)	4.39 ± 0.42	4.29 ± 0.52	4.34 ± 0.48
% Predicted	90.0 ± 6.8	87.2 ± 8.9	88.5 ± 7.9
FEV_1 (l)	3.82 ± 0.37	3.65 ± 0.38	3.77 ± 0.37
% Predicted	97.0 ± 6.1	92.2 ± 7.0	95.5 ± 7.0
$FEF_{25-75\%}$ (l/min)	4.2 ± 0.36	4.1 ± 0.37	4.2 ± 0.42
% Predicted	100.4 ± 8.7	98.8 ± 8.7	100.6 ± 11.7
FEV_1/FVC	87.1 ± 3.2	87.1 ± 3.9	87.9 ± 2.6
% Predicted	79.1 ± 1.2	79.1 ± 4.2	79.4 ± 6.2

Definition of abbreviations: FVC, forced vital capacity; FEV_1, forced expiratory volume in 1-s; $FEF_{25-75\%}$, forced expiratory flow at 25–75% of FVC.
Values are mean ± SD. There were no significant difference ($p > 0.05$) for any variables between diets.

Indiana University Human Subjects Committee Institutional Review Board.

Study design and protocol

The study was conducted as double-blind, randomized, crossover trial over 5 consecutive weeks with each subject serving as their own control. All subjects entered the study on their usual diet. Subsequently, they were randomly assigned to either a pharmaceutical grade ascorbic acid supplement [1500 mg/day (3×500 mg capsules), NOW foods, Bloomingdale, IL] or a matched (color/size) placebo [(3 capsules/day of sucrose), NOW foods, Bloomingdale, IL] for a 2-week period. Thereafter, they followed a 1-week washout period. Afterward, subjects were then assigned the alternate treatment/placebo for the remaining 2 weeks. Subjects were advised to avoid foods that were high in Vitamin C during the study period so that any effects on dependent variables could be attributed to the ascorbic acid supplement.

Upon entering the study on their usual diet and following each 2-week supplementation period, pulmonary function was assessed pre- and post-exercise at 1, 5, 10, 15, 20, and 30 min. The fraction of exhaled nitric oxide (F_ENO), an indirect marker of airway inflammation, was measured pre-exercise and at 30 and 120 min post-exercise. Urine samples were taken pre-exercise and 90 min post-exercise and analyzed for the presence of proinflammatory mediators [cysteinyl leukotriene (LT) C_4–E_4 and 9α, 11β-prostaglandin (PG)F_2)]. Urine samples were stored at $-80\,^{\circ}C$ until analysis. Twenty-four hour dietary recalls were administered upon entry into the study and during each supplementation period. At the end of the 1-week washout period pulmonary function was assessed pre- and post-exercise to ensure that values had returned to baseline.

Exercise challenge testing

Each subject ran on a motorized treadmill (Quinton, Bothell, WA) which was elevated 1% per minute until 85% of age predicted maximum heart rate and ventilation exceeding 40–60% of predicted MVV (MVV estimated by $35 \times FEV_1$).[16] Subjects maintained this exercise intensity for 6 min.[16] Following the 6-min steady state exercise, the grade of the treadmill continued to increase at 1% per minute until volitional exhaustion. Heart rate was monitored using a F1 Polar Heart Rate Monitor (Polar, Helsinki, Finland). Breath-by-breath analysis of expired gases was accomplished by indirect open circuit calorimetry (SensorMedics Vmax 22 metabolic cart, SensorMedics Corp., Yorba Linda, CA).

Pulmonary function

Spirometry was performed in all subjects using a computerized pneumotachograph spirometer (SensorMedics Vmax 22, SensorMedics Corp., Yorba Linda, CA) according to American Thoracic Society recommendations.[17] The maximum percentage fall in FEV_1 from the baseline (pre-exercise) value was calculated using the following equation: (pre-exercise FEV_1 − lowest post-exercise FEV_1)/(pre-exercise FEV_1). In addition, bronchoconstriction was assessed using area under the curve of the percentage fall in post-exercise FEV_1 plotted versus time for 30 min (AUC_{0-30}). The AUC_{0-30} was computed using trapezoidal integration.

