

Improved lung function following dietary antioxidant supplementation in exercise-induced asthmatics

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ABSTRACT

Introduction: Oxidative stress is a characteristic of exercise-induced asthma (EIA), however antioxidant supplementation may attenuate EIA. The purpose of this study was to determine if ascorbic (AsA) and α -tocopherol supplementation would improve airway function in subjects with EIA.

Methods: A single-blind randomized crossover design with eight clinically diagnosed EIA subjects (22.0 ± 0.7 year) and five healthy control subjects (28.2 ± 1.4 year) was used. Subjects consumed vitamins (V) (AsA 500 mg; α -tocopherol 300 IU) or placebo (PLA) daily for three weeks, followed by a three week washout period and then three weeks of the alternative treatment. Ten-minute treadmill tests ($90\% \text{VO}_{2\text{peak}}$) were performed with pulmonary function testing (forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and between 25 and 75% ($\text{FEF}_{25-75\%}$), and peak expiratory flow rates (PEFR)) measured pre-exercise and 1, 5, 15, and 30 min post-exercise.

Results: Supplementation led to significant improvements at minute 5 and minute 15 in FVC; FEV_1 ; PERF; $\text{FEF}_{25-75\%}$ and minute 30 in FEV_1 and $\text{FEF}_{25-75\%}$ post-exercise.

Conclusion: AsA and α -tocopherol may aid the recovery of pulmonary function in subjects with EIA.

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1. Introduction

Exercise-induced asthma (EIA) is a subcategory of asthma and is exhibited by a hyper-reactive, intermittent narrowing of the airways triggered by physical activity (McFadden and Gilbert, 1994). Although EIA occurs in approximately 90% of asthmatics, about 10% of individuals solely experience bronchospasms post-exercise (Gotshall, 2002). Symptoms of an acute asthma attack are wheezing, chest tightness, coughing, decreased forced vital capacity (FVC) and difficulty breathing (Weiler, 1996). These symptoms occur due to the narrowing of the airways, which is known as bronchoconstriction. Typically the amount of bronchoconstriction is quantified by a decrease $>10\%$ in the amount of air forcefully exhaled in one second following a maximal inhalation (FEV_1 ; forced expiratory volume in 1-s) or a decrease of $>15\%$ in peak expiratory flow recorded by a flow meter (Godfrey and Bar-Yishay, 1993; Anderson and Scheffel, 1985). This reduced lung function may impact both the ability to perform physical activity in asthmatics trying to maintain an exercise program as well asthmatics that are already active.

Bronchoconstriction during or after exercise may be caused by several different mechanisms including heat loss, water loss and excessive drying of the airways, airway inflammation and inflammatory mediator release (Anderson and Kippelen, 2005; Anderson and Daviskas, 2000). Rapid rewarming of the airways following exercise may lead to vascular hyperemia and airway edema (McFadden et al., 1986). Although multiple causes contribute to the bronchoconstriction during, and/or after exercise, there is still speculation to the predominant cause of EIA. It is well established that excess reactive oxygen species (ROS) are produced in asthmatics (Malmgren et al., 1986). This may be in part due to reduced antioxidant defenses (Grievink et al., 1999; Wood et al., 2012) that leave asthmatics susceptible to the excess ROS production, and also a reduced antioxidant status to combat the excessive ROS. Wood et al. (2005) reported that asthmatics with a normal dietary intake still presented reduced antioxidant compounds (i.e., lycopene). These deficiencies have been reported in plasma (Misso et al., 2005), decreased ascorbic acid (vitamin C) from bronchoalveolar lavages (Kelly et al., 1999), and decreased α -tocopherol (i.e., vitamin E) in the airways (Wood et al., 2008).

Although these deficiencies reduce the protection against oxidative stress in the airways, there is a growing body of literature suggesting increasing the antioxidant status via dietary supplementation decreases the inflammatory burden, possibly

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attenuating the decline in exercise-induced bronchoconstriction. Supplementation of vitamin C has been shown to have attenuated bronchoconstriction post-exercise (Tecklenburg et al., 2007). Increasing dietary carotenoids (Hu and Cassano, 2000) and lutein (Schunemann et al., 2002) has been reported to improve respiratory status and lung function (Grievink et al., 2000). Although several studies have reported improved post-exercise lung function after taking vitamin C supplementation (Tecklenburg et al., 2007); other studies do not support these findings (Schachter and Schlesinger, 1982; Malo et al., 1986; Nadi et al., 2012). Similarly, there are reported improvements in pulmonary function with vitamin E supplementation in combination with vitamin C (Chatham et al., 1987; Dow et al., 1996) but not with vitamin E alone (Hackney et al., 1981; Pearson et al., 2004). These studies were performed to investigate EIB either in the elderly or in the presence of ozone. No studies, to our knowledge, have investigated supplementation with simultaneous vitamin C and vitamin E as a therapeutic option to reduce bronchoconstriction in exercise-induced asthmatics. Therefore the purpose of this study was to determine if ascorbic (AsA) and α -tocopherol supplementation would improve airway function in subjects with EIA. We were also interested in determining the time course of improvements over the supplementation period. It was hypothesized that daily supplementation of AsA and α -tocopherol for three weeks in subjects with EIA would reduce the airway bronchoconstrictor response to exercise.

