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Lycopene-rich treatments modify noneosinophilic airway inflammation in asthma: Proof of concept

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Abstract
Antioxidant-rich diets are associated with reduced asthma prevalence. However, direct evidence that altering intake of antioxidant-rich foods affects asthma is lacking. The objective was to investigate changes in asthma and airway inflammation resulting from a low antioxidant diet and subsequent use of lycopene-rich treatments. Asthmatic adults ($n = 32$) consumed a low antioxidant diet for 10 days, then commenced a randomized, cross-over trial involving 3×7 day treatment arms (placebo, tomato extract (45 mg lycopene/day) and tomato juice (45 mg lycopene/day)). With consumption of a low antioxidant diet, plasma carotenoid concentrations decreased, Asthma Control Score worsened, %FEV_1 and %FVC decreased and %sputum neutrophils increased. Treatment with both tomato juice and extract reduced airway neutrophil influx. Treatment with tomato extract also reduced sputum neutrophil elastase activity. In conclusion, dietary antioxidant consumption modifies clinical asthma outcomes. Changing dietary antioxidant intake may be contributing to rising asthma prevalence. Lycopene-rich supplements should be further investigated as a therapeutic intervention.

Keywords: *Lycopene, carotenoids, diet, asthma, neutrophilic inflammation*

Abbreviations: *AHR, Airway hyper-responsiveness; CRP, C-Reactive Protein; %FEV1, %predicted Forced Expiratory Volume in 1 s; %FVC, %predicted Forced Vital Capacity; GINA, Global Initiative for Asthma; HPLC, High Performance Liquid Chromatography;* PD_{15}, *Provocation dose resulting in 15% drop in baseline* FEV_1; *TCC, Total cell count*

Introduction

Asthma is a chronic inflammatory disorder of the airways, involving variable airflow obstruction and increased airway responsiveness to a variety of stimuli. Asthma is known to involve a heterogeneous inflammatory response [1]. Allergen-specific responses lead to activation of the acquired immune system, via a predominantly IL-5 mediated, eosinophilic pathway. Stimuli such as viruses, bacteria and air pollutants activate the innate immune system, via a predominantly IL-8 mediated, neutrophilic pathway [2]. Both of these pathways involve the respiratory burst of activated inflammatory cells and production of reactive oxygen species (ROS) [3]. Additionally, the IL-8 mediated neutrophil pathway involves the transcription factor NFκB, which itself is activated by ROS [4].

Host defence against the potentially damaging effects of ROS is provided by a range of antioxidants. These may be endogenous, such as the antioxidant

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enzymes (superoxide dismutase, glutathione peroxidise, catalase), thiols (glutathione) and metal-binding proteins (lactoferrin, transferrin, ceruloplasmin) or exogenous, including a variety of antioxidants obtained from the diet such as tocopherols, carotenoids, flavonoids and ascorbate. However in asthma, host antioxidant defences are overwhelmed by excessive production of ROS and oxidative damage occurs [5]. Detrimental effects of oxidative stress on airway function include: airway smooth muscle contraction, airway hyper-responsiveness and epithelial shedding, each of which contribute to the airway obstruction that is characteristic of asthma [3]. Thus, it is likely that the use of antioxidants, to restore the oxidant-antioxidant balance, may be effective in the treatment of asthma [6].

A growing body of epidemiological evidence has indicated that dietary antioxidants may be important to respiratory health. Fresh fruit intake has been shown to be inversely associated with wheeze [7], chronic lung disease onset [8] and has been positively associated with percentage predicted forced expiratory volume in 1 s (%FEV_1) [9,10]. Total fruit and vegetable intake has been inversely related to asthma prevalence [11] but not related to %FEV_1 [12] or airway obstruction [13]. Tomato products (juice, sauce, pizza) have been shown to be protective against asthma onset in a large longitudinal study [14] and vegetables have been shown to be protective against chronic bronchitis, bronchial asthma [15] and wheeze [16].

While this epidemiological data suggests that antioxidants may be important in protecting against asthma, supplementation trials using isolated antioxidants, mostly vitamin C, have been disappointing [17,18]. One possible explanation for this is that the protective effect of antioxidants is dependent on other closely associated nutrients [17,18]. Thus, experts in the field have hypothesized that dietary modifications using whole foods, rather than supplementation with individual nutrients, may be the most effective strategy [18]. To our knowledge, there have been no studies to date looking at whether altering the intake of antioxidant-rich foods affects asthma outcomes.

