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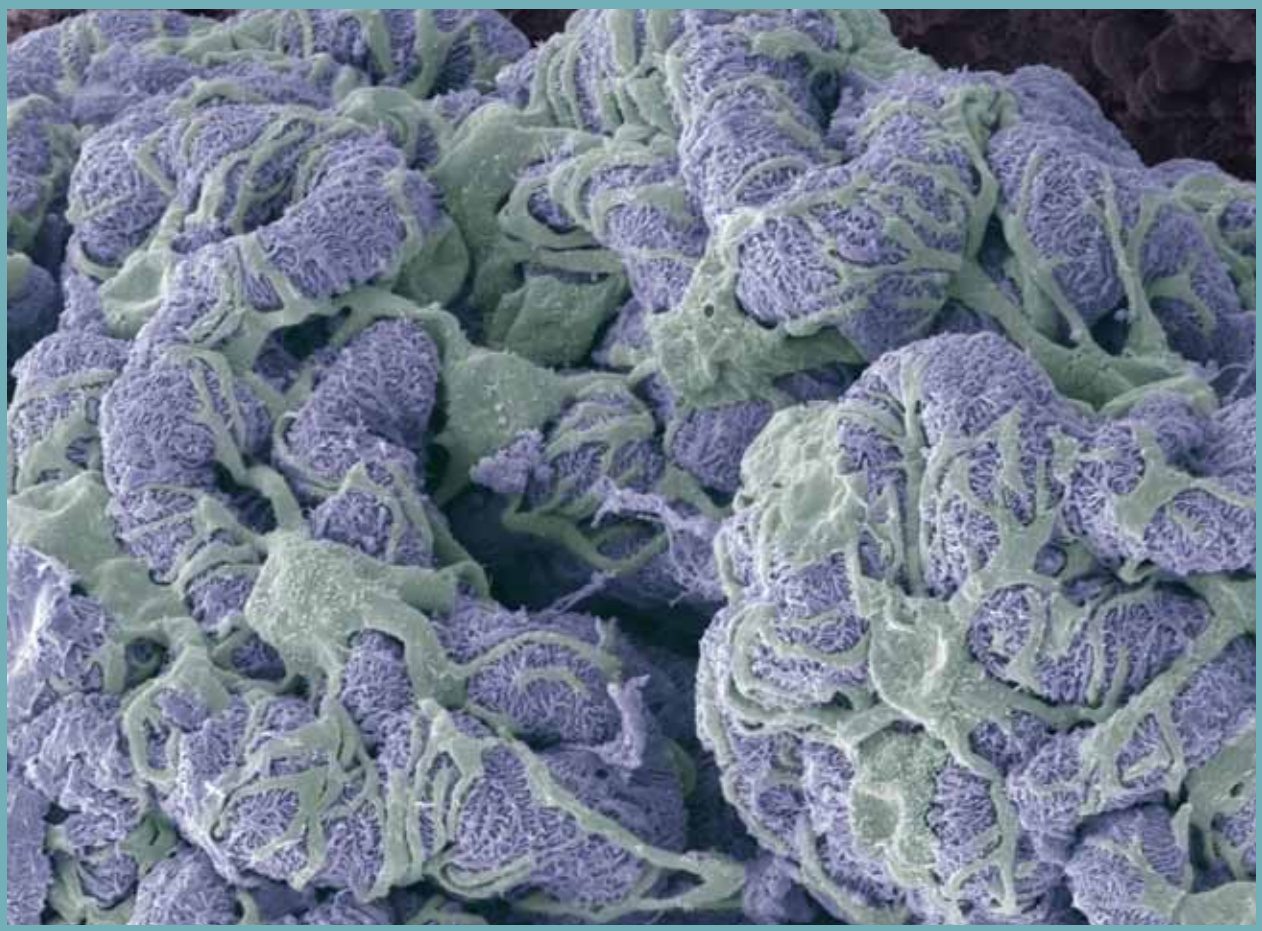

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Nutritional Supplement Therapy Improves Oxidative Stress, Immune Response, Pulmonary Function, and Quality of Life in Allergic Asthma Patients: An Open-Label Pilot Study

Chih-Hung Guo, PhD, Po-Jen Liu, MD, Kuan-Pin Lin, RN, MSN, Pei-Chung Chen, PhD

Abstract

OBJECTIVE: To examine the effects of nutritional supplement therapy on oxidant-antioxidant status, inflammation and immune system responses, pulmonary function, and health-related quality of life in patients with mild to moderate allergic asthma. METHODS: Adult asthma patients (n=30) received daily multiple nutrient supplements for two months. Age- and gender-matched healthy controls (n=30) did not receive any supplements. Enzymatic and non-enzymatic antioxidant status, malondialdehyde (MDA), high-sensitivity C-reactive protein (hs-CRP), immunoglobulin E (IgE) and T-lymphocyte subsets, pulmonary function indices, as well as scores for asthma control and quality of life, were assessed at baseline, at one month of treatment, and at two months of treatment, which was also the end of the study. RESULTS: At baseline, asthma patients had significantly higher IgE, MDA, copper (Cu), hs-CRP, and CD19 and CD4/CD8 lymphocyte ratios, and decreased selenium (Se), zinc (Zn), β-carotene, vitamins C and E, and catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) activities compared to healthy controls ($p < 0.05$). During the study period, asthmatics showed non-significantly increased pulmonary function and a trend toward lower IgE levels, markedly reduced MDA, Cu, hs-CRP, and CD19 and CD4/CD8 ratios, and increases in levels of Se, Zn, β-carotene, vitamins C and E, and enzymatic antioxidant activities. Also, their asthma control and health-related quality-of-life scores increased significantly by the end of the study. CONCLUSION: Our results indicate that nutritional supplement therapy may improve dysregulated oxidant and antioxidant status, inflammation and immune responses, pulmonary function, and health-related quality of life in patients with mild to moderate allergic asthma. (*Altern Med Rev* 2012;17:42-56)

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Introduction

Asthma is a major public health concern that affects the lives of more than 300 million people worldwide.[1] Allergic asthma (also referred to as 'extrinsic asthma') is a condition of chronic allergic airway inflammation, often due to a dysregulation in the immune system. 'Intrinsic asthma', in contrast, is generally triggered by non-allergic factors such as extreme emotions, exercise, or certain chemicals. In allergic asthma (the focus of this study), a variety of inflammatory cells typically infiltrate the airway epithelium and release different mediators that can influence inflammatory responses and airway remodeling.[2]

T lymphocytes (T cells) are one of the critical mediators of the airway inflammation in allergic asthma. Activated CD4+ Th2-type lymphocytes can exacerbate airway inflammation and the airway hyper-responsiveness associated with asthma. These cells also produce signals that activate B lymphocytes and stimulate the production of Immunoglobulin E (IgE) antibodies.[3] However, an association between total IgE and asthma is less clear.