2. Methods

Eight subjects with clinically diagnosed exercise-induced asthma (EIA) and five healthy controls (CON) volunteered for this study. Subjects were recruited from the Kansas State University campus. All subjects completed a medical health questionnaire and signed informed consent prior to participation, which was approved by the Kansas State Institutional Research Board (Protocol #2125). All subjects were non-smokers, and were at various fitness levels. Standard pulmonary function tests were performed on all subjects as part of the initial screening process (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA). Bronchoconstriction was verified in our EIA subjects as determined by at least 10% decrease in FEV₁ following the first exercise-challenge (Anderson et al., 1985). All subjects (EIA and CON) had not been taking AsA or α -tocopherol supplementation in the prior six months to testing.

2.1. Experimental design

A randomized single blind cross over design was used over a consecutive six-week period. Subjects were instructed to refrain from any therapeutic medications and vigorous exercise 12 h prior to testing. First, subjects performed an incremental treadmill test to exhaustion to determine peak aerobic capacity ($\dot{V}O_{2peak}$). Then, EIA and CON subjects were randomly assigned to either a placebo (PLA) or vitamin (VIT) supplementation group for three weeks. An exercise challenge was performed each week of the supplementation period. The exercise challenge consisted of subject running at a workload eliciting 90% $\dot{V}O_{2peak}$ until exhaustion. Prior to and following the exercise challenge pulmonary function was assessed. After a 3 week washout period, subjects were then given the alternate regimen for the remaining three weeks, such that subjects served as their own control. For all exercise testing sessions, an emergency bronchodilator was in the laboratory if necessary. No subjects needed to use the inhaler at any time point during or after the exercise tests.

2.2. Vitamin supplementation

The vitamin treatment included 250 mg of AsA and 100 IU of α -tocopherol with breakfast, and 250 mg of AsA, 200 IU of α -tocopherol, and a multivitamin to be consumed with dinner. The dosage of AsA is the same as previous studies (Kordansky et al., 1979; Schachtner et al., 1982), and the dosage of α -tocopherol is similar to what has previously been investigated in the presence of ozone (Trenga et al., 2001). Additionally, a three week wash-out period and three weeks of supplementation is more conservative than previous studies investigating AsA (Tecklenburg et al., 2007) and α -tocopherol (Trenga et al., 2001) supplementation where a one week washout period was used. These supplements are known to completely washout in less than three weeks (Podmore et al., 1998; Dimitrov et al., 1991). Including the multivitamin (Standard One-A-Day multivitamin: 60 mg AsA; 22.5 IU α -tocopherol), total dietary intake was 933% of the DRI for AsA and 2444% for vitamin E. Both quantities are within healthy limits and are considered non-toxic (Levine et al., 2005; Weber et al., 1997). Daily vitamins were given at the beginning of each week to be self-administered by the subject at breakfast and dinner meals. Vitamin supplementation also included 500 mg AsA and 200 IU of α -tocopherol one-hour prior to exercise. Acute supplementation was taken with a candy bar containing 10 g of fat for maximal absorption of α -tocopherol. Subjects not undergoing vitamin treatment were supplemented with placebo (lactose) capsules throughout the three weeks and acutely one hour prior to exercise.

2.3. Peak aerobic capacity ($\dot{V}O_{2peak}$)

All subjects initially underwent a graded incremental treadmill test (Quinton Instrument Company, Model Q65, Seattle, WA) until exhaustion to determine maximal aerobic capacity ($\dot{V}O_{2peak}$). The exercise protocol was individualized for each subject to choose a speed that they felt was comfortable on a 0% grade. Subjects were allowed to complete a self-determined warm-up (5–10 min). Subsequently, testing began at 0% grade and increased 2% every 90 s until volitional fatigue. Breath-by-breath metabolic data were collected at baseline and throughout exercise (SensorMedic 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA). Heart rate (HR) was assessed using ECG leads. Arterial oxygen saturation (SpO₂) was continuously monitored via a pulse oximeter (Datex-Ohmeda 3900P) attached to the earlobe. Criteria for a successful $\dot{V}O_{2peak}$ test included RER ≥ 1.15 , heart rate within 10% predicted max, and/or a plateau in oxygen consumption (150 mL/min) with an increase in workload.

