

Effects of Cysteine Donor Supplementation on Exercise-Induced Bronchoconstriction

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ABSTRACT

BAUMANN, J. M., K. W. RUNDELL, T. M. EVANS, and A. M. LEVINE. Effects of Cysteine Donor Supplementation on Exercise-Induced Bronchoconstriction. *Med. Sci. Sports Exerc.*, Vol. 37, No. 9, pp. 1468–1473, 2005. **Purpose:** Reactive oxygen/nitrogen species (ROS/RNS) in resident airway cells may be important in bronchoconstriction following exercise. Glutathione (GSH) is a major lung antioxidant and could influence pathological outcomes in individuals with exercise-induced bronchoconstriction (EIB). This study examined the effects of supplementation with undenatured whey protein (UWP) in subjects exhibiting airway narrowing following eucapnic voluntary hyperventilation (EVH), a surrogate challenge for diagnosis of EIB. UWP is a cysteine donor that augments GSH production. **Methods:** In a randomized, double-blind, placebo-controlled study, 18 EIB-positive subjects (age: 25.2 ± 9.01 yr; weight: 77.3 ± 18.92 kg; height: 1.7 ± 0.09 m) with post-EVH falls of $\geq 10\%$ in FEV₁ received 30 g UWP (TX) or casein placebo (PL)/d. Subjects performed 6-min EVH challenges before and after 4 and 8 wk of supplementation. Exhaled nitric oxide (eNO) was measured serially before spirometry and at 1-wk intervals. Spirometry was performed pre- and 5, 10, and 15 min postchallenge. **Results:** Subjects exhibited significant mean improvement in postchallenge falls in FEV₁ from 0 wk ($-22.6 \pm 12.22\%$) with TX at 4 ($-18.9 \pm 12.89\%$, $P < 0.05$) and 8 wk ($-16.98 \pm 11.61\%$, $P < 0.05$) and significant mean reduction in post-EVH peak falls in FEF_{25–75} from 0 wk ($-40.6 \pm 15.28\%$) with TX at 4 ($-33.1 \pm 17.11\%$, $P < 0.01$) and 8 ($-29.7 \pm 17.42\%$, $P < 0.05$) wk. No changes in FEV₁ or FEF_{25–75} were observed in the PL group at any time point. Mean eNO for PL and TX groups at 0, 4, and 8 wk (46.8 ± 31.33 , 46.5 ± 35.73 , 49.3 ± 37.12 vs 35.2 ± 26.87 , 29.1 ± 17.26 , 34.7 ± 21.11 ppb, respectively) was not significantly different. **Conclusions:** UWP may augment pulmonary antioxidant capacity and be therapeutically beneficial in individuals exhibiting EIB, as postchallenge pulmonary function improved with supplementation. The lack of significant change in eNO suggests that the pulmonary function improvements from UWP supplementation are independent of eNO. **Key Words:** ASTHMA, INFLAMMATION, PULMONARY FUNCTION, WHEY PROTEIN, GLUTATHIONE.

Oxidative stress in asthma most likely results from both an increase in reactive oxygen species/reactive nitrogen species (ROS/RNS) and depletion of antioxidants in the airways, and a variety of antioxidant deficiencies exist (16,28). Lipid peroxidation is high (30) in the airways of asthmatics. The lipid-derived leukotrienes formed through metabolism of arachidonic acid are potent contractile mediators in airway hyperresponsiveness (14), and it has been demonstrated that 5-lipoxygenase activity is increased by endogenous ROS (29). Glutathione (GSH), a sulfur-containing thiol, is a cellular antioxidant effective in scavenging ROS and augments the antioxidant activity of vitamins C and E by participating in the regeneration of these vitamins from their radical form to their reduced active form. Pro-GSH agents (4,6) and GSH (9) have shown potential for beneficial effects on airway function both in humans and animals.

High concentrations of GSH are found in pulmonary epithelial cells (24) and alveolar epithelial lining fluid (8,13). Reduced levels of GSH in resident airway cells (10) and erythrocytes (28) and increased levels of oxidized glutathione (GSSG) in bronchoalveolar lavage fluid (16) are indicative of oxidative stress in asthmatics. GSH is a substrate for the enzyme glutathione peroxidase (GSH-Px), which catalyzes the reduction of ROS. It has been suggested that low GSH in the lung may amplify inflammation and hyperresponsiveness (24), and GSH may be a therapeutic option for lung disease associated with bronchospasm (9). Oxidative stress alters the GSH/GSSG redox buffer and directly influences the activation of nuclear factor- κ B (NF- κ B) through degradation of the inhibitor of NF- κ B (7), thus up-regulating the gene expression of proinflammatory cytokines.