Inflammation in the airways increases the risk of oxidative damage, principally due to the activity of immune cells such as macrophages, neutrophils, eosinophils, and lymphocytes. These cells release reactive oxygen species, which, in turn, can enhance the inflammatory response in the airways via the production of pro-inflammatory cytokines.[4,5] There is increasing evidence that the oxidative stress associated with features of asthma,

Key words: asthma, airway hyper-responsiveness, antioxidants, superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, inflammation, immune response, pulmonary function, quality of life

such as decreased antioxidant enzyme status and airway hyper-responsiveness, contributes to a shift in the Th1/Th2 lymphocyte balance and induces cytokine-mediated neutrophil chemoattraction.[6] Oxidative damage to mitochondrial respiratory chain complexes further increases allergic airway inflammation.[7] Oxidant-antioxidant imbalance may lead to vascular permeability, mucus hypersecretion, smooth muscle contraction, and epithelial shedding.[8] Imbalanced antioxidant enzyme activity has been found to be significantly associated with asthma symptoms, suggesting the presence of mitochondrial dysfunction and oxidative stress in allergic asthma.[9,10] Thus, the development of asthma involves complex interactions between oxidative stress and immunologic responses.

Relative to healthy people, asthmatics have been found to have decreased serum concentrations of vitamin D and folate, as well as low plasma B6 in patients treated patients with theophylline.[11-13] Although some studies have been negative, low blood levels of the antioxidants, β-carotene, and vitamins C and E, trace elements zinc (Zn) and selenium (Se), lycopene, and coenzyme Q10 (CoQ10) have also been noted in these patients, and bioflavonoids such as β-carotene and lycopene have been shown to correlate with pulmonary function and asthma severity.[14-20]

Because oxidative stress plays a key role in propagating the inflammatory response in asthma, it has been proposed that a combination of antioxidants may represent an effective strategy in the treatment of the disorder.[4] Some studies have found that patients who received antioxidant and/or omega-3 (n-3) fatty acid treatment showed decreases in certain asthma symptoms or reduced inflammatory responses;[21-23] however, a failure of antioxidants or n-3 fatty acids to improve asthma symptoms has also been reported.[24,25]

Micronutrients have synergistic effects and some of these nutrients are critical for mitochondrial support.[26,27] Only single-nutrient antioxidant treatment has often been used in attempts to mitigate asthma symptoms, which may be one reason for the limited benefits observed in some studies. Multi-nutrient supplementation may more effectively ameliorate the pathological features of asthma. Furthermore, the effects of nutritional supplement therapy on overall micronutrient status, immune system response, pulmonary function, and quality of life in asthma have thus far not been examined.

In the present study, we investigated the effects of two months of multi-nutrient supplementation on micronutrient status, oxidative stress and inflammation, immune response, pulmonary function, and health-related quality of life in patients with mild to moderate asthma.

Methods

Subjects

Between March and November 2007, 30 patients with mild to moderate allergic asthma were enrolled in the study. These patients (9 men and 21 women, mean age 38 ± 2 years) had been diagnosed in the otolaryngology unit of the Cheng-Ching Hospital (Taichung, Taiwan) according to the criteria recommended by the American Thoracic Society.[28] Pulmonary function tests included forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1), measured by using a Jaeger MasterScreen (VIASYS Healthcare GmbH; Hoechberg, FRG). Exclusion criteria included evidence of severe persistent asthma, cancer, chronic diseases such as diabetes, liver or kidney pathology, body mass index (BMI) >27 kg/m^2, and supplementation with natural herbs, antioxidants, vitamins/minerals, n-3 fatty acids, or anti-leukotriene agents used within the previous six months.

Thirty healthy volunteer subjects of similar age and gender served as the control group. All subjects signed informed consent statements. The study protocol was approved by the Ethics in Human Research Committee of the Cheng-Ching Hospital.

Nutritional Supplementation

Asthma patients received daily dietary supplements for two months. Supplements included Oxy Rich™ (containing CoQ10, vitamins A, C, E, and B6), Omega Rich™ fish oil (containing eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], and vitamin E), Healthy Green Barley™ powder (known to be rich in the antioxidant enzymes, superoxide dismutase and catalase[29]), Bio Shield™ (an antioxidant blend of vitamins, citrus bioflavonoids, extracts of green tea and grape seed, Zn, Se, and calcium), Prime C Complex™ (ascorbic acid & calcium ascorbate), and Healthy Life™ (a multivitamin & mineral complex). All formulas were supplied by New Health Enterprise (Irvine, CA, USA). The daily doses of these supplements were guided by our previous clinical trials and internationally recommended daily intakes. (See Table 1 for supplement details). For this particular investigation, there was no placebo group, and the asthmatics served as their own controls.

Table 1. Ingredients and daily doses of multi-nutrient formulas

Nutrient	Daily Dose	Nutrient	Daily Dose
Green Barley powder™	15 g	**Healthy Life™**	
Oxy Rich™		Vitamin A (palmitate/β-carotene)	6,000 IU
Coenzyme Q10 (ubiquinone)	90 mg	Vitamin B1	4.2 mg
Vitamin A (acetate)	3,000 IU	Vitamin B2	4.5 mg
Vitamin C (ascorbic acid)	60 mg	Vitamin B3 (nicotinamide)	60 mg
Vitamin E (d-α-tocopheryl acetate)	22.5 IU	Vitamin B6 (pyridoxine HCl)	6 mg
Vitamin B6 (pyridoxine HCl)	6 mg	Biotin	450 µg
Omega Rich™,[1]		Pantothenic acid	15 mg
Vitamin E (d-α-tocopheryl acetate)	9.0 IU	Folic acid	900 µg
EPA	990 mg	Vitamin B12	9 µg
DHA	660 mg	Vitamin C (ascorbic acid)	225 mg
Prime C Complex™		Vitamin D3	7.5 µg
Ascorbic acid/calcium ascorbate	1,500 mg	Vitamin E (d-α-tocopheryl acetate)	30 mg
Bio Shield™		Choline	45 mg
Green tea extract	336 mg	Inositol	75 mg
Grape seed extract	75 mg	Selenium (yeast)	150 µg
Citrus bioflavonoid complex	54 mg	Zinc (gluconate)	24 mg
Vitamin A (palmitate/β-carotene)	15,000 IU	Magnesium (oxide)	300 mg
Vitamin C (ascorbic acid)	246 mg	Copper	3 mg
Vitamin E (d-α-tocopheryl acetate)	80.6 IU	Calcium (carbonate)	450 mg
Calcium (carbonate)	165 mg	Chromium (yeast)	150 µg
Zinc (gluconate)	15 mg	Lycopene	3 mg
Selenium (yeast)	150 µg		

[1] Concentrated fish oil: Eicosapentaenoic acid (20:5, n-3) and Docosahexaenoic acid (22:6, n-3)

Biochemical Analysis

Blood was drawn in the morning after an overnight fast of 12 h. All specimens were collected at baseline, at one month during treatment, and at the end of the two-month study period. Hemoglobin concentrations, total serum IgE, eosinophil counts, and eosinophil cationic protein (ECP) were determined using routine laboratory methods.