2.4. Exercise challenge

Within one week of initial testing, subjects performed an exercise challenge. Exercise challenges were repeated on day 7 (week 1), 14 (week 2), and 21 (week 3) of each treatment group. Ambient temperature did not differ significantly from trial to trial ($23.5 \pm 0.1^\circ\text{C}$). Subjects warmed-up for 5 min at a 4 mph walk before the exercise challenge. The exercise challenge consisted of exercising at a workload that corresponded to 90% of $\dot{V}O_{2peak}$ (EIA = $92.0 \pm 0.1\% \dot{V}O_{2peak}$; CON = $94.0 \pm 0.1\% \dot{V}O_{2peak}$) for 10 min. If subjects were unable to complete the 10 min of exercise, they were encouraged to exercise as long as possible. All subjects went at least six minutes for each of the exercise challenge tests. Prior to testing, a 3 mL venous blood draw was taken from the antecubital vein of the arm to determine hemoglobin ([Hb]). Maximum flow-volume loops measurements were taken prior to and immediately upon cessation of exercise and at 5, 15, and 30 mins post-exercise. Rate of perceived exertion (RPE) and dyspnea ratings were recorded at minutes 5, 8, and 10 of exercise. Plasma levels for AsA and α -tocopherol determi-

Table 1

Subject characteristics mean $\pm$ standard error. (EIA $n=8$; Control $n=5$) M/F, male to female; FEV₁, forced expiratory volume in 1-s; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; Hb, hemoglobin. No significant differences between groups ($p > 0.05$).

	EIA	Control
Age (yrs)	22.0 $\pm$ 0.7	28.2 $\pm$ 1.4
M/F ratio	3/5	2/3
V O _{2peak} (mL/kg/min)	44.9 $\pm$ 1.7	47.9 $\pm$ 0.5
Height (cm)	175.0 $\pm$ 1.5	175.8 $\pm$ 1.5
Weight (kg)	71.2 $\pm$ 1.9	70.8 $\pm$ 2.8
FEV ₁ (%predicted)	92.3 $\pm$ 5.7	95.0 $\pm$ 2.9
FVC (%predicted)	93.5 $\pm$ 4.3	104.8 $\pm$ 4.4
TLC (%predicted)	100.4 $\pm$ 4.8	96.8 $\pm$ 6.4
RV (%predicted)	78.1 $\pm$ 6.3	97.7 $\pm$ 10.1
Hb (g/dL)	14.2 $\pm$ 0.1	13.6 $\pm$ 0.2

nation were collected from venous blood samples for each testing session. However, due to technical difficulties associated with the assays these values were not included in the analysis.

2.5. Pulmonary function tests

Pulmonary function tests were assessed according to American Thoracic Society/European Respiratory Society guidelines (Miller et al., 2005). Maximum flow-volume loops (MFVL) (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA.) were performed in triplicate at each time period. The MFVL gave measures of forced expiratory volume in one second (FEV₁), forced vital capacity (FVC), forced expiratory flow at 50% (FEF_{50%}), and between 25 and 75% (FEF_{25–75%}) of FVC, and peak expiratory flow (PEF). Total lung capacity was measured via nitrogen washout technique (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA.).

2.6. Statistical analysis

Data are reported as mean $\pm$ SE. Changes in pulmonary function are represented as % change from baseline values. Data were analyzed using SigmaStat (Jandel Scientific Software). Independent samples *t*-tests were used to measure baseline differences between EIA and CON. A two-way repeated measure ANOVA (time $\times$ condition) was used to determine differences between treatments in each subject population. A Tukey post hoc test was used to determine where differences existed. Significance was set at $p < 0.05$.

3. Results

3.1. Subject characteristics

Subjects demographics are reported in Table 1. The male to female ratio was similar between both groups (EIA=3 males/5 females; CON=2 males/3 females). The mean age, height, and weight for the subjects were not significantly different between EIA and controls. V O_{2peak} values were not different ($p > 0.05$) between EIA (44.9 $\pm$ 1.7 mL/kg/min) and control (47.9 $\pm$ 0.5 mL/kg/min) subjects. There were no significant differences found between EIA subjects and control populations in regard to lung volume measures.