Fraction of exhaled nitric oxide

Fraction of exhaled nitric oxide (F_ENO) was measured with an online measurement of resting values using a restricted exhaled breath protocol (NOA 280i Nitric Oxide Analyzer, Accurate NO Breath Kit, Thermal Mass Flowmeter, NO Analysis software Version 3.21, Sievers Instruments, Boulder, CO). Measurements were conducted as outlined by American Thoracic Society guidelines.[18] Three exhalations were performed with nose clips at each test with at least 30 s between exhalations. The procedure entailed maximal inhalation to total lung capacity and immediate exhalation against expiratory resistance for at least 6 s to obtain a NO plateau lasting at least 3 s. Subjects were instructed to maintain a flow rate of 50 ± 10 ml/s as monitored by a computer display.

Asthma symptom questionnaire

In order to document asthma symptoms during the course of the study, subjects were required to complete the symptoms section (12 items) of the self-administered Asthma Quality of Life Questionnaire (AQLQ) at the beginning of the study and at the end of each treatment period. The symptoms section of the questionnaire asks subjects to rank the severity of their symptoms over each 2-week treatment period, and to respond to each question on a 7-point interval scale (e.g., 1 = a very great deal of discomfort or distress to 7 = no discomfort or distress). Results are analyzed directly from the scores recorded on the questionnaire, with the overall score being expressed as a mean of all the items. The minimum important difference, for both quality of life and individual domains, has been calculated as a change of 0.5 per item; a change of 1.0 is considered moderate and a change of 1.5 is considered large.[19]

Urinary 9α, 11β PGF_2 and LTC_4–E_4 quantification

The urinary concentrations of inflammatory mediators (LTC_4–E_4 and 9α, 11β-PGF_2) were determined by enzyme-linked immunoassay (Neogen, Lexington, KY). The plates were washed using an EL $\times$ 405 Automated Plate Washer (Bio-Tek Instruments, Winooski, VT) and read at 650 nm using a Powerwave XS Spectrophotometer (Bio-Tek Instruments, Winooski, VT). Inter-assay and intra-assay coefficients of variation for both eicosanoid kits were $<10\%$. Inflammatory mediator concentration was adjusted for creatinine concentration (Cayman Chemicals, Ann Arbor, MI). The intra-assay and inter-assay coefficient of variation for creatinine was 2.7% and 3%, respectively.

Dietary analysis and compliance

Dietary intake was assessed for three 24-h periods during the study for each subject. Twenty four hour dietary recalls were collected by a registered dietitian in a personal

interview. Recalls were collected using Nutrition Data System for research software (University of Minnesota, School of Public Health, St. Paul, MN). All subjects participated in one interview at the beginning of the study to reflect their usual dietary intake. The second and third interviews occurred during the ascorbic acid supplementation and placebo periods. These recalls were conducted to see if the subjects were indeed complying with the restriction instructions and that diet patterns were similar over the study period. Adherence to the supplementation regimen was assessed by asking the subjects to document the dose of capsules consumed daily. In addition, subjects were asked to return any unused capsules, another indicator of adherence.

Statistical analysis

Data were analyzed using SPSS version 13.0 statistical software. A repeated-measures ANOVA was used to analyze the data with both treatment and time as the "within-subject" effects. A Mauchly's test was conducted to determine if sphericity was violated. In cases where sphericity was violated, a Gieser-Greenhouse correction was applied. Where a significant *F*-ratio was found ($p<0.05$), Fisher's protected least-square difference post hoc test was used to isolate differences in group means ($p<0.05$). Diet recalls were compared between study periods using 2×2 repeated-measures ANOVA. Dietary vitamin intake such as ascorbic acid and other antioxidant nutrients, as well as macronutrient intake, were compared. A pairwise *t*-test was used to assess differences in symptom scores between diets. Data were analyzed for the presence of carryover effects between treatments using a 2×2 ANOVA. Statistical significance was set at $p<0.05$. Data are expressed as mean and their 95% confidence intervals (95% CI). Effect size (ES) estimates were calculated using omega-squared.[20] With eight asthmatic EIB patients, the data had at least 80% power to detect statistical significance. Our power calculation was based on upon the work of Neuman et al.[13,14] who demonstrated an ES of 0.38 and 0.50 , for a study power of 0.97 and 0.98, respectively.