N-acetyl-L-cysteine (NAC) and nalcystelyn (NAL), cysteine donors and precursors of GSH, have been used therapeutically to attenuate pulmonary inflammation in various lung diseases with promising results. Significant increases of GSH in bronchoalveolar lavage fluid (BALF) (22), improved lung function (5), and decreased oxidant-mediated human bronchial muscle contraction (11) have been observed with NAC supplementation. NAC reduces lipid peroxidation, NF- κ B binding activity, and inducible nitric oxide synthase (iNOS) expression (6). NAL has been shown to increase lung epithelial cell GSH levels and inhibit ROS production (4,15); like NAC, NAL inhibits NF- κ B (4). Undenatured whey protein (UWP) rich in glutamylcysteine

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and cystine increases lymphocyte GSH (18), and UWP improved pulmonary function and increased plasma GSH in a single patient chronic obstructive pulmonary disease case study (21).

The benefits of UWP for enhancing pulmonary antioxidant status and as a treatment for inflammatory asthma and EIB are unknown. The present study examined the efficacy of supplemental UWP in attenuating airway narrowing following eucapnic voluntary hyperventilation (EVH), a surrogate challenge for identification of EIB (26). We hypothesized that 8 wk of UWP treatment would attenuate the bronchoconstrictive response to EVH and would reduce resting exhaled nitric oxide.

METHODS

Subjects. Eighteen healthy individuals (7 male, 11 female) participated in this study. Mean subject characteristics are presented in Table 1. The Marywood University institutional review board approved research procedures, and subjects provided written informed consent for participation. Subjects were recruited through local advertisement and asked to complete a medical history questionnaire; those identified as positive for EIB by a fall of $\geq 10\%$ in FEV₁ after a 6-min EVH challenge were asked to continue the study. Five subjects reported using an inhaled beta₂-agonist before exercise; one subject was receiving fluticasone in combination with an inhaled beta₂-agonist; one subject was receiving formoterol in combination with an inhaled beta₂-agonist; one subject was receiving montelukast in combination with an inhaled beta₂-agonist; and two subjects were receiving fluticasone/salmeterol, montelukast, and an inhaled beta₂-agonist. History of atopic response to environmental stimuli was reported in 14 subjects; allergy diagnosis was reported in 12 subjects.

Study design. This study employed a randomized, placebo-controlled, double-blind, parallel design. Subjects were randomly assigned to one of two groups receiving 30 g·d⁻¹ UWP or placebo (casein) for 8 wk. Both UWP (TX) and casein placebo (PL) were supplied by Immunotec Research Corporation Ltd. (Vaudreuil-Dorion, Quebec, Canada). Subjects were provided with canisters containing a 10-d supply and returned each week for new canisters. Canister weight change was used as an indicator of compliance.

Participants with prescribed asthma medications were asked to temporarily abstain from use before testing. Medications and time frames for suspension were as follows: short-acting bronchodilators, sodium cromoglycate, nedocromil sodium, and ipratropium bromide: 4 h before testing; long-acting or sustained-release bronchodilators,

antihistamine medications: 48 h before testing; leukotriene antagonists and combined long-acting bronchodilators: 4 d before testing (3). Additionally, subjects were asked to abstain from caffeine and vigorous exercise on the day of testing and to discontinue the use of dietary supplements beginning 2 wk before and during the study.

Anthropometric measures. Height of subjects was measured without shoes, and weight was obtained with subjects wearing light clothing using a calibrated balance-beam scale (Healthometer, Sunbeam Products, Inc., Purvis, MS). Body composition was assessed via dual energy x-ray absorptiometry (DEXA) (Lunar Prodigy Axial DEXA, General Electric Medical Systems, Waukesha, WI; enCORE Software, Version 6.60).