3.2. Pulmonary function

No changes ($p > 0.05$) were observed in pre-exercise pulmonary function measurements (FEV₁, FVC, PEF, FEF_{25–75}) in the EIA subjects following vitamin or placebo supplementation, except for a significantly lower in FEF_{25–75} for VIT compared to PLA at week

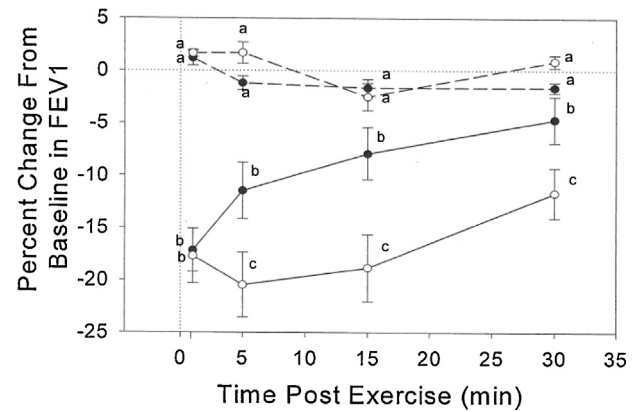


Fig. 1. Forced expiratory volume in one second after the third week of treatment. Dotted lines represent control subjects ($n=5$) and straight lines represent EIA subjects ($n=8$) White circles show placebo condition while solid black circles represent vitamin supplements. Significant is shown as $a \neq b \neq c$ within the same time period ($p < 0.05$).

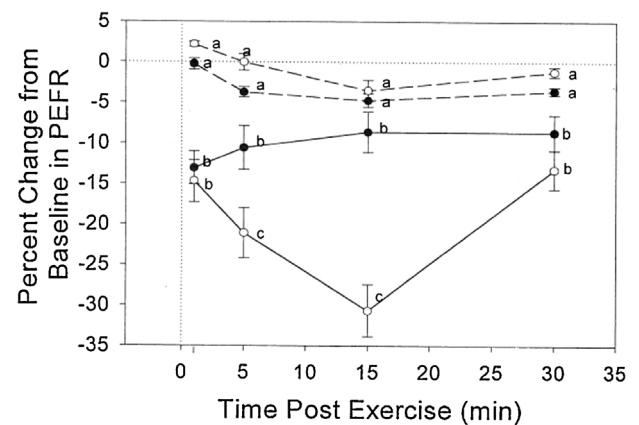


Fig. 2. Peak expiratory flow rate after the third week of treatment. Dotted lines represent control subjects ($n=5$) and straight lines represent EIA subjects ($n=8$) White circles show placebo condition while solid black circles represent vitamin supplements. Significant is shown as $a \neq b \neq c$ within the same time period ($p < 0.05$).

3. Weekly changes in pulmonary function expressed as percent change (% Δ) from pre-exercise values over three weeks of VIT and PLA supplementation for EIA are shown in Table 2. There were no significant changes in PFTs one minute post-exercise across the three weeks. In the second week of VIT supplementation, EIA subjects demonstrated significant improvements for FEV₁ and FEF_{25–75} at minute 5, 15, and minute 30 post-exercise. FVC and PEF also were improved ($p < 0.05$) at minute 5 and 15 post-exercise. After three weeks of VIT supplementation, FEV₁, FVC, PEF, FEF_{25–75} at minute 5, 15, and 30 post-exercise were significantly improved compared to PLA.

Post-exercise pulmonary function data including FEV₁, PEF, FEF_{25–75}, and FVC are shown for the third week of treatment in Figs. 1–4, respectively. Immediately post-exercise (1 min post-exercise) there were no significant differences found in pulmonary function between treatments for either the EIA or control groups. EIA subjects demonstrated a significantly lower pulmonary function on all measures (FEV₁, PEF, FEF_{25–75}, and FVC) at the end of exercise compared to control subjects (Figs. 1–4). CON subjects did not demonstrate a difference in ($p > 0.05$) in FEV₁, PEF, and FEF_{25–75} measures with vitamin supplementation.

Figs 1 and 2 also show the percent change in FEV₁ and PEF measures post-exercise. The EIA group was significantly lower than the CON at all post-exercise times. With VIT supplementation, the EIA

Table 2
Percent change (% Δ) from pre-exercise values in weekly pulmonary function measures in EIA.