Results

The intake of dietary ascorbic acid in the subjects' usual diets ranged from 1.4 to 230.6 mg (mean; 133.6 mg), but the ascorbic acid intake during the supplementation and placebo periods ranged from 16.4 to 86.8 mg (mean; 49.4 mg), and from 3.41 to 170.29 mg (mean; 49.9 mg), respectively. The lower dietary ascorbic acid intake during supplementation period indicated compliance to the restriction instruction. The 2×2 repeated measure ANOVA also indicated no significant difference ($p>0.05$) in dietary ascorbic acid intake between supplementation and placebo groups. Intake of carbohydrates, protein, and fat were similar among periods, suggesting diet patterns were consistent. Also, intake of antioxidants did not differ, thus, dietary intake did not confound supplementation effects.

A 2×2 ANOVA used to test for the presence of carryover effects between diets indicated that none were present for all dependent variables; this was further supported by pre- and post-exercise pulmonary function values at the end of the washout period returning to baseline values established upon entry into the study (usual diet). The absence of a significant difference ($p>0.05$) in minute ventilation ($\dot{V}_E$) between any of the treatment periods suggests that differences in dependent variables between treatments were not due to changes in $\dot{V}_E$ but rather due to changes in diet.

No significant difference ($p>0.05$) was observed in baseline (pre-exercise) pulmonary function between diets (Table 1). The maximum percentage drop in FEV_1 post-exercise on the ascorbic acid diet was −6.4% (95% CI, −12.0 to −0.8%; ES, 0.40) which is indicative of an attenuated EIB response. This was significantly different ($p<0.05$) from the maximum drop of −12.9% (95% CI, −18.6 to −12.3%) and −14.3% (95% CI, −18.1 to −10.5%) on the placebo and usual diet (Fig. 1). The post-exercise bronchoconstrictor response, as determined by the AUC_{0-30}, was significantly less ($p<0.05$) on the ascorbic acid diet (−72.5; 95% CI, −214.5 to 69.4; ES, 0.36) compared to the placebo (−255.8; 95% CI, −385.2 to −126.3) or usual diet (−220.9; 95% CI, −306.2 to −135.7).

A significant difference ($p<0.05$) was observed for post-exercise F_ENO on the ascorbic acid diet compared to usual and placebo diet. Mean F_ENO for the ascorbic acid diet was 25.0 ppb (95% CI, 7.3–40.3 ppb) at 30 and 22.6 ppb (95% CI, 8.1–42.3) at 120 min post-exercise as compared to 38.3 ppb (95% CI, 17.5–50.7 ppb) at 30 and 38.0 ppb (95% CI, 15.3–51.7) at 120 min post-exercise for the placebo diet, and 42.5 ppb (95% CI, 13.8–83.0 ppb) at 30 and 47.1 ppb (95% CI, 15.8–85.3) at 120 min post-exercise for the usual diet. Post-exercise F_ENO was not significantly different ($p>0.05$) between the placebo and usual diet (Fig. 2).

A significant improvement ($p<0.05$) in mean asthma symptom scores was observed (6.3; 95% CI, 5.8–6.8) on the ascorbic acid diet compared to the placebo diet (5.8; 95% CI, 5.1–6.2) and usual diet (5.6; 95% CI, 5.0–6.3). However, no significant difference ($p>0.05$) was observed in mean asthma symptoms scores between the usual and placebo diet.