We have recently examined carotenoids, as accurate markers of fruit and vegetable intake [19], and shown that, despite normal dietary intake, peripheral blood concentrations of carotenoids, in particular lycopene, are low in asthmatics [20]. Furthermore, we have demonstrated that, with supplementation, circulating levels of lycopene increase and this is reflected in the airway lining fluid [20], thus directly affecting antioxidant protection in the lungs. Previously only one supplementation trial has been published using lycopene in asthma [21], with promising results. Short-term (1 week) supplementation with tomato extract (containing 30 mg lycopene/day) led to a reduction in exercise-induced bronchoconstriction [21].

We hypothesized that reducing antioxidant intake would reduce airway antioxidant defences, thereby increasing susceptibility to oxidative damage and worsening asthma status. We further hypothesized that this effect would be reversed by dietary supplementation with lycopene-rich treatments. The aim of this study was, first, to determine whether reducing the intake of antioxidant-rich foods affects asthma outcomes. Secondly, we aimed to improve asthma outcomes using lycopene-rich treatments.

Methods

Subjects

Adults with stable asthma ($n = 32$) were recruited from the John Hunter Hospital Asthma Clinic, NSW, Australia, commencing May 2004. All study visits were completed by December 2005. Asthma was diagnosed based upon current (past 12 months) episodic respiratory symptoms, doctor's diagnosis of asthma (ever) and airway hyper-responsiveness to hypertonic saline. Clinical asthma pattern was categorized according to GINA guidelines [22]. Exclusion criteria included: recent (past month) respiratory tract infection, recent asthma exacerbation, recent unstable asthma or change in maintenance therapy, current smoking and/or use of vitamin or mineral supplements. The study was approved by the Hunter New England and University of Newcastle Human Research Ethics Committees and subjects gave written informed consent.

Protocol

Following collection of baseline data, subjects were instructed to consume a diet low in antioxidants [23]. This diet included no more than one piece of fruit and two serves of vegetables per day and avoidance of tea, coffee, red wine, fruit juices, nuts, seeds, vitamin or mineral supplements and aspirin. Following the initial 10 days on the low antioxidant diet, subjects commenced a randomized cross-over supplementation trial, which included three treatment arms (placebo, tomato juice (45 mg lycopene/day) or tomato extract capsules (45 mg lycopene/day)). Each treatment was used for 7 days, with a 10 day wash-out period between each treatment [24,25]. For the duration of the study, subjects consumed the low antioxidant diet as described. Compliance with the dietary recommendations and supplements was monitored by 24-h food recalls at each visit, plasma carotenoid assessments and pill countback. Asthma medications were kept constant for the duration of the study. Spirometry, exhaled nitric oxide (NIOX®, Aerocrine AB), the validated Asthma Control Questionnaire [26], plasma and induced sputum were collected at baseline, after

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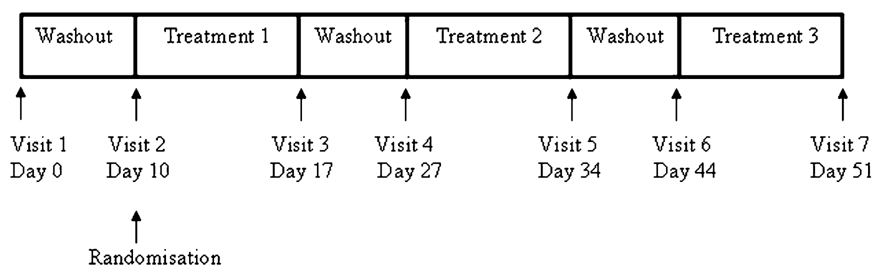

Figure 1. Study protocol.

10 days on the low antioxidant diet and again after each treatment and wash-out phase (Figure 1). Subjects fasted for 12 h before each visit.

The order in which subjects received the treatments was determined by computer generated random allocation, derived by a statistician and given to the clinical research officers, who allocated the next available number as participants entered the trial. Initial treatment allocation was concealed from clinical staff. During the treatment phase, neither the subjects or the investigators were blinded to the treatments, however outcome assessments (induced sputum) were conducted blind to both subjects and investigators.

Participant flow

Thirty-two subjects were recruited for the study. Twenty-two of these subjects completed the initial washout period on the low antioxidant diet and entered the randomized trial. Seventeen of 22 subjects completed the trial. The progress of participants through the trial is described in Figure 2.