Determination of Oxidative Stress Status and Inflammation

Malondialdehyde (MDA) is an end product of lipid peroxidation by reactive oxygen species. Higher plasma MDA levels indicate a greater degree of oxidative stress. To perform this assay, plasma samples were mixed with 3% sodium dodecyl sulfate, 0.1N HCl, 10% phosphotungstic acid, and 0.7% thiobarbituric acid, and then incubated at 95°C. MDA was extracted into n-butanol and the fluorescence of the n-butanol layer was measured.[30]

Plasma concentrations of high-sensitivity C-reactive protein (hs-CRP) were measured using the human CRP ELISA kit (E-80CRP, Immunology Consultants Laboratory, Inc, Newberg, OR, USA). The absorbance at 450 nm was measured, and readings were interpolated into the standard curve.

Catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and superoxide dismutase (SOD) are endogenous antioxidant enzymes that serve to neutralize oxygen free radicals and hydrogen peroxide, thereby helping to control oxidative damage. Erythrocyte catalase, GPx, GR, and SOD enzyme activities were thus determined as indices of anti-oxidative capacity. Catalase activity was estimated using a commercial EIA kit (Cayman Chemical Corporation, Ann Arbor, MI); one unit was defined as the amount of enzyme that caused the formation of 1.0 nmol of formaldehyde per minute at 25°C. GPx activity was measured using a kit from Cayman Chemical (cat #703102); the rate of decrease in absorbance at 340 nm was directly proportional to the GPx activity. GR activity was assessed by monitoring the oxidation of NADPH to NADP+ after the addition of oxidized glutathione (GSSG); one unit was defined as the amount of enzyme that catalyzed the reduction of 1 mmoL of GSSG. SOD activity was determined with a RANSOD kit (Randox, San Diego). This procedure is based on competitive reduction of p-iodonitrotetrazolium (INT) salts between SOD in the sample and superoxide anions generated during the test. One unit was defined as the amount of enzyme necessary to produce a 50% inhibition in the rate of INT reduction.

Determination of Trace Minerals

The concentrations of plasma Zn and Cu were measured with a flame atomic absorption spectrophotometer (932 plus, GBC, Australia) using an air-acetylene flame without background correction at 213.9 and 324.71 nm, respectively. Samples were digested in a H_2O_2/HNO_3 mixture in a START D microwave-assisted digestion system (Milestone Microwave Labstation ETHOSD), and subsequently brought up to volume with double deionized water.

Accessory hydride formation system (HG 3000) from GBC was also used for determining Se concentrations. Samples were digested in a $HNO_3/HClO_4$ mixture for a total of 6.5 h starting at an initial temperature of 60°C for 0.5 h, followed by increasing the temperature in 10°C increments, and finally heated to 200°C for 0.5 h in a mixture of 0.5 mL nitric acid (16N). Accuracy of this method was confirmed by comparing to serum reference materials (level 2, NO0371, Seronorm, Nycomed, Oslo, Norway).[30]

Determination of β-carotene, and Vitamins C and E

Plasma concentrations of β-carotene and α-tocopherol were measured by reversed-phase HPLC (LabAlliance, HPLC Pumps, Systems & Accessories), using a modification of the method of Borel et al.[31] Briefly, samples were extracted with n-hexane/ethanol containing α-tocopheryl acetate as an internal standard. The n-hexane layer was separated and evaporated to dryness. The residue was re-dissolved in the methanol/acetonitrile/tetrahydrofuran mobile phase. A Gemini C18 column (250 x 4.6 mm, 5 μm)(Phenomenex) was used and peaks were detected at wavelengths of 450 nm for β-carotene and 295 nm for α-tocopherol.

For ascorbic acid determination, plasma samples were immediately treated with 4% metaphosphoric acid/dithiothreitol as a stabilizer. The product was then coupled to o-phenylenediamine to produce a chromophore for which the absorbance was measured at 340 nm.[30]

Determination of EPA and DHA

Plasma samples were dissolved in a mixture of methanol/benzene (4:1, v/v), and then mixed with acetyl chloride. The reaction mixture was stirred at 40°C for 1 h and then heated at 100°C for 1 h. The mixture was cooled to room temperature, and 3 mL of a 6% (wt/v) K_2CO_3 solution and 0.5 mL benzene were added and thoroughly mixed. The samples were then centrifuged for 10 min (900 x g at 4℃). The benzene layer was removed and added to a gas

chromatography vial ready for analysis.[32] The extract was injected into a Hewlett-Packard 5890 gas-liquid chromatogram fitted with a 30 m x 0.25 nm x 0.25 µm silica capillary column (SP-2560; Supelco Inc.); detection was accomplished using a flame ionization detector.

Determination of Immunologic Variables

Peripheral blood B- and T-lymphocytes of all subjects were stained with the following monoclonal antibodies, which were conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE): CD3- FITC/ CD4- PE/ CD8- PE/ CD19- PE (eBioscience). T-helper cells (CD4+) are T lymphocytes that direct immune responses via the production of various cytokines. Cytotoxic T cells (CD8+) are T lymphocytes that can induce the death of infected cells or tumor cells. Both CD4 and CD8 are subsets of mature T lymphocytes (CD3). CD19 is an antigen expressed on the surface of B lymphocytes and is involved in regulatory signaling. Abnormalities in CD4, CD8 and CD19 percentages are well documented in asthmatics. Briefly, 100 µL of whole blood were incubated with 20 µL of monoclonal antibody reagent for 15 min in the dark at room temperature. Following leukocyte fixation and erythrocyte lysis with a CyLyse lysing reagent kit (Partec, GMBH, Münster, Germany), the percentages of lymphocyte subsets were determined using a Partec CyFlow ML flow cytometer (Partec, GmbH).

Health-Related Quality of Life (QoL)]

The SF-36 Health Survey™ was used to assess the health-related quality of life for all subjects. The SF-36, the most frequently used health-related QoL scale,[33] is a short-form survey that includes 36 items that incorporate four physical and four psychological dimensions: physical functioning (10 items), role limitations due to physical health problems (4 items), bodily pain (2 items), general health (5 items), vitality (4 items), social functioning (2 items), role limitations due to emotional problems (3 items), and mental health (5 items). These scales can be further aggregated into two summary scores: Physical Component Summary (PCS) score and Mental Component Summary (MCS) score.[34] For the eight dimension scores and two summary scores, higher scores indicate better health and functioning.

