A comprehensive evaluation of the enzymatic and nonenzymatic antioxidant systems in childhood asthma

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Background: Even though there is ample evidence on the oxidative stress in asthma, there is limited information on the antioxidant defense systems.

Objectives: To conduct a comprehensive evaluation of various components of both enzymatic and nonenzymatic antioxidants in a large group of children with asthma.

Methods: A total of 164 children with mild asthma and 173 healthy children were included in the study. Levels of the enzymes glutathione peroxidase and superoxide dismutase were measured by using ELISA, whereas reduced glutathione, ascorbic acid, α-tocopherol, lycopene, β-carotene, amino acids participating in glutathione synthesis, and amino acids susceptible to oxidation were measured by HPLC. All comparisons were adjusted for atopy, body mass index, smoke exposure, and pet ownership.

Results: Levels of the enzymes glutathione peroxidase and superoxide dismutase and of the nonenzymatic components of the antioxidant system including reduced glutathione, ascorbic acid, α-tocopherol, lycopene, and β-carotene were significantly lower in children with asthma compared with healthy controls (P < .001 for each). Of the amino acids contributing to glutathione synthesis, glycine and glutamine were significantly lower in children with asthma (P < .001). The majority of the amino acid susceptible to oxidative stress displayed lower levels in children with asthma (P < .05).

Conclusion: Childhood asthma is associated with significant decreases in various components of both enzymatic and nonenzymatic antioxidant defenses. (J Allergy Clin Immunol 2008;122:78-85.)

Key words: Amino acid, antioxidant, ascorbic acid, asthma, glutathione, glutathione peroxidase, lycopene, malondialdehyde, oxidative stress, superoxide dismutase, α-tocopherol, β-carotene

The lung, with its high surface area and high exposure to atmospheric oxygen, is highly susceptible to oxidative injury and is equipped with strong antioxidant defenses to counteract the oxidant insult. An imbalance between the oxidative forces and the antioxidant defense systems favoring an oxidative injury has been implicated in many lung diseases, including asthma.1,2

Increased levels of reactive oxygen species such as superoxide (O$_2^-$), hydrogen peroxide H$_2$O$_2$, and hydroxyl radical (OH) inactivate antiproteases, induce apoptosis, and lead to increased airway reactivity and secretions, production of chemoattractant molecules, and increased vascular permeability, which collectively augment the existing inflammation that is a hallmark of asthma.3-5 The antioxidant pathways that form the major line of defense against the oxidative insult within the lung can be categorically divided into enzymatic and nonenzymatic systems.1,4 The enzymatic antioxidants include superoxide dismutases, catalase, and glutathione peroxidase, whereas the major nonenzymatic antioxidants of the lungs are glutathione, some specialized proteins such as thioredoxins, and some vitamins such as ascorbic acid, α-tocopherol, and carotenoids including lycopene and β-carotene.4,7

Among the various superoxide dismutases, the extracellular superoxide dismutase (ECSOD) is a major defense system lining the pulmonary fluids and interstitial spaces of the lung and is present in blood vessels and airways.5 ECSOD reduces O$_2^-$ to H$_2$O$_2$, which is then converted to H$_2$O by the action of glutathione peroxidase through oxidation of glutathione.9 Reduction of the oxidized form of glutathione is then accomplished by glutathione reductase through the glutathione cycle.10

Glutathione is abundant in the epithelial lining fluid of the respiratory tract, where its concentration exceeds plasma levels by 100-fold. More than 95% of glutathione is in the reduced form.11,12 Glutathione is a key molecule in the antioxidant system not only because it is a critical substrate in the enzymatic machinery, as discussed, but also because it is a major constituent of the nonenzymatic antioxidant defense. Therefore, amino acids that are the precursors in the glutathione synthesis are also critical in the defense against the oxidative stress. Glutathione is synthesized in a cycle from 3 amino acids, beginning with the combination of cysteine and glutamic acid and ending with the addition of glycine. The amino acids involved in the glutathione synthesis (cycle) can be divided into 2 groups: (1) amino acids involved in γ-glutamyl cycle: glycine and glutamic acid; and (2) sulfur-containing amino acids: cysteine and its metabolic precursors, methionine and cystathionine. Among these, cysteine, in addition to being a precursor for glutathione synthesis, has direct antioxidant properties, because the thiol group of cysteine is capable of interacting with the electrophilic groups of reactive oxygen species.13,14

Abbreviation used
ECSOD: Extracellular superoxide dismutase
We have recently shown that systemic levels of reduced glutathione are significantly decreased in children with asthma. However, the amino acid changes in the glutathione cycle that may potentially lead to the observed decrease in the reduced glutathione have not been investigated in detail. In one study involving 89 adult patients with asthma and controls, Fogarty et al.6 found that only glycine was associated with a reduced risk of asthma.

In addition to glutathione, another arm of the nonenzymatic antioxidant defense system is formed by vitamins such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), and carotenoids (lycopene and β-carotene). They have important roles, especially against lipid peroxidation.17

Even though there is ample evidence about the oxidative stress in asthma, less is known about the antioxidant defense systems, and there is little information concerning the effect of oxidative stress on amino acids. Free amino acids and amino acid residues in proteins are highly susceptible to oxidation by reactive oxygen species. Reactive oxygen species can react directly with the peptide bond or the side chain within the protein.18,19 The modification of amino acids such as histidine, tyrosine, phenylalanine, tryptophan, valine, leucine, lysine, arginine, proline, glutamic acid, and threonine result in oxidation products. The relationship of oxidation products of amino acid residues to asthma is not well known.