Pulmonary function test procedure. Pulmonary function was measured by spirometry using a calibrated computerized pneumotachograph spirometer (Jaeger Masterscope PC, Hoechberg, Germany; LAB Manager Software version 4.53.2, 2002). Forced vital capacity (FVC), FEV₁, FEV₁/FVC ratio, forced expiratory flow through the mid-portion of the vital capacity (FEV₂₅₋₇₅), and peak expiratory flow (PEF) were obtained immediately before and for 15 min after EVH. Pulmonary function test maneuvers were performed according to ATS criteria (2). The procedure for all pulmonary function tests included 1) three normal tidal volume breaths, followed by 2) inhalation to total lung capacity, and 3) forced maximal exhalation lasting at least 6 s, terminating with 4) a final maximal inhalation. Post-challenge pulmonary function was measured at 5, 10, and 15 min after the completion of the 6-min EVH challenge. If any postchallenge measurement was technically unacceptable, it was repeated.

EVH protocol. Subjects were evaluated for the presence of EIB using EVH at screening, baseline, and 4 and 8 wk. Ambient laboratory conditions were 22°C and 50% RH (VWR Digital Hygrometer/ Thermometer, VWR International, Inc.). The EVH challenge required each subject to breathe room temperature, dry (<3 mg H₂O·L⁻¹ air), compressed gas consisting of 21% oxygen, 5% carbon dioxide, and nitrogen balance for 6 min at a minute ventilation (\dot{V}_E) equal to 30 × FEV₁, an estimate of 85% of maximal voluntary ventilation (MVV) (3). Compressed gas flowed from a cylinder through a calibrated rotameter (Model 1110DK41XMGAA, Brooks Instrument Division, Emerson Electric Co., Hatfield, PA) into a 300-g meteorological balloon via high-pressure tubing and was delivered to subjects through a 35-mm breathing tube and a two-way non-rebreathing valve and mouthpiece (Hans Rudolf, Kansas City, MO). Expired gas passed through a flow sensor and \dot{V}_E was measured and recorded as verification of respiration intensity (Vmax Spectra Metabolic Measurement Cart, SensorMedics, Yorba Linda, CA; CPX2 Software version 10-1a, June 2002).

Exhaled nitric oxide (eNO) protocol. Online visual measurement of resting eNO using a restricted exhaled breath (REB) protocol (NOA 280i Nitric Oxide Analyzer, Accurate NO Breath Kit, Thermal Mass Flowmeter, NO Analysis software Version 3.21, Sievers Instruments, Boul-

TABLE 1. Subject characteristics.

	Age (yr)	Height (m)	Weight (kg)
All (N = 18)	25.22 ± 9.01	1.70 ± 0.09	77.32 ± 18.92
Placebo (N = 7)	28.57 ± 9.88	1.70 ± 0.09	70.76 ± 15.04
Treatment (N = 11)	23.09 ± 8.15	1.70 ± 0.09	81.50 ± 20.58

Mean ± SD. No significant differences.

der, CO) was applied serially at 1-wk intervals; eNO was measured before spirometry and EVH at weeks 0, 4, and 8 and on separate trips to the lab at weeks 1 through 3 and 5 through 7. Measurement techniques were employed as outlined by the American Thoracic Society (1). Three exhalations were performed without nose clips at each test, with at least 30 s elapsing between exhalations. The procedure was 1) maximal inhalation to total lung capacity over 2–3 s, and 2) immediate exhalation against increased expiratory resistance for at least 6 s to obtain a NO plateau lasting at least 3 s. During exhalation, subjects were instructed to monitor a visual computer display to maintain a flow rate of 50 mL·s⁻¹ ± 10%.

Statistical analysis. Data were analyzed using repeated-measures ANOVA, followed by *post hoc* paired *t*-tests for significant *F* values (SPSS version 11.5). Independent samples *t*-tests were used to determine differences between groups. Pearson product moment correlations were used to evaluate relationships between resting measurements and postchallenge falls in FVC, FEV₁, or FEF_{25–75}. For all statistical comparisons, the alpha level was set at *P* ≤ 0.05.

RESULTS

Twenty-one individuals enrolled in the study; three dropped out before study commencement. All remaining subjects completed the 8-wk study. No differences were observed between PL and TX groups in subject height, weight, or age (Table 1). No changes in weight, body mass index, body fat (%), or grams of lean mass were observed over the 8-wk study period. Gender effect was not observed for any variable.

Pulmonary function. Mean resting lung function at 0 wk is presented in Table 2. No differences were noted in resting lung function parameters between PL and TX groups at study onset, at 4 or 8 wk. No difference in resting lung function was observed across time within groups. Mean FEV₁ and FVC were within normal range. No subject had below 85% of predicted values for FEV₁ or less than 90% of predicted values for FVC. Two subjects in the TX group and four subjects in the PL group exhibited less than 70% of predicted values for FEF_{25–75}, suggestive of mild asthma. No significant correlations were identified between resting lung function and postchallenge falls in FVC, FEV₁, or FEF_{25–75} for any trial.