Analysis

Key end points of this study were Asthma Control Score and airway inflammation. With 17 subjects, the study had 80% power to detect a clinically significant change in Asthma Control Score of 0.5 and 96% power to detect 1 SD in sputum %neutrophils. Statistical analysis of the initial washout phase was performed using Minitab version 13.32 for Windows (Minitab Inc., State College, USA). Data were tested for normality using the Anderson-Darling Test. Data is reported as mean $\pm$ standard error for normal data and median (interquartile range) for non-parametric data. PD_{15} was log transformed and presented as the geometric mean (log SD). Statistical comparisons were performed using the paired Student *t*-test for normally distributed data and the Wilcoxon test for non-parametric paired data. Associations were examined using Pearson's correlation and Spearman's rank correlation coefficients for normal and non-parametric data, respectively. Significance was accepted if $p < 0.05$. Supplementation analysis was conducted using GraphPad Prism4 (GraphPad Software, Inc, San Diego, CA). Difference between treatment groups was calculated by repeated measures ANOVA for normally distributed data and Friedman's analysis for non-parametric data. Dunn's post-hoc testing was performed on significant variables. Two-sided significance tests were used throughout.

Treatments

The tomato extract capsules used were Lyc-o-Mato®, supplied by LycoRed Natural Products Industries, Ltd (Beer Sheva, Israel), containing 15 mg lycopene per capsule. One capsule of Lyc-o-Mato® was taken three times per day resulting in a total daily dose of 45 mg lycopene. For the tomato juice arm, a commercial juice was used (Spring Valley, Spring Valley® Beverages, Australia). The concentration of lycopene in the tomato juice was

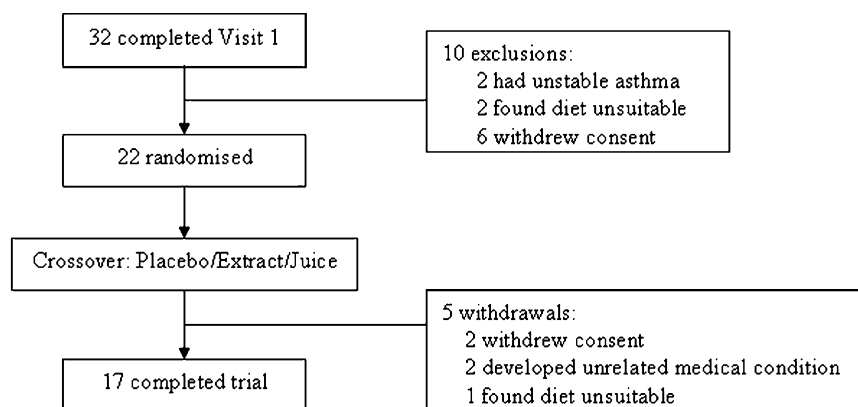

Figure 2. Flow diagram showing flow of participants through the study.

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determined by HPLC analysis and the volume of juice required to provide an equivalent dose of 45 mg lycopene was 840 mL per day. This was also taken three times per day in 280 mL volumes. The placebo was identical in appearance to the Lyc-o-Mato® capsules, but contained only soybean oil. Both treatments and the placebo were taken three times per day with a slice of white bread spread with olive oil margarine to ensure maximum carotenoid bioavailability.

Sputum induction and analysis

Spirometry (Minato Autospiro AS-600; Minato Medical Science, Osaka, Japan) and combined bronchial provocation and sputum induction with hypertonic saline (4.5%) were performed as described [27] by trained respiratory research officers. Lower respiratory sputum portions were selected from saliva and processed as described [27]. The dispersed suspension was filtered and a total cell count (TCC) of leucocytes and viability was performed. Cytospins were prepared, stained (May-Grunwald Geimsa) and a differential cell count obtained from 400 non-squamous cells. The remaining solution was centrifuged (400 g, 10 min, 4°C) and the cell-free supernatant was aspirated and stored at −70°C. Neutrophil elastase activity was detected in sputum supernatant samples using the Human Neutrophil Elastase Immunocapture Activity Assay Kit (Calbiochem, Darmstadt, Germany).