Asthma Control Test

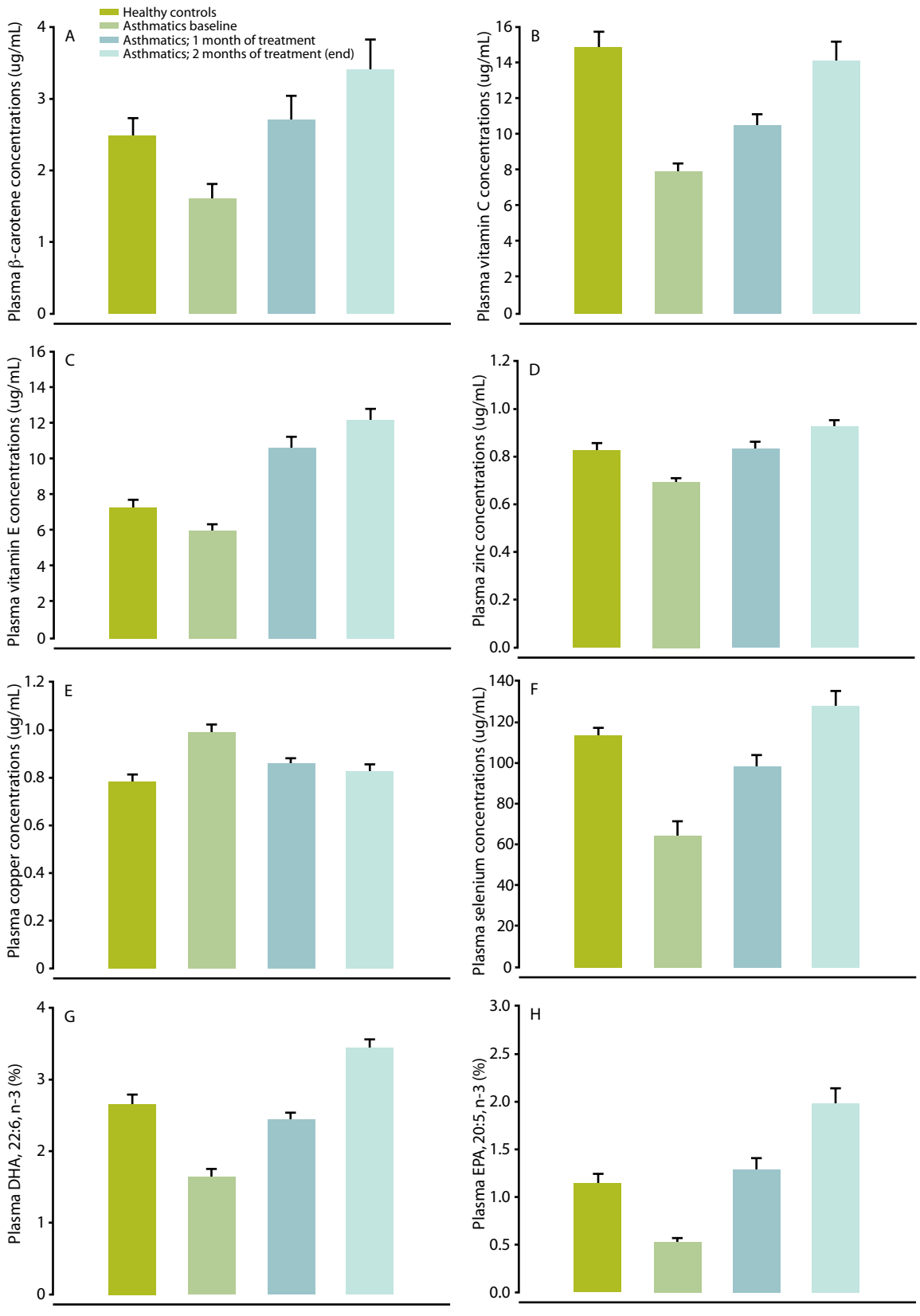
The Asthma Control Test™ (ACT) is a 5-question health survey used to measure a patient's degree of asthma control over the previous four weeks. Scores range from 5 to 25; higher scores indicate better control. If a patient scores <19, asthma symptoms are considered to be poorly controlled, whereas scores between 20 and 24 indicate partially controlled asthma.

Table 2. Characteristics of subjects

	Healthy controls (n = 30)	Asthmatics (n = 30)	
		Pre-treatment	End-of-treatment
Gender (M/F)	10/20	9/21	
Age (yrs)	35 ± 1	38 ± 2	
BMI[3] (kg/m²)	23 ± 1	21 ± 2 (M) / 23 ± 2 (F)	
Eosinophil count (µL)	215.8 ± 31.4	275.1 ± 27.7*	-
ECP (µg/L), median (IQR)[4]	9.2 (5.1-14.8)	15.2 (5.5-24.2) *	-
Hemoglobin (g/L)	136.5 ± 3.4	166.8 ± 9.9*	141.3 ± 4.0

[1] Values represent the mean (standard error of the mean, or SEM) unless stated otherwise. [2] *$p < 0.05$, comparing pre-treatment asthmatics with healthy controls. [3] BMI = body mass index; [4] ECP = Eosinophil cationic protein

Figure 1. Plasma concentrations of non-enzymatic antioxidants and plasma EFAs in healthy subjects and asthmatics



Statistical Analysis

Results are listed as means (standard deviation of the mean, or SEM) or as medians (the average of the interquartile range, or IQR). A Kruskal-Wallis test was used to evaluate the distribution of each data set. Comparisons of different variables between asthma patients and healthy individuals were made by chi-square test, student's t-test, pair t-test or Mann-Whitney test, as appropriate. In addition, one-way ANOVA and Duncan's multiple range tests were used to evaluate differences at the different time points for asthma patients. A two-tailed p-value < 0.05 was considered statistically significant.

Results

Clinical Characteristics

A total of 32 asthma patients were initially included; however, 2 patients were not available at the end of treatment. All data were collected for the remaining 30 asthma patients at two months, with the exception of eosinophil counts and ECP. There were no significant differences in age, sex, and BMI between asthma patients and healthy subjects ($p > 0.05$) (Table 2).

Compared to the healthy subjects, asthma patients had significantly higher baseline total blood eosinophil counts, ECP levels, and concentrations of hemoglobin and IgE at baseline (Table 2 and Figure 4).

At the end of the two-month treatment period, asthma patients showed a trend toward decreased IgE levels compared to their baseline values, and a return to normal concentrations of hemoglobin, comparable to the healthy subjects (Table 2).

Plasma Elements and Vitamins

Asthma patients had significantly lower baseline concentrations of plasma Zn and Se, β-carotene, ascorbic acid and α-tocopherol, and higher Cu concentrations than the healthy control group (Figure 1).