No differences in mean \dot{V}_E over 6 min of EVH were observed within or between groups. At 0, 4, and 8 wk, mean \dot{V}_E was 111.4 ± 29.85, 115.1 ± 34.20, and 114.2 ± 28.03

TABLE 2. Resting lung function of placebo and treatment groups at 0 wk expressed as a percentage of predicted values.

Baseline lung function	Placebo (N = 7)	Treatment (N = 11)
FVC (%)	106.88 ± 9.89	117.72 ± 11.40
FEV ₁ (%)	101.91 ± 9.91	109.25 ± 11.67
FEV ₁ /FVC (%)	95.57 ± 7.36	93.12 ± 9.42
FEF _{25–75} (%)	77.62 ± 19.69	85.60 ± 24.59

Mean ± SD. No significant differences.

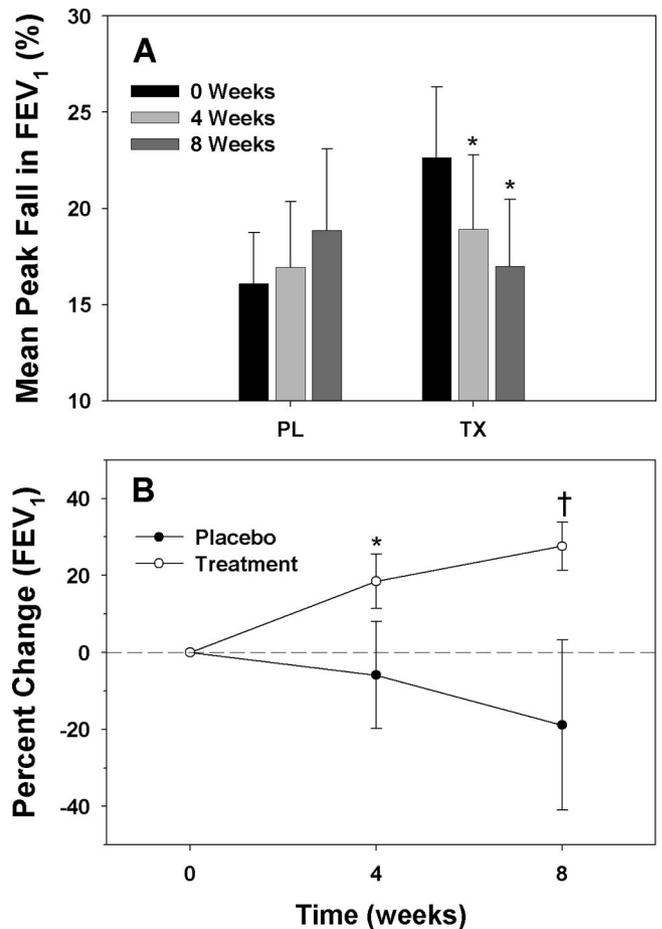


FIGURE 1—A. Post-EVH peak fall in FEV₁. * Significant difference (*P* < 0.05) from 0 wk in UWP TX group. Values are presented as mean ± SE. B. Percentage of improvement in post-EVH falls in FEV₁, expressed as the percentage of change from post-EVH falls in FEV₁ at 0 wk. * Significant difference between 0 and 4 wk in the UWP TX group (*P* < 0.05). † Significant difference between 0 and 8 wk in the UWP TX group (*P* = 0.001). Values are presented as mean ± SE.

L·min⁻¹ for the TX group and 123.3 ± 26.87, 120.8 ± 29.66, 119.7 ± 31.98 L·min⁻¹ for the PL group.

Post-EVH mean peak fall in FEV₁ was significantly attenuated in the TX group at 4 wk (*P* < 0.05); this improved lung function was maintained over the 8-wk supplementation period (Fig. 1A) (*P* < 0.05), though no significant differences were apparent between 4 and 8 wk. No difference in post-EVH fall in FEV₁ was noted between 0, 4, and 8 wk for the casein PL group. Significant improvements in post-EVH FEV₁, expressed as the percentage of change from post-EVH falls in FEV₁ at study onset, was observed in the TX group at 4 (18.5 ± 23.52%) and 8 (27.6 ± 20.81%) wk (Figs. 1 and 2B) (*P* < 0.05 and *P* = 0.001, respectively); no improvements were observed in the PL group at either 4 (−5.9 ± 36.76%) or 8 (−18.9 ± 58.47%) wk. Individual changes in FEV₁ are presented in Table 2.