Carotenoid analysis

High performance liquid chromatography (HPLC) was used to determine carotenoid (β-carotene, lycopene, α-carotene, β-cryptoxanthin and lutein/zeaxanthin) and tocopherol (α- and γ- tocopherol) concentrations in plasma [28]. All extractions were carried out in a darkened laboratory under red light. Ethanol:ethyl acetate (1:1) containing internal standards (canthaxanthin and butylated hydroxyanisole (BHA)) was added to the sample. The solution was sonicated using a probe sonicator, centrifuged (3000 g, 4°C for 5 min) and the supernatant was collected. This process was repeated three times, adding ethyl acetate twice, then hexane to the pellet. Ultra pure water was then added to pooled supernatant and the mixture was vortexed and centrifuged. The supernatant was decanted, the solvents evaporated with nitrogen and the sample reconstituted in dichloromethane:methanol (1:2 v/v). Chromatography was performed on a Hypersil ODS column (100 mm × 2.1 mm × 5 μm) with a flow rate of 0.3 mL/min. Carotenoids were quantified by calibrating with a standard (canthaxanthin) run over a range of known concentrations, under the same HPLC conditions. Analysis was done using a mobile phase of acetonitrile:dichloromethane:methanol 0.05% ammonium acetate (85:10:5 v/v). Carotenoids and Tocopherols were detected at 470 nm and 290 nm, respectively, using a photodiode array detector.

Results

Twenty-two subjects completed the initial washout period on the low antioxidant diet and entered the randomized trial (Figure 2). They had a mean age (±SEM) of 52.1 (±2.4) years. This included eight males and 14 females, with 16/22 subjects (73%) being atopic. Asthma pattern was classified as intermittent ($n=4$, 18%), mild ($n=6$, 27%), moderate ($n=5$, 23%) or severe persistent ($n=7$, 32%). Median (Q1–Q3) daily dose of inhaled corticosteroids was 1000 (1000–1800) μg beclomethasone equivalents/day.

Following 10 days on a low antioxidant diet, plasma carotenoid concentrations decreased, with a 42% reduction in plasma lycopene. Plasma concentrations of α- and γ-tocopherol did not change significantly (Table I). Several markers of asthma control also deteriorated after 10 days on the modified diet, including %predicted FEV_1, %predicted FVC and Asthma Control Score (Figure 3 and Table II). Airway inflammation, assessed as%neutrophils in induced sputum, increased significantly over the 10 day period (Table II).

Seventeen of 22 subjects completed the cross-over trial and their responses were analysed (Figure 2). Following treatment with both tomato juice and tomato extract capsules, there was a significant increase in plasma concentrations of total carotenoids, predominantly due to increases in lycopene (Table III). Plasma lycopene concentrations after supplementation increased 3–3.5-fold. Airway inflammation was modified by both the tomato juice and the tomato extract capsules, with a decrease in%neutrophils resulting from both treatments compared to placebo (Figure 4). Neutrophil elastase activity was also reduced following treatment with the tomato extract. Exhaled nitric oxide, a marker of eosinophilic inflammation, showed no change. No changes in clinical parameters were observed (Table IV).

Discussion

This is the first study to provide proof of concept that altering the intake of antioxidant-rich foods directly affects asthma outcomes. Placing subjects with asthma on a low antioxidant diet for 10 days led to a significant worsening of lung function and asthma control score. This finding is highly significant for subjects with asthma, as it indicates that omitting antioxidant-rich foods from the diet, for even a short time frame, will have a detrimental effect on asthma

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Table I. Plasma concentrations of carotenoids/α-tocopherol at baseline and following 10 days on low antioxidant diet.

Antioxidant (mg/L)	Baseline	Following 10 days on low antioxidant diet	p-value
Total Carotenoids	0.878 (0.454–1.353)	0.616 (0.343–1.014)	0.026
Lutein/Zeaxanthin	0.191 (0.117–0.259)	0.140 (0.105–0.208)	0.025
β-cryptoxanthin	0.106 (0.054–0.151)	0.060 (0.038–0.102)	0.001
Lycopene	0.198 (0.038–0.477)	0.114 (0.047–0.305)	0.059
α-carotene	0.015 (0.010–0.036)	0.016 (0.005–0.028)	0.022
β-carotene	0.209 (0.094–0.418)	0.149 (0.087–0.297)	0.055
α-tocopherol	8.991 (7.162–10.483)	7.902 (7.190–8.963)	0.076
γ-tocopherol	0.399 (0.305–0.504)	0.396 (0.290–0.512)	0.808

Values are median (interquartile range).

symptoms. Furthermore, while airway inflammation, assessed by %neutrophils in induced sputum, worsened on the low antioxidant diet, lycopene-rich treatments reversed this trend. This demonstrates the potential for lycopene-rich treatments to influence asthma outcomes.