Mean urinary 9α, 11β PGF_2 and cyst-LTC_4–E_4 levels are shown in Figs. 3 and 4, respectively. Pre-exercise urinary 9α, 11β PGF_2 and cyst-LTC_4–E_4 concentrations were not significantly different ($p>0.05$) between diets. However, the urinary concentration of post-exercise 9α, 11β PGF_2 was significantly less ($p<0.05$) on the ascorbic acid diet (5.2 ng mmol creatinine^{-1}; 95% CI, 4.1–6.6 ng mmol creatinine^{-1}) compared to the placebo (9.9 ng mmol creatinine^{-1}; 95% CI, 6.2–15.3 ng mmol creatinine^{-1}) and usual diet (13.8 ng mmol creatinine^{-1}; 95% CI, 4.7–18.7 ng mmol creatinine^{-1}) (Fig. 3). The post-exercise urinary concentration of LTC_4–E_4 on the ascorbic acid diet was 5.8 ng mmol creatinine (95% CI, 1.1–9.4 ng mmol creatinine), which was significantly lower ($p<0.05$) than the placebo (14.3 ng mmol creatinine; 95% CI, 5.4–16.4 ng mmol creatinine) and usual diet (17.1 ng mmol creatinine; 95% CI, 6.5–19.3) (Fig. 4).

Discussion

This double-blind, randomized, crossover, placebo-controlled study has demonstrated that 1500 mg/d of ascorbic

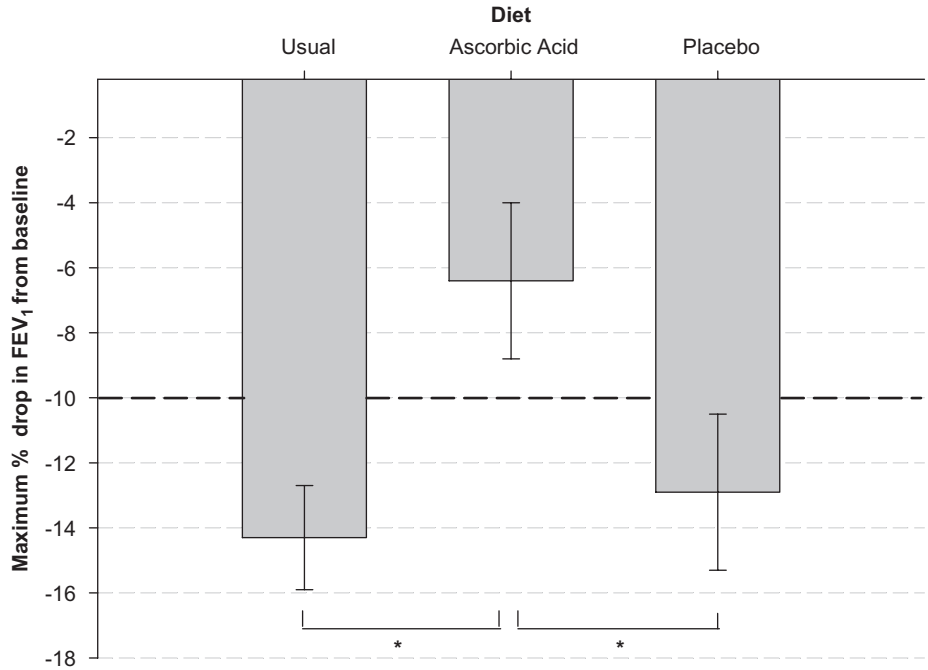

Figure 1 The percent change in FEV_1 from pre- to post-exercise across the three diets. Reductions in post-exercise FEV_1 in excess of 10% represent a positive diagnosis of exercise-induced bronchoconstriction. *Ascorbic acid diet significantly different ($p<0.05$) from usual and placebo diet. No significant difference ($p>0.05$) was observed for percent change in FEV_1 pre- to post-exercise between usual and placebo diet.