The TX group exhibited significant reductions in mean post-EVH peak falls in FEF_{25–75} at 4 and 8 wk compared with that at study onset (Fig. 3, *P* < 0.05 and *P* < 0.01, respectively; −40.6 ± 15.28%, −33.1 ± 17.11%, and −29.7 ± 17.41% for 0, 4, and 8 wk, respectively). Significant reductions in post-EVH peak falls in FEF_{25–75} were

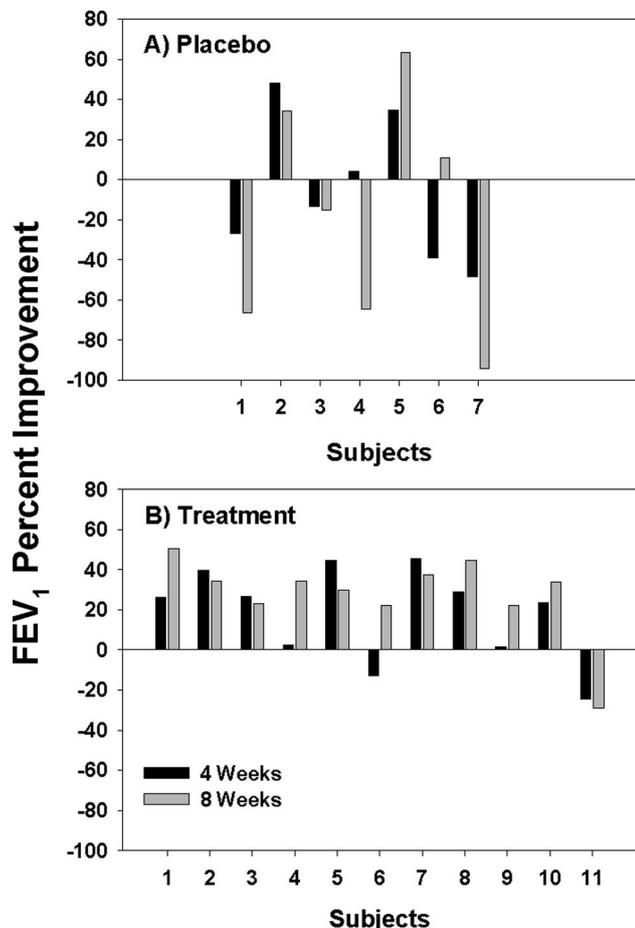


FIGURE 2—Individual change in FEV₁ for PL (A) and UWP TX (B). Data are expressed as the percentage of change from post-EVH falls in FEV₁ at 0 wk.

also observed between 4 and 8 wk in the TX group ($P < 0.05$). No changes in post-EVH peak falls in FEV₂₅₋₇₅ were observed in the PL group at any time point ($-30.6 \pm 10.29\%$, $-32.4 \pm 13.95\%$, and $-31.7 \pm 19.17\%$ for 0, 4, and 8 wk, respectively).

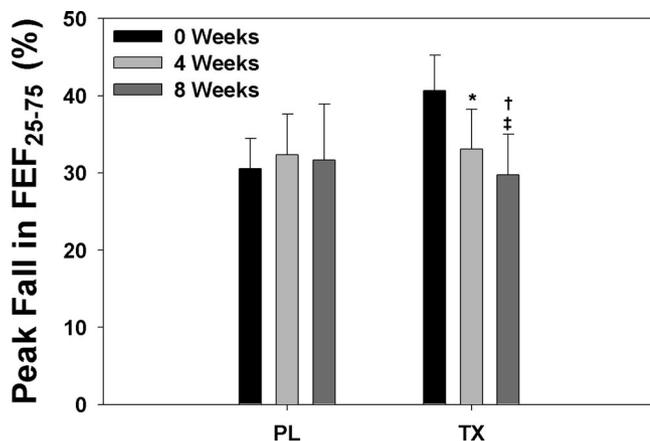


FIGURE 3—Post-EVH peak fall in FEF₂₅₋₇₅. * Significant difference between 0 and 4 wk in the UWP TX group ($P < 0.05$). † Significant difference between 0 and 8 wk in the UWP TX group ($P < 0.01$). ‡ Significant difference between 0 and 8 wk in the UWP TX group ($P < 0.01$). Values are presented as mean \pm SE.