This study provides an insight into the mechanism by which dietary changes lead to loss of asthma control. The onset of asthma has traditionally been considered to occur via a predominantly eosinophilic pathway, involving activation of the acquired immune response. However, recently, a neutrophilic inflammatory sub-type of asthma has been identified, involving persistence of symptoms and AHR in the presence of increased sputum neutrophils and activation of the innate immune response [29–32]. In our group of asthmatics, the withdrawal of antioxidants from the diet enhanced neutrophil influx into the airways, suggesting that altering antioxidant intake affects the innate immune response. This is supported by another recent study in healthy men that showed a daily intake of eight vs two serves of fruit and vegetables led to an increase in plasma carotenoid levels and a reduction in plasma C-reactive protein (CRP) concentration [33] in the high intake group. CRP is another marker of innate immune

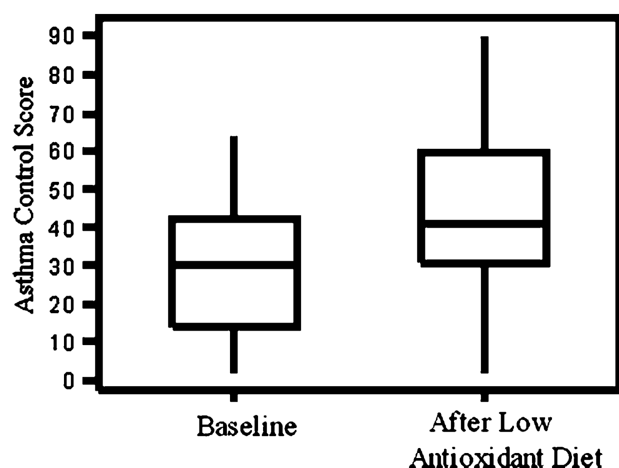

Figure 3. Change in asthma control score following 10 days on the low antioxidant diet ($p = 0.032$).

activation, that has been associated with non-allergic asthma [34]. An important first-line host defence molecule, CRP recognizes pathogens and damaged cells and promotes their elimination by activating the complement system and mediating phagocytic clearance [35]. Thus, the study by Watzl et al. [33] supports our finding that altering intake of antioxidant-rich foods has an effect on innate immunity.

The response to the supplements used in our study provides evidence for the role of dietary antioxidants in protecting against neutrophil responses in asthma. Following each of the lycopene-rich treatments, plasma lycopene concentrations increased 3-fold, corresponding with a reduction in the proportion of airway neutrophils. Furthermore, the tomato extract also reduced neutrophil elastase activity in the airways. Neutrophil elastase is a proteolytic enzyme, present in excess when neutrophil numbers surge, thereby contributing to destruction of lung tissue and accelerating airway inflammation. Our data thus demonstrates the anti-inflammatory properties of lycopene-rich treatments in reducing neutrophil proportions and reducing neutrophil elastase activity, indicating both a reduction in neutrophil influx and neutrophil activation. It should be noted that if subjects had not been depleted of antioxidants, the effect of the supplements may not have been as significant, or seen at all. Nonetheless, this study achieved its aim of proving the concept that lycopene-rich supplements can affect airway inflammation. It is likely that this will be most relevant in subjects with severe asthma, who have higher levels of inflammation and oxidative stress, or in subjects with dietary deficiency, which may further impair antioxidant defences. Our data also raises the possibility that lycopene, which modifies airway neutrophil levels, may also be relevant to other diseases involving airway neutrophilia, such as COPD. This would be an interesting area for future research.

A possible mechanism by which enhanced neutrophilic inflammation may cause a loss of asthma control may involve oxidative damage, as activated neutrophils produce reactive oxygen species, which can lead to oxidative stress [3]. Furthermore, oxidative stress

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Table II. Clinical characteristics and induced sputum inflammatory markers at baseline and following 10 days on low antioxidant diet ($n = 22$).