However, compared to their baseline concentrations, asthma patients showed an increase in trace elements (Zn, Se) and the vitamins, and a decrease in Cu, at one month of treatment. At the end of the 2-month treatment period, concentrations for all of these nutrients were significantly higher or comparable to those in healthy controls.

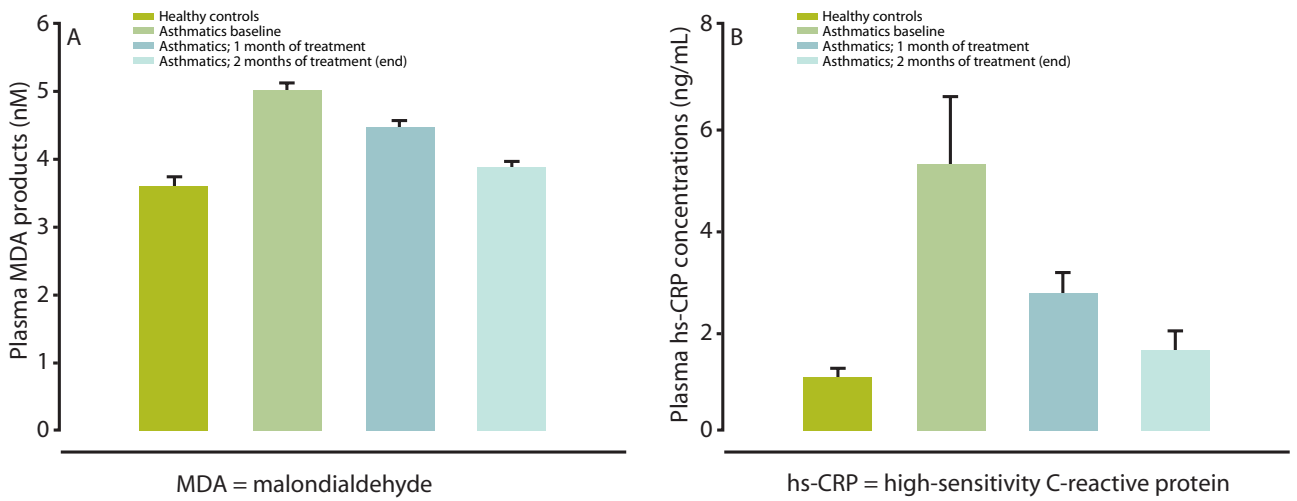
Plasma n-3 Fatty Acids

At baseline, plasma concentrations of EPA and DHA were significantly lower in asthma patients compared to healthy subjects. However, after one month of nutritional intervention, levels of both fatty acids had increased significantly among patients. After the end of treatment, patients showed maximum levels of DHA and EPA, which were considerably higher than those of healthy subjects (Figure 1).

Oxidative Stress Status

Baseline levels of plasma oxidative products (MDA) in asthma patients were considerably higher than those of healthy subjects, indicating increased oxidative stress. At one month of treatment,

Figure 2. Plasma lipid peroxidation and inflammatory markers in healthy subjects and asthmatics



asthma patients showed significantly decreased concentrations of MDA compared to baseline values. By the end of treatment, MDA levels had declined further among patients and were not significantly different from those of healthy controls (Figure 2).

Relative to the healthy control group, asthma patients showed significantly lower erythrocyte antioxidant enzyme activity for catalase, GPx, and GR. During intervention with dietary supplements, the activity of these enzymes gradually increased. By the end of treatment, antioxidant enzyme activities were similar to those of the healthy controls. Conversely, SOD activity in patients steadily decreased during the two months of treatment (Figure 3).

hs-CRP and Immunologic Variables

At baseline, asthma patients had significantly higher concentrations of the inflammatory marker, hs-CRP, compared to the healthy control group. However, concentrations gradually declined during treatment and by two months, hs-CRP levels approximated those of controls (Figure 2).

Among asthma patients, the baseline ratio of CD4/CD8 T cells and the percentage of CD19 were significantly higher than in healthy subjects. After completing treatment, there was no significant difference in CD3 (total mature T-lymphocytes), nor in CD4/CD8 ratios, between asthma patients and healthy controls (Figure 4).

Figure 3. Erythrocyte enzymatic antioxidant activities in healthy subjects and asthmatics