		Week 1		Week 2		Week 3	
		Placebo	Vitamin	Placebo	Vitamin	Placebo	Vitamin
FEV ₁ (% Δ)	5 min	−15.8 ± 2.8	−14.2 ± 2.8	−21.3 ± 3.3	−12.8 ± 3.2*	−20.4 ± 3.1	−11.5 ± 2.7*
	15 min	−10.7 ± 3.2	−11.0 ± 2.6	−14.2 ± 3.3	−6.9 ± 3.2*	−18.9 ± 3.2	−7.9 ± 2.5*
	30 min	−8.9 ± 2.7	−10.6 ± 2.4	−10.2 ± 2.5	−4.6 ± 2.8*	−11.7 ± 2.4	−4.7 ± 2.2*
FVC (% Δ)	5 min	−8.4 ± 2.2	−7.2 ± 1.8	−13.3 ± 2.0	−7.2 ± 2.7*	−12.1 ± 1.9	−7.4 ± 1.7*
	15 min	−6.5 ± 2.2	−11.6 ± 4.4*	−7.0 ± 1.8	−2.0 ± 2.9*	−8.8 ± 1.8	−5.3 ± 1.6*
	30 min	−5.6 ± 2.1	−4.0 ± 1.2	−3.7 ± 1.0	−2.9 ± 1.9	−3.2 ± 1.1	−1.1 ± 1.0*
PEFR (% Δ)	5 min	−17.7 ± 2.4	−12.2 ± 3.6	−21.7 ± 3.4	−15.1 ± 2.8*	−21.1 ± 3.4	−10.6 ± 2.5*
	15 min	−15.4 ± 2.6	−11.6 ± 3.4	−18.4 ± 3.2	−11.5 ± 2.6*	−30.7 ± 4.6	−8.6 ± 2.2*
	30 min	−12.6 ± 2.6	−10.3 ± 2.9	−13.9 ± 2.2	−11.4 ± 2.5	−13.3 ± 2.6	−8.7 ± 2.1*
FEF _{25–75} (% Δ)	5 min	−20.8 ± 4.7	−15.8 ± 5.0	−27.4 ± 4.7	−16.9 ± 5.4*	−27.7 ± 4.7	−10.2 ± 5.3*
	15 min	−14.0 ± 5.1	−13.8 ± 4.1	−20.4 ± 4.5	−8.7 ± 5.3*	−28.3 ± 4.5	−2.4 ± 5.0*
	30 min	−9.6 ± 4.0	−13.6 ± 3.5	−13.7 ± 3.6	−4.5 ± 4.7*	−18.4 ± 3.8	−3.8 ± 3.7*

Data expressed as Mean ± SE. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; PEFR, peak expiratory flow rate; FEF_{25–75}, forced expiratory flow at 25–75% of forced vital capacity.

* Significantly different between conditions within each week ($p < 0.05$).

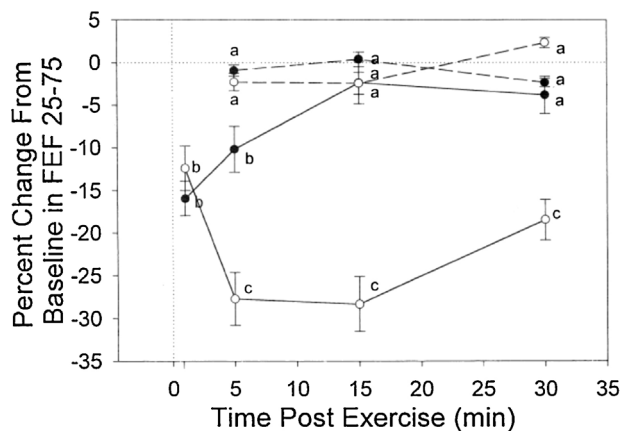


Fig. 3. Forced expiratory flow between 25 and 75% of forced vital capacity after the third week of treatment. Dotted lines represent control subjects ($n = 5$) and straight lines represent EIA subjects ($n = 8$) White circles show placebo condition while solid black circles represent vitamin supplements. Significant is shown as $a \neq b \neq c$ within the same time period ($p < 0.05$).

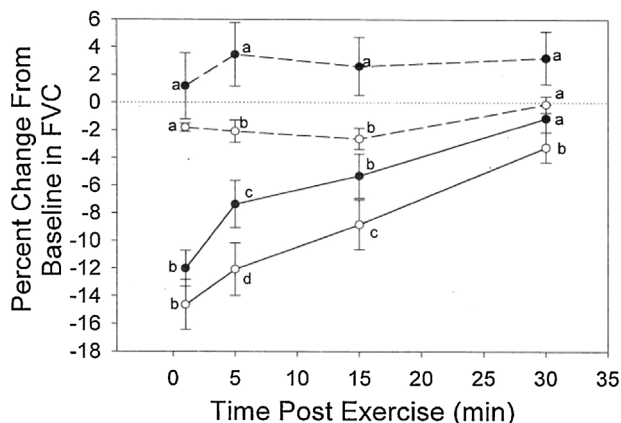


Fig. 4. Forced vital capacity after the third week of treatment. Dotted lines represent control subjects ($n = 5$) and straight lines represent EIA subjects ($n = 8$) White circles show placebo condition while solid black circles represent vitamin supplements. Significant is shown as $a \neq b \neq c$ within the same time period ($p < 0.05$).