	Baseline	Following 10 days on low antioxidant diet	p-value
%predicted $FEV_1^{a,d}$	79.4 (71.6–87.2)	76.5 (68.9–84.1)	0.004
%predicted $FVC^{a,e}$	93.0 (87.1–98.9)	90.4 (84.3–96.5)	0.002
%FEV_1/FVC^b	71.5 (57.8–75.3)	70.5 (61.0–75.0)	0.407
Asthma Control Score [a]	1.0 (0.6–1.4)	1.4 (1.0–1.8)	0.035
PD_{15} (mLs)[c]	0.66 (0.74)	1.02 (0.70)	0.838
Total cell count ($\times 10^6$/mL)[b]	2.70 (1.1–5.3)	2.34 (1.1–8.5)	0.401
%Neutrophils[b]	31.0 (13.1–45.9)	41.0 (24.2–56.6)	0.038
%Eosinophils[b]	1.3 (0–8.1)	1.0 (0–3.1)	0.894
%Macrophages[b]	50.5 (44.6–62.3)	39.2 (31.6–51.8)	0.060
%Lymphocytes[b]	0.75 (0.1–2.0)	0.25 (0–1.3)	0.834
Exhaled nitric oxide (ppb)[b]	21.2 (14.9–37.0)	19.9 (16.4–27.5)	0.975
Neutrophil elastase (ng/mL)[b]	856 (113–3224)	1114 (119–3202)	0.961

[a]Values are mean (95% CI); [b]Values are median (interquartile range); [c]PD_{15} is provocation dose resulting in 15% drop in baseline FEV_1 expressed as geometric mean (log SD); [d]FEV_1 is forced expiratory volume in 1 s; [e]FVC is forced vital capacity.

which may further induces the nuclear transcription factor NFκB, which mediates neutrophil influx and activation and may perpetuate the cycle of neutrophilic inflammation [36]. Several previous interventions in healthy people using antioxidant-rich foods, specifically fruit and vegetables, have resulted in changes in markers of oxidative stress. For example, a study in healthy controls demonstrated that a daily intake of fruit and vegetables of nine serves vs four serves resulted in a lower concentration of breath ethane in the group with the higher intake [37]. Another study saw a reduction in urinary 8-iso-$PGF_{2\alpha}$ and 8-hydroxydeoxyguanosine as healthy subjects increased their daily fruit and vegetable intake from six to 12 serves per day [38].

Our study showed no changes in exhaled nitric oxide as a result of antioxidant manipulation. This is not surprising, however, as nitric oxide is a marker of eosinophilic disease and not general inflammation in asthma [39]. While eNO has been shown to correlate with oxidative stress markers in some asthma studies [40,41], this relationship is not consistently observed [42], which probably reflects the heterogeneous nature of inflammation in asthma. As the changes we induced using antioxidant manipulation involved neutrophil influx rather than changes in eosinophilic inflammation, corresponding changes in exhaled nitric oxide levels would not be expected.

While fruit and vegetable intake was not the only dietary change that subjects adopted, the decrease in plasma carotenoid levels, which are an accurate marker of fruit and vegetable intake [19], suggests that this was an important component of dietary change. We have previously demonstrated that plasma carotenoid levels correlate with induced sputum carotenoids levels [20]. Thus, we are confident that the changes in antioxidant protection that we measured in plasma reflect events in the airways. Interestingly, the low antioxidant diet consumed by subjects during the initial phase of this study, which incorporated one serve of fruit and two serves of vegetables per day, is typical of Western diets. It is equivalent to the median intake of fruit and vegetables for adult Australian males (all ages) and females (<45 years) [43]. As $\sim 50\%$ of the population usually consume a diet with an intake of fruit and vegetables equivalent to the study diet or less, it appears likely that this dietary pattern, which must be considered suboptimal for lung health, may be having a significant impact on asthma management. While the overall impact on disease severity and prevalence remains to be established, the data suggests that it may be considerable and supports the hypothesis that changes in dietary fruit and vegetable intake may be a factor contributing to the increased burden of illness from asthma seen in Western societies.

Table III. Comparison of plasma carotenoid levels at end of 7 day treatment with placebo vs tomato juice vs tomato extract.