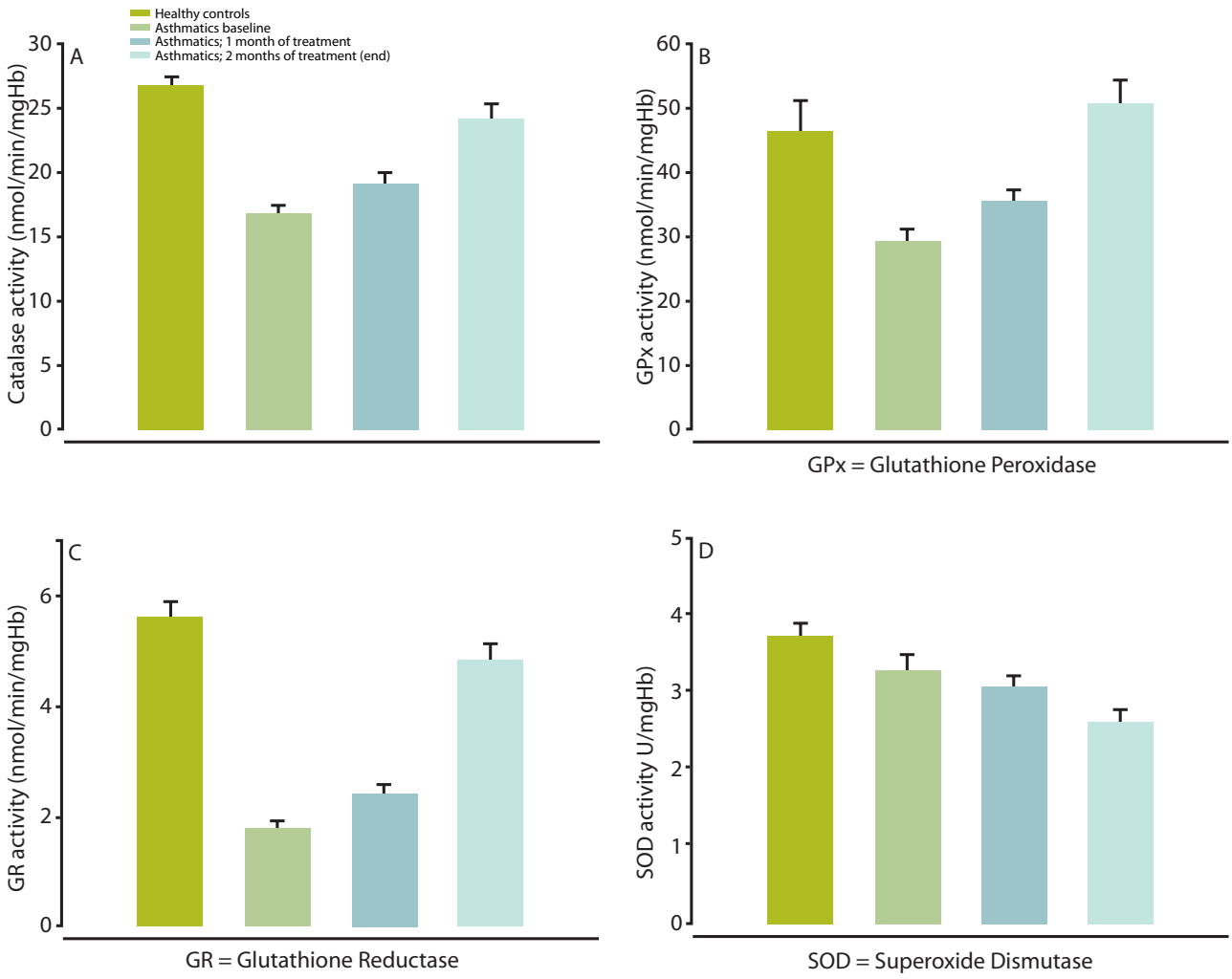


Figure 4. Changes in immune index (CD4/CD8 ratio and %CD19) and total serum IgE in healthy subjects and asthmatics

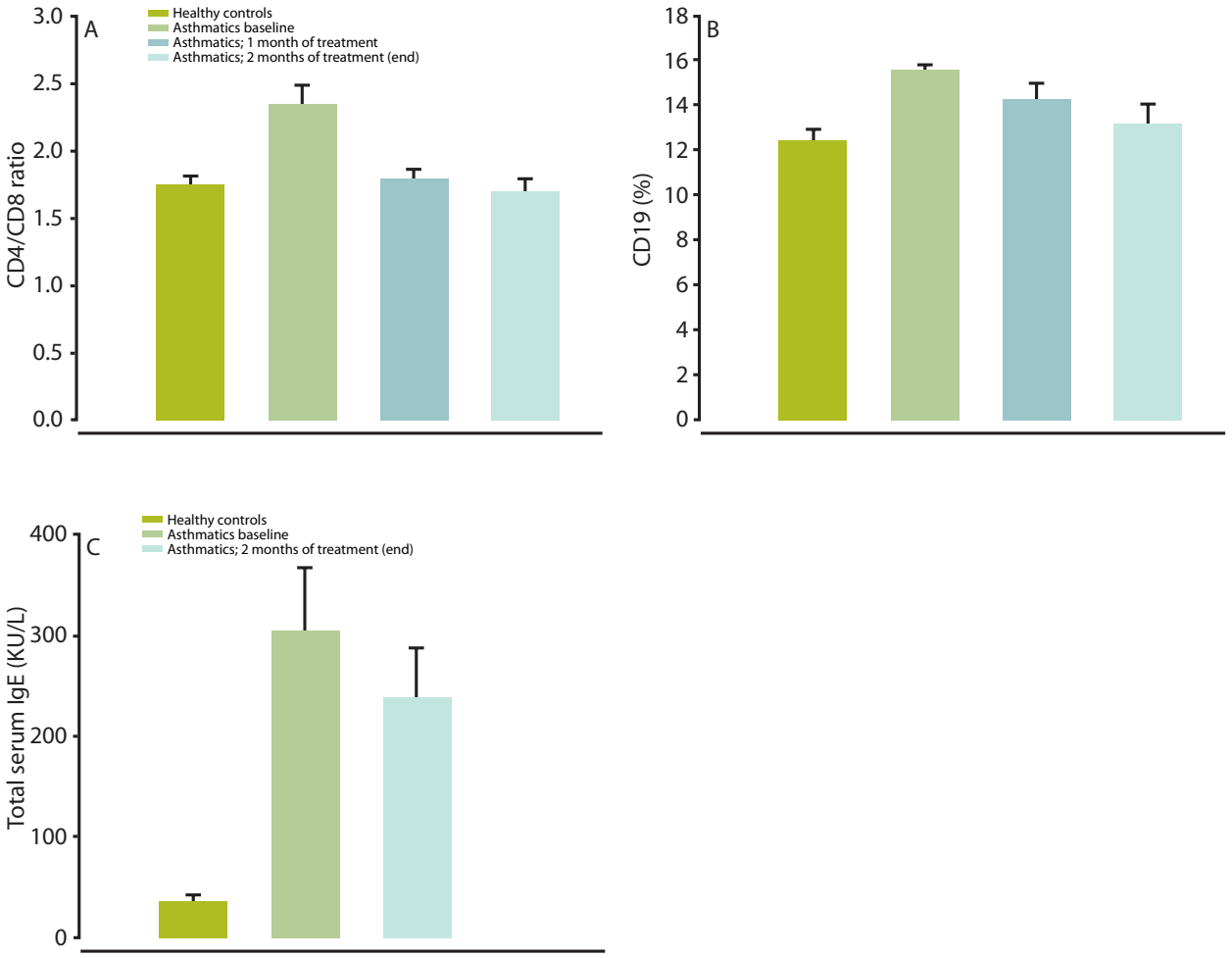
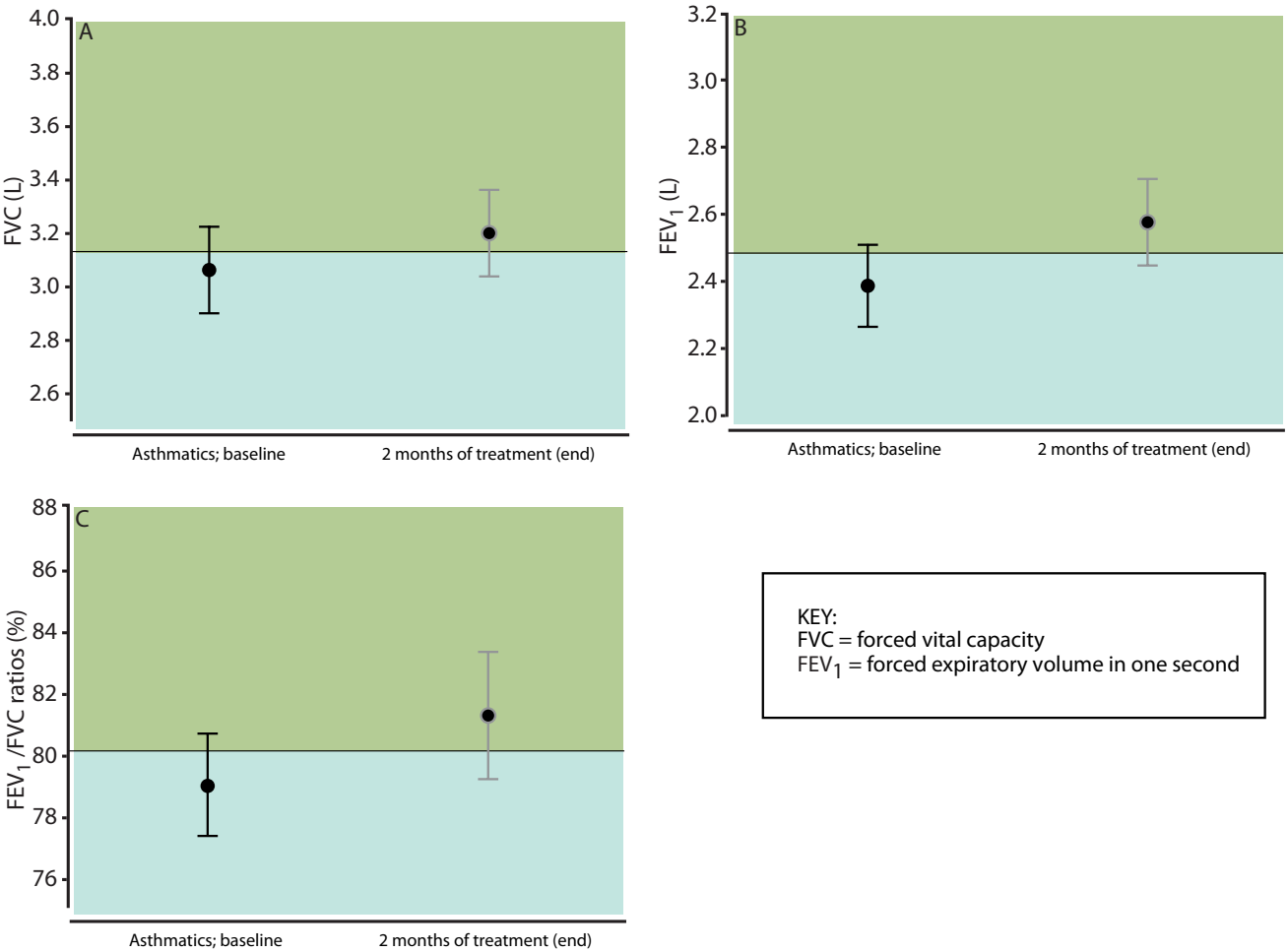