group demonstrated significant improvements in FEV₁ at minute 5, minute 15, and minute 30 post-exercise compared to EIA PLA. PEFR (Fig. 2) was significantly improved at minute 5 and minute 15, but

not minute 30 post-exercise in EIA. FEF_{25–75} (Fig. 3) improved at minute 5, minute 15, and minute 30 post-exercise in EIA. FEF_{25–75} improvements for EIA at minutes 15 and 30 were not significantly different from control subjects ($p > 0.05$). Fig. 4 shows a significant effect of AsA and α-tocopherol supplementation on both EIA and control subjects. EIA subjects demonstrated an improvement at minute 5, minute 15, and minute 30 post-exercise, with minute 30 results no showing a significant difference from control data ($p > 0.05$). Control subjects also demonstrated an improvement in FVC at minute 5 and minute 15.

Baseline pulmonary function measurements were not different ($p > 0.05$) following VIT or PLA supplementation. Weekly changes in pulmonary function expressed as % Δ from pre-exercise values over three weeks of VIT and PLA supplementation for CON are shown in Table 3. There were no significant changes in the % Δ in PFTs one minute post-exercise across the three weeks. One week of VIT supplementation led to no changes ($p > 0.05$) in the % Δ in PFTs at 5, 15, and 30 min following exercise. After two weeks of supplementation, the % Δ was lower ($p < 0.05$) in FEF_{25–75} for VIT compared to PLA at 15 and 30 min. After three weeks of supplementation, the % Δ in FVC was significantly higher for VIT compared to PLA at 5, 15, and 30 min.

3.3. Metabolic and ventilatory data

Table 4 contains data collected during minute 5 of the exercise bout and at end of exercise. Exercise duration did not differ ($p > 0.05$) between EIA (range 9.0–9.1 min) and CON (range 8.9–9.1 min) or between vitamin or placebo treatments. No significant differences were found between EIA control subjects pertaining to HR, ventilation (VE), rate of perceived exertion, or dyspnea.

4. Discussion

4.1. Major findings

Our findings demonstrate a significant effect of antioxidants on pulmonary function post-exercise in subjects with EIA. Specifically, these results support a portion of our hypothesis that daily ingestion of 500 mg AsA and 300 IU of α-tocopherol over three weeks significantly improves FEV₁, PEFR, FVC, and FEF_{25–75} following exercise in this population. Improvements in lung function may be due to the effect of antioxidant loading on several mechanisms of action.

Table 3

Percent change (% Δ) from pre-exercise values in weekly pulmonary function measures in CON.

		Week 1		Week 2		Week 3	
		Placebo	Vitamin	Placebo	Vitamin	Placebo	Vitamin
FEV ₁ (% Δ)	5 min	1.4 ± 0.7	−0.9 ± 0.6	0.9 ± 0.4	−1.1 ± 0.6	−1.7 ± 1.0	−1.2 ± 0.7
	15 min	−0.3 ± 0.4	−0.7 ± 0.7	2.2 ± 0.5	0.9 ± 0.5	−2.5 ± 1.3	−1.7 ± 0.8
	30 min	0.4 ± 0.5	1.3 ± 0.3	3.6 ± 0.9	−0.4 ± 0.4	0.9 ± 0.6	−1.6 ± 0.5
FVC (% Δ)	5 min	0.5 ± 0.6	−2.7 ± 0.7	−1.3 ± 0.2	−1.9 ± 0.7	−2.1 ± 0.8	3.5 ± 2.3*
	15 min	−1.0 ± 0.4	−1.6 ± 0.5	−0.4 ± 0.3	0.0 ± 0.4	−2.6 ± 0.8	2.6 ± 2.1*
	30 min	−0.4 ± 0.7	−0.8 ± 0.1	0.3 ± 0.5	−1.2 ± 0.5	−0.1 ± 0.6	3.2 ± 1.1*
PERF (% Δ)	5 min	0.5 ± 0.5	0.4 ± 0.8	−0.5 ± 0.6	−3.0 ± 0.5	0.0 ± 0.7	−3.7 ± 0.4
	15 min	−2.7 ± 0.5	−2.2 ± 1.2	1.2 ± 1.0	0.1 ± 1.0	−3.5 ± 0.8	−4.7 ± 0.6
	30 min	−0.5 ± 1.1	−1.5 ± 1.1	0.0 ± 0.9	−1.2 ± 0.6	−1.2 ± 0.7	−3.6 ± 0.7
FEF _{25–75} (% Δ)	5 min	4.2 ± 1.4	2.9 ± 0.9	5.1 ± 1.0	0.2 ± 0.9	−2.3 ± 1.5	−0.9 ± 0.4
	15 min	2.5 ± 0.9	0.9 ± 1.1	7.2 ± 1.3	2.6 ± 1.1*	−2.4 ± 2.2	0.4 ± 0.9
	30 min	2.1 ± 0.6	6.6 ± 1.1	10.7 ± 2.4	1.0 ± 0.8*	2.3 ± 1.2	−2.4 ± 0.5

Data expressed as mean ± SE.