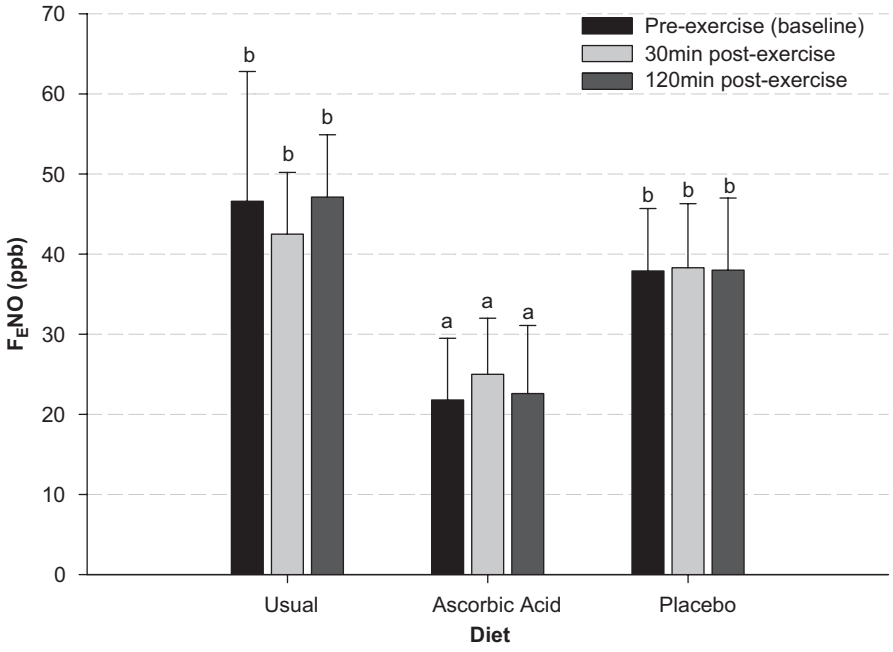

Figure 2 Mean fraction of exhaled nitric oxide (F_ENO) concentration (ppb). A difference in letter (a to b) designates a significant difference ($p<0.05$) across diet within time.

supplementation for 2 weeks attenuates the bronchoconstrictive response to exercise in asthmatic subjects. The ascorbic acid supplemented diet significantly improved post-exercise pulmonary function as demonstrated by the reduction in post-exercise FEV_1, and reduced the severity of EIB as measured by AUC_{0-30}. The maximum post-exercise fall in FEV_1 was approximately halved on ascorbic acid supplemented diet compared to the usual and placebo diet.

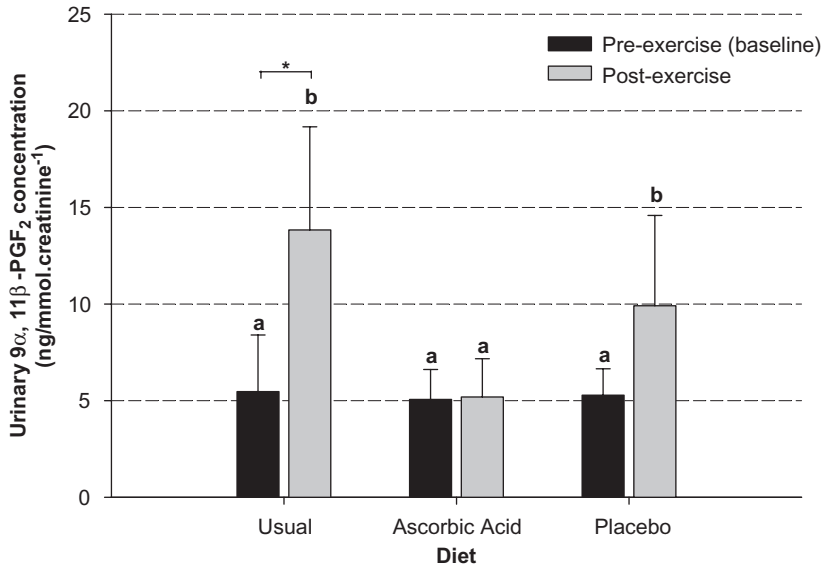

Figure 3 Mean urinary 9α, 11β-PGF_2 excretion (ng mg mmol creatinine^{-1}). A difference in letter (a to b) designates a significant difference ($p<0.05$) across diet within time. *Denotes a significant difference ($p<0.05$) between pre- and post-exercise value.