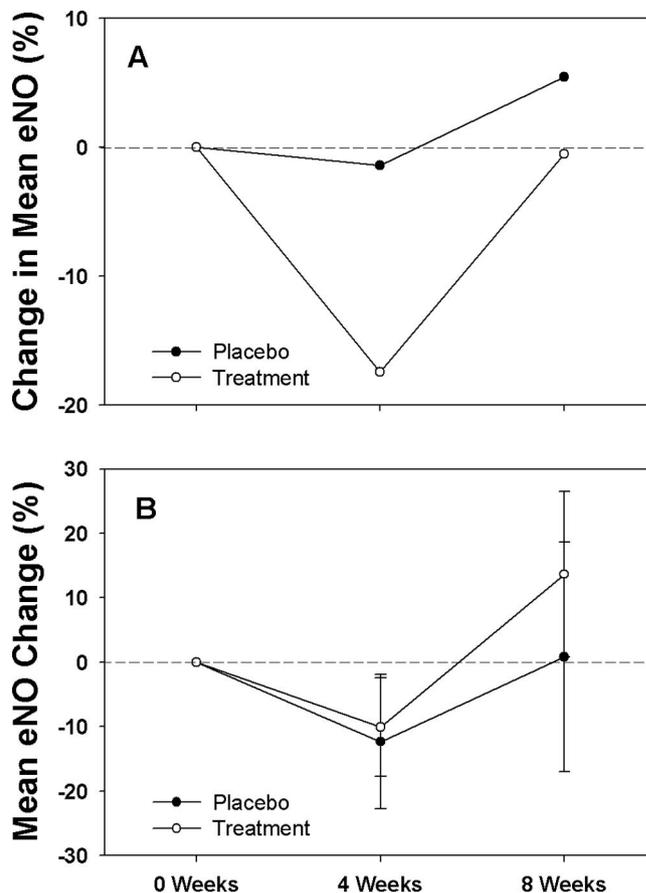


FIGURE 4—A. Change in mean eNO, expressed as the percentage of change from mean eNO (ppb) values obtained at 0 wk. B. Mean percentage of change in eNO, expressed as the mean of the individual percentage of change in eNO from 0 wk. No significant differences. Values are presented as mean \pm SE.

Exhaled nitric oxide. No significant differences were found in mean eNO values at 0, 4, and 8 wk for either PL (46.8 ± 31.33 , 46.5 ± 35.73 , and 49.3 ± 37.12 ppb) or TX (35.2 ± 26.87 , 29.1 ± 17.26 , and 34.7 ± 21.11 ppb) groups. Compared with baseline, mean eNO at 4 wk decreased by 0.5% in the PL group and 17.5% in the TX group; at 8 wk, mean eNO increased by 5.4% in the PL group and decreased by 1.4% in the TX group (Fig. 4A). However, the mean percentage of change in eNO from baseline was not different at any time point for either group (Fig. 4B).

DISCUSSION

The present study examined the effects of UWP supplementation on airway function following EVH. Post-EVH percentage of change in FEV₁ was used as a measure of abnormal pulmonary function response. Eighteen subjects were identified as EIB positive by a fall in FEV₁ of $\geq 10\%$ following EVH; this criterion used to define EIB is consistent with that suggested by others (12). The major finding of the present study was that UWP supplementation resulted in significant attenuation of post-EVH peak falls in FEV₁ and FEF₂₅₋₇₅ at 4 and 8 wk. Interestingly, this occurred without concomitant declines in the indirect marker of airway inflammation, eNO.

FEF₂₅₋₇₅ has been shown to be a sensitive indicator of airway obstruction (20). However, conventional inhaled therapies are best suited to treat the larger proximal airways more effectively than the smaller distal airways due to large diffused particle size of inhalant (27). UWP supplementation resulted in significant attenuation of mean post-EVH falls in FEV₁ and FEF₂₅₋₇₅ at 4 and 8 wk. Whereas no significant improvements were observed in mean fall in FEV₁ between 4 and 8 wk, additional improvements were observed in FEF₂₅₋₇₅, suggesting that long-term UWP supplementation may provide additive protection in the more distal airways. Thus, long-term UWP supplementation beyond 4 wk may prove useful as an adjunctive therapy.