* Significantly different between conditions within each week ($p < 0.05$).**Table 4**

Exercise challenge measures at minute 5 and end exercise.

		Pre-treatment		Week 3—vitamin		Week 3—placebo	
		EIA	Control	EIA	Control	EIA	Control
Exercise time (min)		9.1 ± 0.2	9.1 ± 0.3	9.0 ± 0.3	9.4 ± 0.2	9.0 ± 0.3	8.9 ± 0.3
V O ₂ (mL/kg/min)	5 min	38.1 ± 1.0	43.1 ± 1.0	40.5 ± 1.3	42.7 ± 0.9	40.7 ± 1.4	40.7 ± 0.9
	End	42.9 ± 1.5	44.2 ± 1.0	41.9 ± 1.3	43.1 ± 1.0	42.7 ± 1.3	43.0 ± 1.0
HR (bpm)	5 min	176.3 ± 2.2	178.0 ± 2.4	173.3 ± 2.3	173.8 ± 2.0	169.9 ± 2.0	171.2 ± 1.4
	End	182.3 ± 2.1	184.6 ± 2.8	179.6 ± 2.2	181.6 ± 2.2	179.5 ± 1.7	179.4 ± 1.8
V _E (L/min)	5 min	78.9 ± 2.0	99.1 ± 5.5	86.9 ± 3.2	95.6 ± 5.6	84.2 ± 2.5	95.7 ± 4.9
	End	88.0 ± 2.9	103.1 ± 5.4	92.0 ± 3.4	101.2 ± 6.4	87.1 ± 2.8	103.6 ± 5.5
RPE (1–10)	5 min	4.0 ± 0.1	5.4 ± 0.4	4.2 ± 0.2	5.0 ± 0.3	4.4 ± 0.2	5.8 ± 0.4
	End	4.7 ± 0.2	7.2 ± 0.4	5.8 ± 0.3	6.6 ± 0.4	5.0 ± 0.3	6.8 ± 0.4
Dyspnea (1–10)	5 min	4.8 ± 0.3	5.4 ± 0.4	5.5 ± 0.3	4.6 ± 0.4	5.3 ± 0.3	5.2 ± 0.4
	End	6.0 ± 0.4	7.2 ± 0.4	6.6 ± 0.3	6.2 ± 0.4	6.4 ± 0.3	6.6 ± 0.4

Data are expressed Mean ± SE. HR, heart rate; V_E, ventilation; RPE, rate of perceived exertion. No significant differences found ($p > 0.05$).

4.2. Possible mechanisms of action

It is possible antioxidant loading of AsA and α-tocopherol increased metabolism of histamine (Johnston et al., 1996), increased rate of ROS removal (Barnes, 1990), caused alterations in the metabolism of arachadonic acid (Frei, 1991), and/or decreased direct stimulation of pulmonary smooth muscle contraction (Sipahi and Ercan, 1997). Although we did not take direct measures in the airway or lung, these mechanisms are considered and should be the focus of future investigation.

Researchers have reported that greater histamine release caused greater degree of bronchospasm (Kivity, 1994), corresponding with an EIA attack (Anderson et al., 1981). However there is a dose-dependent relationship between AsA and the relaxation of a histamine challenged trachea (Sipahi and Ercan 1997). Therefore increasing antioxidant status to scavenge free radicals (H₂O₂ and O₂) and reducing histamine and ROS (Frei, 1988) acting upon the pulmonary smooth muscle may have alleviated direct stimulation for bronchoconstriction (Barnes et al., 1990, Kanazawa, 1991, Calhoun 1992).

4.3. Improved pulmonary function in healthy and asthmatic subjects

There were improvements in pulmonary function in the asthmatic group receiving supplementation, and also in healthy subjects. The improvements in post-exercise FVC present in the

healthy subjects were an interesting finding. We have recently found that antioxidant supplementation improved pulmonary function at rest and during exercise in healthy individuals not meeting fruit and vegetable recommendations (Chenoweth et al., 2015). These improvements seen in both healthy and asthmatic individuals may be due to changes in smooth muscle tone and arachadonic acid metabolism. These changes may also explain why epidemiological studies (Britton et al., 1995; Schunemann, 2001; Grievink et al., 1998) have also found significantly higher pulmonary function (i.e., FEV₁ and FVC) in individuals with higher dietary intake of AsA and α-tocopherol.