Outcome	n	Placebo	Tomato Juice	Tomato Extract	p-value
Total carotenoids	16	0.80 (0.42–1.05)	1.31^a (0.85–1.70)	1.24^a (0.90–2.20)	0.0004
Lutein	16	0.14 (0.11–0.21)	0.17 (0.13–0.23)	0.14 (0.08–0.20)	0.144
β-cryptoxanthin	16	0.06 (0.04–0.07)	0.07 (0.04–0.10)	0.06 (0.04–0.10)	0.736
Lycopene	16	0.24 (0.07–0.47)	0.60^a (0.41–1.07)	0.70^b (0.46–1.26)	<0.0001
α-carotene	16	0.015 (0.009–0.023)	0.008 (0.004–0.018)	0.01 (0.004–0.025)	0.214
β-carotene	16	0.20 (0.07–0.24)	0.24 (0.17–0.42)	0.29^c (0.17–0.57)	0.026

Values are median (interquartile range); [a]$p < 0.01$ vs placebo; [b]$p < 0.001$ vs placebo; [c]$p < 0.05$ vs placebo.

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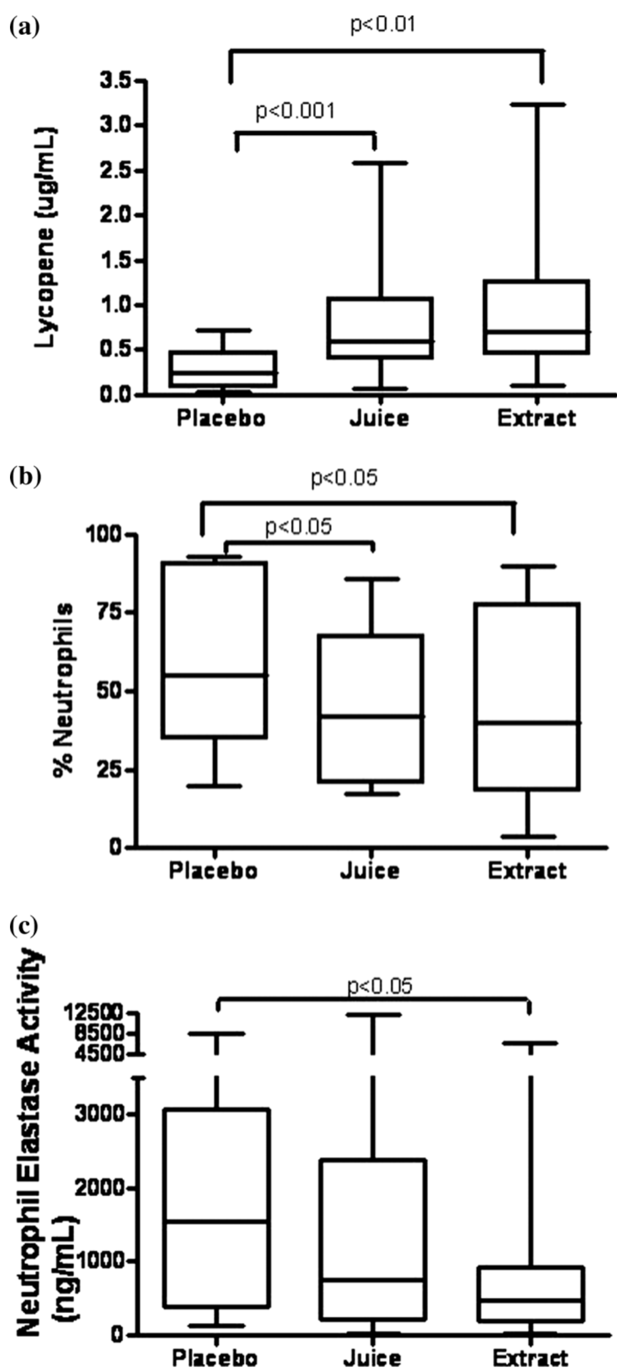

Figure 4. Comparison of (A) plasma lycopene concentration, (B) induced sputum neutrophils and (C) induced sputum neutrophil elastase activity, at end of 7 day treatment with placebo vs tomato juice vs tomato extract.

It is important to note that both of the interventions used in our study contain nutrients, other than lycopene, that may be contributing to the anti-inflammatory effects observed. Two important antioxidants that are present in both tomato juice and tomato extract are β-carotene, which was observed to increase following both supplements (Table III) and vitamin C. While it is impossible to elucidate the contribution of these other nutrients, it is likely that they enhance the effects of lycopene, which is a benefit of supplementing with whole foods, rather than with individual nutrients [18].