Table 3. Asthma control test (ACT) scores among patients

Degree of control	Pre-treatment, n (%)	End of treatment, n (%)	Difference, (%)
Poorly-controlled (ACT < 19)	23 (76.6)	10 (33.3)*	43.3
Partially controlled (ACT = 20-24)	7 (23.3)	17 (56.7)*	33.4
Well controlled (ACT = 25)	0	3 (10.0)*	10.0

[1] Asthma Control Test (ACT) is a 5-question test; scores range from 5 to 25, with higher scores representing better asthma control; *$p < 0.05$.

Figure 5. Changes in pulmonary function (FVC, FEV_1, and FEV_1/FVC ratio) in asthmatics



Asthma Control, Pulmonary Function, and Quality of Life

Relative to baseline scores, asthma patients showed significant improvements in ACT scores by the end of treatment (Table 3).

Patients also showed a non-significant increase in pulmonary function indicators (FVC, FEV_1, and FEV_1/FVC ratio) between baseline and the end of treatment (Figure 5).

As shown in Table 4, asthma patients had significantly lower baseline SF-36 scores for the physical dimensions within the Physical Health domain, compared with controls; however, no significant differences in baseline scores were found between patients and controls for the four psychological dimensions within the Psychological Health domain. After completing treatment, patients showed significantly increased SF-36 scores in both the Physical Health and the Psychological Health domains compared to those at baseline; except for comparatively lower scores for physical function, results for all other scales were restored to those of healthy controls by the end of treatment.

Discussion

This investigation demonstrated that two months of nutritional supplementation significantly improved micronutrient and oxidative stress status, inflammatory responses, immune function, as well as better asthma control and health-related quality of life in patients with mild to moderate allergic asthma.

Relative to healthy subjects, asthma patients have been shown to have markedly elevated MDA and Cu, lower concentrations of non-enzymatic

antioxidants (Zn, Se, β-carotene, and vitamins C and E), and lower levels of enzymatic antioxidants (catalase, GPx, and GR). Studies have found asthmatics to have significantly higher MDA products in blood, breath condensate, or bronchoalveolar lavage,[17,35,36] as well as suboptimal blood concentrations of β-carotene, ascorbic acid, α-tocopherol, lycopene, and/or CoQ10.[19,37,38] Increased oxidative stress can induce chemokine production and pro-inflammatory cytokines in the respiratory system, which causes tissue injury and immune-mediated damage.[39] Decreased GPx activity has been found to be positively correlated with asthma symptoms,[40,41] and blood levels of the antioxidant enzyme, catalase, have been shown to be lower in asthmatics compared to non-asthmatics.[42] Low concentrations of Se and/or Zn in plasma, serum, hair, and nails have also been reported in asthmatics.[43-45] Reduced plasma Se and Zn levels and higher Cu concentrations in asthma patients have also been associated with reduced antioxidant capacity.[17,46] In the present investigation, nutritional supplement therapy significantly reduced oxidative stress (as reflected by a decrease in MDA), thereby improving the oxidant-antioxidant balance.

SOD is also an important part of the enzymatic antioxidant system. However, enhanced dismutation of excess superoxide can result in an accumulation of hydrogen peroxide (H_2O_2); although H_2O_2 is weaker oxidant than superoxide, it is still a reactive oxygen species. Although no difference in baseline SOD activity between asthma patients and healthy controls was observed in the present study, asthma patients had significantly reduced values of SOD by the end of treatment compared to baseline and at one month of treatment. The decrease in SOD levels suggests a reduction in oxidative stress. This finding was accompanied by increased catalase, GPx, and GR activities.

Cu promotes the catalytic activity of cytosolic SOD; however, excess Cu induces mitochondrial and cytosolic oxidative stress.[47] The release of Cu during tissue damage mediated by inflammation may account for increased Cu concentrations.[48] Zn deficiency, on the other hand, can induce inflammation and promote the intestinal uptake for Cu.[49] Elevated Cu levels can increase protein kinase C activity, which has been associated with the progression of inflammation.[50] A positive relationship between plasma Cu and hs-CRP was observed in a previous study.[17] Increased hs-CRP concentrations have also been associated with airflow obstruction and airway inflammation, and thus may serve as a marker of airway inflammation and asthma severity.[51] In the present results, the markedly increased concentrations of plasma Cu and hs-CRP in asthma patients were decreased by the end of treatment and were comparable to controls.