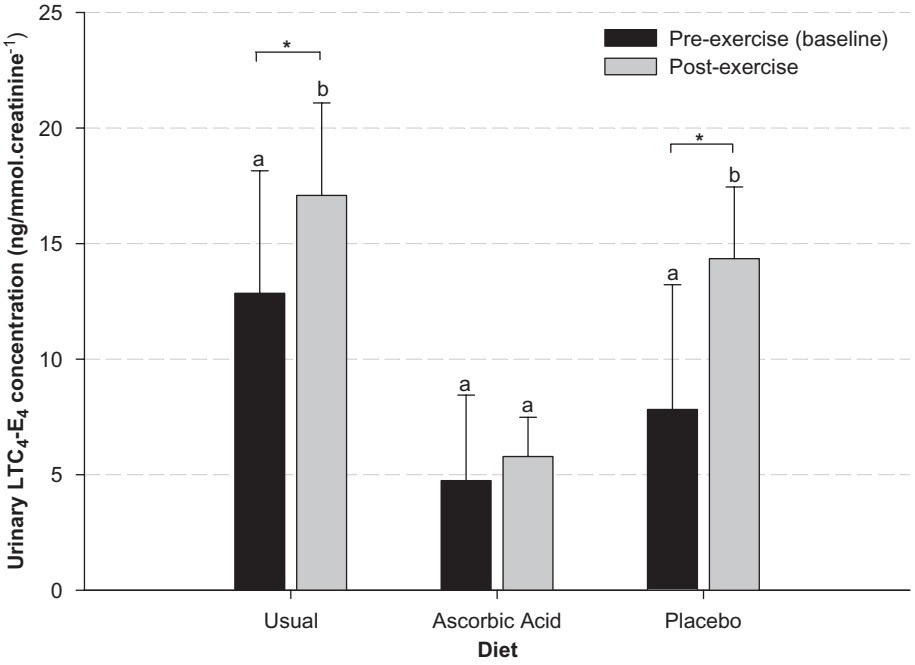

Figure 4 Mean urinary LTC_4–E_4 excretion (ng mg mmol creatinine^{-1}). A difference in letter (a to b) designates a significant difference ($p<0.05$) across diet within time. *Denotes a significant difference ($p<0.05$) between pre- and post-exercise value.

In addition, the intensity of asthma symptoms was significantly reduced on the ascorbic acid diet compared to the usual and placebo diet.

The maximum decrease in FEV_1 post-exercise was −14.3% and −12.9% on the usual and placebo diets, respectively. It is possible that these modest reductions in post-exercise FEV_1 are related to the continuation of pharmacotherapy between tests. Even though usual medications were withheld prior to testing, as per previous studies,[9,10,13,14] it is feasible that taking these medications between tests may have had a protective effect against more severe bronchoconstriction developing following exercise. Nonetheless, we believe that the reduction in severity of EIB found in the subjects following the ascorbic acid-supplemented diet is of clinical significance, since the ascorbic acid diet reduced the maximum post-exercise fall in FEV_1 by about 56% (ES, 0.40), and reduced post-exercise F_ENO, cyst-LTC_4–E_4 and 9α, 11β PGF_2 concentrations by approximately 47%, 66% and 62%, respectively, compared to the usual diet.