There are some methodological features to this study that warrant consideration. The initial antioxidant withdrawal phase used an open design and the supplementation trial used a randomized cross-over design. Open studies can introduce bias, however this is an unlikely explanation of the results because the same pattern of inflammatory response (airway neutrophils) was altered in the withdrawal and supplementation phases. The treatment phase was unblinded, however the significant changes that were observed were in objective measures (%neutrophils, neutrophil elastase) that would not be affected by assessment bias. In the supplementation trial we did not observe changes in the clinical markers, suggesting that a longer duration of supplementation with lycopene-rich treatments may be needed to improve clinical asthma variables. This is supported by the relative magnitude of changes seen, where, after placebo supplementation, the increase in airway neutrophils is further accentuated, whereas this effect is only partially reversed with lycopene-rich treatments. A longer duration of supplementation may be needed to reduce neutrophils further and alter clinical outcomes. It should also be noted that the plasma lycopene levels following the placebo treatment (Table III) are higher than at the end of the initial washout period (Table I), which may also have contributed to the lack of difference in the clinical parameters between the placebo and the treatment groups. The clinical parameters that worsened during the initial antioxidant washout period (i.e. lung function and asthma control score) did not stay at these levels after the placebo treatment but actually became closer to the baseline values. This may mean that the 10-day washout was sufficient to reduce lycopene levels after a regular diet but not after supplementation with the tomato juice or extract. We do not expect that this is the case, however, as the 10 day washout period that was used is based on previous studies which have demonstrated that the plasma half-life of lycopene is 2–3 days, with plasma concentrations of lycopene returning close to baseline values $\sim$3 days after a supplemental dose [24,25]. Another explanation is that compliance with the low antioxidant diet deteriorated as the trial progressed. However, the 24 h diet analysis we conducted throughout the trial did not identify such an effect. While the changes in airway inflammatory markers are exciting, the methodological issues emphasize the need to conduct further research involving greater subject numbers, receiving supplementation for a longer period, with tight control of the diet during the treatments. A longer washout period between the treatment periods or a parallel study design is also recommended.

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Table IV. Comparison of clinical and induced sputum inflammatory markers at end of 7 day treatment with placebo vs tomato juice vs tomato extract.

Outcome	*n*	Placebo	Tomato Juice	Tomato Extract	*p*-value
Asthma Control Score[a]	17	1.10 (0.75–1.44)	0.96 (0.60–1.31)	1.14 (0.76–1.51)	0.287
% predicted FEV_1^a	17	80.9 (72.7–89.2)	80.0 (71.1–88.9)	79.7 (72.0–87.5)	0.645
%predicted FVC[a]	17	92.3 (85.2–99.4)	91.7 (83.6–99.8)	91.3 (83.9–98.7)	0.752
FEV_1/FVC%[a]	17	71.2 (67.5–75.0)	71.0 (67.1–74.9)	71.1 (67.6–74.7)	0.888
Total cell count	10	3.65 (1.13–9.05)	3.33 (2.16–12.74)	2.79 (1.26–4.77)	0.135
Neutrophils%	10	55.1 (35.0–91.1)	42.0[b] (21.0–67.8)	39.8[b] (18.4–77.5)	0.006
Eosinophils%	10	0.4 (0–1.5)	0.9 (0–17.8)	0.9 (0.1–1.8)	0.974
Macrophages%	10	33.9 (6.7–58.8)	46.3 (15.1–66.6)	46.7 (19.5–69.4)	0.187
Lymphocytes%	10	0.9 (0.1–1.5)	0.1 (0–1.1)	0.5 (0.1–2.1)	0.710
Neutrophil elastase (ng/ml)	13	1551 (379–3069)	756 (208–2371)	458[b] (175–924)	0.023
Exhaled nitric oxide	11	19.1 (12.9–31.4)	19.7 (11.0–25.9)	19.6 (13.1–31.6)	0.629

[a]Values are mean (95% CI). All other values are median (interquartile range); [b]$p < 0.05$ vs placebo.

In conclusion, this is the first study to provide proof of concept that reducing the intake of antioxidant-rich foods worsens asthma control and lung function. Furthermore, we have shown that increases in airway neutrophils, resulting from a low antioxidant diet, can be reversed using lycopene-rich treatments. Dietary antioxidant consumption may be an important co-factor that modifies clinical asthma status and, thus, changes in dietary antioxidant intake may be relevant to rising asthma prevalence. Further studies, involving long-term intervention with lycopene-rich supplements in a larger cohort are warranted.

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