CoQ10, is an essential electron carrier in the mitochondrial respiratory chain and is another critical antioxidant.[52] Significant uptake of CoQ10 by lung tissue in a rat model has been demonstrated,[53] and a positive correlation between suboptimal plasma concentrations of CoQ10 and α-tocopherol has been noted in patients with asthma.[37] Administration of corticosteroids has been shown to result in mitochondrial dysfunction; however, concurrent treatment with a combination of CoQ10 and vitamins C and E can result in reduced corticosteroid dosages in asthma patients.[53] Vitamin E administration has also been shown to decrease mitochondrial dysfunction and alleviate asthmatic features in animals.[54] In the present study, CoQ10 concentrations were not determined; however, CoQ10 supplementation might be expected to enhance mitochondrial function and improve antioxidant status in asthma patients.

The asthma patients in this study had markedly lower concentrations of plasma EPA and DHA compared to healthy controls, in agreement with other studies.[55] Both EPA and DHA are potentially anti-inflammatory agents, partly by competing with arachidonic acid as substrates for eicosanoids; whereas arachidonate produces pro-inflammatory eicosanoids, EPA produces non-inflammatory eicosanoids. Omega-3 fatty acids also act to reduce the production of inflammatory cytokines from various immune cells.[56] EPA and DHA supplementation has been shown to significantly reduce pro-inflammatory mediator production, as well as serum eosinophil counts and ECP levels.[57] In this study, nutritional supplement treatment significantly increased the plasma status of EPA and DHA in the asthma patients, which likely contributed to their decreased inflammatory status and improved oxidant-antioxidant balance.

Concentrations of eosinophils in sputum have been correlated with symptom severity, the degree of airflow limitation, and airway responsiveness in asthmatics.[58,59] ECP concentrations have also been shown to be correlated with airflow obstruction and IgE concentrations in asthma patients.[60-62] Increased IgE delays programmed cell death of neutrophils from allergic asthmatics, and this may contribute to inflammation in asthma.[63] Anti-IgE therapy has been found to significantly decrease

the eosinophil count in sputum, IgE cells in airway mucosa, and serum IgE in patients with mild to moderate asthma; however, it did not reduce airway hyper-responsiveness.[64] Nutritional status of β-carotene, vitamins C and E, Se, Zn, or EPA and DHA is associated with the immune response.[65] Although there is little evidence to indicate that nutritional intervention significantly affects total IgE status in asthma patients, patients in this particular study who received two months of nutritional supplementation showed a trend for decreased mean serum IgE levels.

The asthma patients in this study had a higher baseline percentage of B lymphocyte antigen (CD19) and a higher CD4/CD8 T cell ratio than healthy subjects; however, the CD19 percentage and CD4/CD8 ratio had decreased by the end of treatment and were comparable to those of controls. Increased CD4/CD8 ratios in asthma patients have been observed, with the CD4/CD8 ratios being inversely correlated with lung function.[66] Animal studies have shown that Th2 lymphocytes (differentiated from CD4 T-helper cells) and increased production of type 2 cytokines, such as interleukin-4 (IL-4) and IL-13, are involved in airway inflammation, mucus production, and airway hyper-responsiveness in asthma.[67,68] Oxidative stress contributes to an altered Th1/Th2 balance in favor of an augmented Th2 response, also promotes neutrophil migration and the induction of various pro-inflammatory genes.[6] Administration of an antioxidant-rich multi-nutrient formula in the current study reduced oxidative stress in the patients with asthma, likely helping to restore the Th1/Th2 immune balance in the process.

Furthermore, asthma patients in the present study showed marked improvements in asthma control scores and non-significantly higher pulmonary function after nutritional supplementation. Relative changes in pulmonary function variables depend on factors such as a patient's height, age, sex, and stage of pulmonary fibrosis; such factors may have contributed to the

Table 4. SF-36 Quality-of-life scores among patients versus controls

SF-36 Variables	Healthy controls (n = 30)	Asthmatics (n = 30)	
		Pre-treatment	End-of-treatment
Physical Domain	53.4 (0.9)	43.8 (1.9) *	50.7 (1.6) **
Physical functioning	89.4 (1.0)	83.3 (3.2) *	85.7 (2.9)
Physical role limitations	83.3 (4.1)	58.3 (7.9) *	82.5 (5.1) **
Bodily pain	78.6 (3.0)	64.8 (4.3) *	80.5 (3.6) **
General health	60.1 (5.4)	39.4 (3.9) *	56.4 (3.6) **
Psychological Domain	46.5 (1.4)	42.8 (2.4)	48.8 (1.6) **
Vitality	57.5 (2.7)	56.0 (3.9)	64.3 (3.0) **
Social functioning	78.0 (2.4)	74.2 (3.7)	81.3 (2.9) **
Emotional role limitation	75.4 (5.8)	61.1 (7.7)	85.6 (4.8) **
Mental health	63.5 (2.3)	64.3 (3.2)	67.6 (2.5)

[1]Values represent the mean (standard error of the mean, or SEM); [2] SF-36, 36-Item Short Form Health Survey; [3]* $p < 0.05$, comparing pre-treatment asthmatics with healthy controls; ** $p < 0.05$, comparing end-of-treatment with pre-treatment scores

statistically non-significant results. Various studies have shown Se, Zn, vitamin C, lycopene, or n-3 fatty acid concentrations to be positively associated with FEV1/FVC ratios,[14,17,23] consistent with improved pulmonary function, whereas MDA and Cu/Zn ratios have been inversely associated with FEV1/FVC ratios.[17] Supplementation with vitamin C or Se has been shown to decrease asthma frequency, as well as increase FEV_1 and FVC.[69-71]

Our results showed that nutritional supplement therapy had beneficial effects on measures from a quality-of-life instrument, SF-36, in asthma patients. Asthma attack frequency is associated with quality of life;[72,73] not surprisingly, an association between lung function and quality of life in asthma has been found.[71] Thus, one of the main objectives of health care in asthma is preserving a satisfactory health-related quality of life.[34] Increased asthma severity is significantly related to a reduced quality of life.[73] Although one study found asthma severity to predict scores for the physical component, but not the mental component, of the SF-36,[74] another study demonstrated a trend toward lower scores for both domains in asthma patients.[34] Following nutritional supplement therapy, the asthmatics in the current study showed significantly higher physical and psychological scores. This suggests that nutritional supplement intervention can positively influence quality of life in asthma.

In conclusion, nutritional supplement therapy was found to not only improve micronutrient homeostasis, oxidant-antioxidant balance, and inflammatory status in patients with mild to moderate allergic asthma, but also to provide benefits in the areas of pulmonary function and the quality of life in these patients. Further large-scale studies are needed to better understand the full impact of nutritional supplement therapy on asthma patients.

Conflict of interest: The authors do not have any conflicts of interest to declare.

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