The present study is the first to measure markers of airway inflammation on ascorbic acid supplementation in

asthmatic subjects with EIB. The ascorbic acid diet caused a significant suppression of the proinflammatory urinary eicosanoids, LTC_4–E_4 and PGD_2 metabolite 9α, 11β PGF_2. Measurement of inflammatory mediators in urine has been used extensively in clinical and investigative monitoring of asthmatic patients, and previous studies have indicated that urinary concentrations of LTE_4 and 9α, 11β PGF_2 increase after exercise in adults with asthma[21–23] and elite athletes with EIB.[24] 9α, 11β-PGF_2, the initial urinary metabolite of prostaglandin D_2, is a sensitive marker of mast cell activation in the airways, and a potent bronchoconstrictor.[23] Metabolism of inhaled doses of bronchoconstrictive mediators LTC_4 and LTE_4 in asthmatic patients strongly supports the use of urinary cysteinyl-LTs as an index specifically reflecting cysteinyl-LT release in airways of patients with asthma.[25] Cysteinyl-LTs are important in the pathogenesis of EIB since they have been shown to increase following exercise in asthmatic subjects,[26] and to directly increase eosinophilic airway inflammation and cause bronchial smooth muscle contraction.[27]

Previous studies have shown that F_ENO can be used as an indirect marker of asthmatic airway inflammation and can be correlated to asthma severity, airway hyperresponsiveness[28] and severity of EIB.[29] Exhaled NO, which is derived from NO synthase (NOS), can be detrimental because peroxynitrite, a major metabolite of NO, causes airway epithelial damage and airway hyperresponsivess,[30] or conversely, is bronchoprotective in asthma through a direct action on bronchial smooth muscle.[31] The increased levels of F_ENO observed in asthmatic compared to nonasthmatic individuals is thought to be secondary to increased airway expression of inducible NO (iNOS), which can be found in a number of cells within the respiratory epithelium such as monocytes and macrophages, and can be stimulated by endogenous cytokines.[32] In the present study baseline and post-exercise F_ENO was significantly reduced on the ascorbic acid diet, suggesting an amelioration of airway inflammation. Mohsenin et al.[33] have demonstrated a protective effect of oral ascorbic acid (500 mg qd) on NO_2-induced airway hyperresponsiveness in humans, suggesting that oral doses of ascorbic acid supplementation are capable of reaching the respiratory tract.

In pulmonary diseases such as asthma, oxidant stress induced mainly via inflammatory mechanisms inflicts tissue injury, sensitizes cells in the lung to proinflammatory mediators, and consequently aggravates the disease process.[34] In asthmatic subjects, cytokines released from activated eosinophils and additional inflammatory cells can initiate ROS/RNS generation by pulmonary macrophages, interstitial cells and leukocytes infiltrating lung tissue.[35] Excess ROS/RNS may overwhelm antioxidant defense, and consequently lead to bronchoconstrictor mediators responsible for EIB. The genes for these inflammatory mediators are regulated by redox-sensitive transcription factors, nuclear factor (NF)-kappa(κ)B and activator protein (AP)-1.[36] NF-κB has also been shown to upregulate the gene for iNOS resulting in increased F_ENO,[37] and has been implicated in the up-regulation of pro-inflammatory cytokines, and the release of proinflammatory eicosanoids, such as cysteinyl-LTs and prostaglandins.[37] Importantly, it has been demonstrated that ascorbic acid blocks TNF-α-mediated activation of NF-κB.[38]

Prior studies indicate that there may be a link between ascorbic acid in the diet and asthma. It has been established that asthmatics tend to have lower serum antioxidants, including vitamin C, and that low vitamin C intake can be correlated with asthma severity.[39,40] These data suggest that asthmatic subjects may have an antioxidant deficiency and/or that they have increased oxidative stress that requires higher levels of ROS/RNS scavenging antioxidants. Thus, it seems plausible that antioxidant supplementation could be effective in reducing the effects of this disease. Indeed, Fogarty et al.[41] have recently shown that vitamin C supplementation may have a modest corticosteroid sparing effect in asthmatic patients. However, a recent Cochrane systematic review[42] evaluated the evidence for the efficacy of vitamin C supplementation in the treatment of asthma. The review concluded that evidence is inconclusive to recommend a specific role for vitamin C in the treatment of asthma.