Studies have also reported vitamin E may slow or prevent radical formation from ozone (O₃) exposure (Trenga et al., 2001). Considering vitamin E increases the absorption of vitamin C, incorporating the two dietary antioxidants may be more beneficial than consumption of one supplement; however this idea needs to be validated. Previous literature that has investigated the two dietary antioxidants together reported the attenuation of ozone-induced bronchoconstriction, and found beneficial results post-exercise when taking both vitamin E and vitamin C compared to controls receiving the placebo (Trenga et al., 2001). Several other studies that have investigated EIA without measuring ozone have utilized vitamin C (Miric and Haxhu, 1991; Cohen et al., 1997; Techlenburg, 2007), a cysteine donor (Baumann et al., 2005), beta carotene (Neuman et al., 1999), lycopene supplementation (Neuman et al., 2000), or dietary fish oil (Mickleborough et al., 2006) in asthmatics and have reported various degrees of protectiveness after

supplementation. However more recently in the Cochrane reports and metaanalyses, vitamin C supplementation alone has recently been found to limit effectiveness in treatment of asthma and have various (minor to moderate) improvements in bronchoconstriction (Milan et al., 2013). Therefore more recent studies in the past five years have investigated combined treatments, (e.g. dietary fish oil and vitamin C (Chatham et al. (2004); Mickleborough and Lindley, 2014); dietary fish oil and vitamin D (Price et al., 2015)) to aid in improvements in post-exercise bronchoconstriction. In the existing literature, the combination of both vitamin C and vitamin E has never been investigated in regards to EIB in asthmatics. Our goal was to determine if improvements were present with a combination of dietary antioxidants.

4.4. Time course for changes in pulmonary function

Inflammation of the lung tissue is a common symptom associated with EIA, and may be due to both mast cell release of histamine as well as free radical release in the inflammatory process. AsA and α -tocopherol may have an impact on inflammation in EIA population due to AsA's ability to increase the metabolism of histamine. Although we are not sure if AsA and α -tocopherol may reduce initial levels of histamine from leukocytes, it is possible higher levels present in the lung may increase the disappearance of oxidants at minute five post-exercise in the second and third week of supplementation. We are not surprised benefits from supplementation occurred at two weeks, as this has previously been reported with improvements in lung function (Tecklenburg et al., 2007). Additionally, the increased anti-oxidant status two and three weeks after supplementation likely decreased extracellular ROS. Frei et al. (1988) showed that AsA and α -tocopherol act to decrease the release of cellular radicals. If there were a decrease in cellular radicals then there would likely be a decrease in direct stimulation for bronchoconstriction in our EIA subjects (Barnes, 1990; Kanazawa et al., 1991; Calhoun et al., 1992). This likely also reduced a chemotactic effect of other leukocytes in the area (Perez et al., 1980) and lowering histamine and ROS producing leukocytes in the lung (Lopez et al., 1988).

4.5. Exercise performance

Although our primary purpose of this study was to determine if supplementation of vitamin C and vitamin E improve post-exercise pulmonary function, many athletes as well as recreationally active individuals express EIA and experience difficulty with performance itself (Fitch et al., 1976). Therefore if there were improvements in pulmonary function present after dietary antioxidant supplementation, we were interested in determining whether these translated to improvements in exercise performance. Interestingly, the time that subjects were able to maintain 90% VO_2 max until exhaustion did not differ between CON and EIA or between the placebo and supplementation condition. There were also no significant differences in ratings of perceived exertion of dyspnea, indicating subjects did not feel exercise or breathing was different with supplementation. Pharmacological agents (corticosteroids) as well as several non-pharmacological agents (i.e., warming-up) and pharmacological agents have been studied with regards to improvements in symptoms if EIA (Kippelen et al., 2012), however we are not aware of any literature that has reported if improvements in exercise performance were associated with lessened airway narrowing after dietary antioxidant supplementation. Interesting we did not find a significant effect in dyspnea rating during exercise. It is possible our exercise-induced asthmatics did not experience significant reactivity during exercise; or possible EIA have increased reactivity but do not always notice asthma symptoms due to remodeling of the lung parenchyma. Another possibility is subjects with EIA

have psychologically adapted to exercise such that perceptions of effort, both whole body and dyspnea are not different from control subjects.

5. Conclusion

EIA is an increasing public concern, specifically because of the high healthcare costs associated with asthma treatment. Therefore alternative treatments are important to explore. Our finding suggests that chronic supplementation of AsA and α -tocopherol for up to three weeks appears to improve the pulmonary function in EIA subjects. The importance of these findings may be significant for patients who rely on medications in order to exercise. Future research should be focused on determining the relative contribution of each component of the supplementation, as well as if supplementation for people with EIA might help them become less dependent on medications and provide them alternative treatments of EIA.

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