UWP supplementation provided protection against falls in FEV₁ in 10 of 11 subjects. Eight weeks of UWP supplementation provided approximately a mean 28% improvement in postchallenge falls in FEV₁. Although this is somewhat less than the approximate 44% improvement in post-EVH falls in FEV₁ afforded by the leukotriene receptor antagonist montelukast (25), the present study overlapped the onset of the spring allergy season, which could have affected our results. History of allergic symptoms was reported in 78% of our subjects. Although not statistically significant, the post-EVH mean percentage of change in FEV₁ demonstrated a trend to worsen between 4 and 8 wk in the PL group (cf. Fig. 1B), and eNO increased in both groups during the same time period (cf. Fig. 4), supporting the notion that the onset of the allergy season affected our subjects' airways.

Recruitment and activation of proinflammatory cells in the airway leads to production of ROS/RNS. Excess ROS/RNS may overwhelm antioxidant defense, lead to oxidative stress, and subsequently initiate mediators leading to EIB. The tripeptide GSH is accepted as a key protective airway antioxidant (8,13,24). Overwhelming the antioxidant capacity through increased ROS and RNS can cause the oxidation of GSH to GSSG without the capacity for recycling to GSH, and asthmatics have been noted to have increased GSSG levels in bronchoalveolar lavage fluid (16). Products of the AA 5-lipoxygenase pathway are upregulated by ROS (29) and are potent stimulators of airway hyperresponsiveness (14). Additionally, disruptions in the balance of the GSH/GSSG redox buffer activate NF- κ B (7), a transcription factor responsible for upregulation of pro-remodeling and proinflammatory genes. Though the present study did not measure GSH levels, we suggest that the observed pulmonary function improvements are most likely due to increased pulmonary GSH concentrations and concomitant downregulation of oxidant-induced proinflammatory mediators.

Given the influence of ROS/RNS in the asthmatic cascade, augmenting the antioxidant capacity of the lungs may be therapeutically beneficial. In fact, several studies have suggested that treatment with GSH precursors may be beneficial in inflammatory lung disorders (4,5,6,15,21). Studies have shown that the GSH precursors NAC (22) and NAL

(15) increase GSH concentration in the BALF and lung epithelial cells. Further, other studies have shown improvements in lung function (5) and beneficial effects of NAC on lung inflammation (6,11). UWP contains high concentrations of glutamylcysteine and cystine, GSH precursors previously shown to augment whole blood GSH levels (21) and lymphocyte GSH (18), which may reflect pulmonary GSH status (24).

The lack of a reduction in mean eNO in the UWP TX group over the 8-wk supplementation period is difficult to resolve. The higher level of NO in exhaled breath of asthma sufferers than that of nonasthmatics is believed to be secondary to increased airway expression of iNOS (23). The pro-GSH agent NAC has been shown to reduce iNOS expression in rats (6). Based on this observation, we hypothesized that supplementation with UWP would reduce iNOS expression and eNO in those exhibiting post-EVH bronchoconstriction.

The onset of the allergy season between 4 and 8 wk may have interfered with detection of significant eNO changes (cf. Fig. 4). Recent evidence suggests that atopic individuals exposed to allergen exhibit elevated levels of eNO (19). However, the nonsignificant changes in eNO observed with UWP supplementation over the 8-wk treatment period in the TX group could be a result of an increased antioxidant role of GSH, thus preserving NO from active ROS scavenging and subsequent degradation to peroxynitrite (ONOO⁻). NO has a defined role as a ROS scavenger, and NAC supplementation ameliorates pulmonary tissue damage associated with ONOO⁻, most likely through decreased NO scavenging of superoxide radicals (O₂⁻) and/or upregulation of antioxidant systems (17). Like NAC, UWP may inhibit the formation of ONOO⁻ through enhanced antioxidant status.

In conclusion, our results demonstrate that UWP may provide protection against EIB, independent of eNO. Although the protection against post-EVH falls in FEV₁ afforded by UWP supplementation was less than that seen with the pharmacological intervention montelukast, UWP may provide therapeutic benefits through enhanced pulmonary GSH in those with EIB and be used as adjunctive therapy. Because biochemical measures were not employed in this study, elucidation of the precise mechanism of UWP action is speculative. Findings of the present research provide a basis for the use of UWP as a nonpharmacological therapeutic strategy for airway dysfunction. Additional supporting research and further clarification of the mechanism by which UWP attenuates post-EVH declines in pulmonary function is necessary.

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