Interventional studies using *extended* supplementation periods with other antioxidants such as β-carotene, lycopene, and undenatured whey protein have been effective in improving pulmonary function in subjects with EIB.[9,12–14] Neuman et al.[13] found a protective effect on post-exercise pulmonary function (post-exercise drop $<$15%) in EIB subjects with a daily dose of 64 mg β-carotene supplementation for 1 week. All subjects on the placebo diet demonstrated a greater than a 15% reduction in post-exercise FEV_1. In a subsequent study, the same authors[14] found that 1 week of 30 mg of lycopene (LYC-O-MATO™) supplementation also improved post-exercise pulmonary function. EIB subjects demonstrated a $>$14% reduction in post-exercise FEV_1 (average 26.5%), while 7 days of LYC-O-MATO™ supplementation resulted in a significant improvement in post-exercise FEV_1, with an average decrease in FEV_1 of 14.7%. More recently, Baumann et al.[9] found a reduction in the severity of EIB when subjects were supplemented with a cysteine donor whey protein for 8 weeks. Eighteen EIB-positive subjects demonstrated a significant mean improvement in post-exercise FEV_1 from baseline ($-22.6 \pm +12.2\%$), 4 weeks ($-18.9 \pm 12.9\%$) and 8 weeks ($-16.9 \pm 11.6\%$), with concomitant reductions in $FEF_{25–75\%}$, on the undenatured whey protein diet. No changes in FEV_1 or $FEF_{25–75\%}$ were observed for any time points on the placebo diet. Murphy et al.[12] reported in abstract form, that 500 mg/day of ascorbic acid combined with α-tocopherol (300 mg/day) for 3 weeks improved in post-exercise FEV_1 compared to placebo.

Conversely, Falk et al.[43] found that a daily dose of LYC-O-MATO™ did not affect pulmonary function following exercise in 19 adolescent athletes. The authors attributed their negative findings, which contrasts with previous research,[9,12–14] to an exercise intensity which may not be sensitive enough to document EIB in an athletic population, a reduced environmental stress (testing took place in a warm, humid environment) and not accounting for other dietary factors (i.e., high dietary intake of natural antioxidants such as fruits and vegetables). Interestingly, Grievink et al.[44] reported a beneficial effect of 3-month supplementation of an ascorbic acid and tocopherol combination, with or without β-carotene, which reduced the acute bronchoconstriction induced by ozone in healthy cyclists following exercise.

At present, only 3 interventional studies have investigated the efficacy of a *single dose* of ascorbic acid supplementation on the severity of EIB. Schachter and Schlesinger[15] studied 12 asthmatics with EIB and found significant improvement in post-exercise FEV_1 with 500 mg of ascorbic acid taken 90 min prior to exercise, while the placebo treatment had no effect on post-exercise pulmonary function. Cohen et al.[10] studied 20 asthmatics with EIB in which they were given either 2 g of ascorbic acid or placebo before an exercise challenge. Nine of the 20 subjects exhibited a protective effect on post-exercise pulmonary function on the ascorbic acid diet. Five patients in the protected group continued with 500 mg/d ascorbic acid for 2 weeks and demonstrated continued protection. The studies by Schachter and Schlesinger[15] and Cohen et al.[10] was supported by evidence presented by Miric and Haxhiu,[11] who demonstrated that pretreatment with ascorbic acid prevented a significant alteration in airway geometry induced by exercise in asthmatic subjects.

In conclusion, this study has shown that 2 weeks of ascorbic acid supplementation provides protection against exercise-induced airway narrowing in asthmatic subjects. The improvement in post-exercise pulmonary function on the ascorbic acid diet was accompanied by significant suppression of urinary measures of airway inflammation, F_ENO and a significant reduction in the intensity of asthma symptoms compared to the usual and placebo diet. Blocking oxidative stress, with ascorbic acid supplementation, is unlikely to lead to complete resolution of bronchoconstriction following exercise but might be useful as an adjunct therapy in asthma patients. Additional research should be aimed at establishing what combination of antioxidants will be most effective in correcting airway dysfunction, and to assess whether dietary manipulation with natural foods, high in antioxidants (i.e., fresh fruits and vegetables,) is as effective as ascorbic acid supplementation in providing protection against EIB.

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