Therefore, we decided to conduct a comprehensive evaluation of various components of both enzymatic and nonenzymatic antioxidants that are functional in the biological systems. For this purpose, we measured glutathione peroxidase and superoxide dismutase levels as the enzymatic antioxidants with extracellular activities and glutathione, ascorbic acid, α-tocopherol, and carotenoids as the nonenzymatic antioxidants in a large group of children with asthma and healthy controls. In an effort to have a more complete analysis of the glutathione cycle, we measured the levels of amino acids involved in glutathione synthesis. Finally, to complement our measurement on glutathione-related amino acids and extend our understanding of the role of amino acids on oxidative stress, we measured plasma levels of various amino acids susceptible to oxidation such as histidine, proline, lysine, threonine, leucine, isoleucine, tyrosine, phenylalanine, tryptophan, and valine in these children.18-20

METHODS

Study population

Patients with asthma. Patients with asthma have been detailed previously. Briefly, children with mild asthma age 6 to 16 years who were not receiving any controller medication and who had not had any symptoms of respiratory infection or asthma exacerbation within the previous 4 weeks were included in this study. Spirometric measurements, total IgE, and eosinophil counts were obtained. Skin testing was performed as detailed previously.15 From this cohort of white children with mild asthma, results are presented on 164 children whose samples were available.

Healthy controls. The control group was detailed previously and was composed of 173 white Turkish school children who responded negatively to an established and validated asthma questionnaire, never had any diagnosis of asthma or allergic bronchitis, and never had a history of wheezing. All children underwent skin testing, and IgE was measured in serum. All children in this control group underwent a hypertonic saline challenge. Children without a history of respiratory symptoms or asthma diagnosis but who showed a positive response (>15% fall in FEV₁) were also included in the control group. All children in the control group had normal pulmonary function tests. All study procedures were performed in accordance with a protocol previously approved by the Ethics Committee of Hacettepe University. All parents provided written informed consent.

Sample collection and processing

Samples were collected from patients with asthma between October 2002 and September 2003 and from healthy controls between October 1999 and April 2000. Peripheral blood samples were drawn into EDTA-containing tubes between 9:00 and 12:00 AM in children with asthma and any time during the day from healthy children. Plasma was separated within 2 hours in children with asthma and within 8 hours after collection in healthy children. All plasma samples were kept at –80°C until analysis. All measurements were performed at the same time in March 2005. Thus, plasma was separated after a longer time and was stored at –80°C for a longer period in healthy children than children with asthma.

Study measurements

Malondialdehyde. Plasma malondialdehyde levels were analyzed using an HPLC-based method as described previously.25 Nonenzymatic antioxidants. Reduced glutathione. Plasma levels of reduced glutathione were determined by using a previously described methodology,26,27 in which sulfhydryl groups of the reduced glutathione react with 5,5′-dithio-bis-2-nitrobenzoic acid (Ellman reagent) and form 5-thio-2-nitrobenzoic acid. The concentration is calculated from the OD measured at 405 nm. Plasma levels of antioxidant vitamins and carotenoids. Plasma levels of vitamin C and α-tocopherol were extracted and respectively measured by absorption at 300 and 254 nm by using an HPLC kit from Immundiagnostic AG (Bensheim, Germany). β-Carotene was measured by reversed-phase HPLC.28 β-Carotene and lycopene were first extracted by using hexane and then measured by absorption at 450 nm. The mobile phase consisted of a mixture of acetonitrile, methylene chloride, and methanol (70:20:10, vol/vol/vol). A C18 column (5 μm, 150 × 4.6 mm; Phenomenex, Inc, Torrance, Calif) was used. The measurements were performed by using an HPLC system (ThermoQuest, Waltham, Mass) with UV detector (ThermoQuest).

Enzymatic antioxidants. Plasma glutathione peroxidase and superoxide dismutase levels were analyzed by using an ELISA kit (Cayman Chemical, Ann Arbor, Mich) with adaptation on a Molecular Device, Thermomax analyzer (Sunnyvale, Calif).29-31

Stability of the biomarkers. To investigate the stability of the markers over time, samples were analyzed for the same biomarkers a second time in February 2008—that is, almost 3 years after the first analysis. A power calculation showed that a sample size of 16 achieves 90% power to detect a difference of 0.5 between the means with a significance level of .05 using a 2-sided paired samples t test. In these samples, reduced glutathione, malondialdehyde, glutathione peroxidase, superoxide dismutase, ascorbic acid, α-tocopherol, lycopene, and β-carotene were measured using the same protocols, and the results were again compared between healthy children and children with asthma.

Amino acids. The EZ-Faast gas chromatographic analysis kit (Phenomenex, Inc) was used to measure the plasma levels of the amino acids involved in glutathione synthesis and of those amino acids that are highly susceptible to oxidative stress by a flame ionization detector attached to a gas chromatography system (Trace GC; ThermoQuest). The person performing the assays was blind to the group to which the patient belonged. Results of the malondialdehyde and reduced glutathione measurements were given in our previous study,15 but are briefly presented here again.

Spirometry, total IgE level, and eosinophil counts were determined as previously described.15

Statistical analyses

All data including age, eosinophil count, IgE levels, and FEV₁% showed nonnormal distribution; therefore, data are given as medians and interquartile ranges, and all statistical comparisons were performed by using the
RESULTS

The study population consisted of 164 children with mild asthma and 173 healthy children. Characteristics of the study groups are summarized in Table I. As expected, asthma and atopy–related findings including IgE, skin prick tests, FEV₁, eosinophil counts and family history of allergic diseases were significantly different between the 2 groups (P < .001).

Malondialdehyde

Malondialdehyde, a marker for the oxidant stress, was significantly higher in children with asthma compared with healthy controls (P < .001; Fig 1).

Nonenzymatic antioxidants

Reduced glutathione was significantly lower in children with asthma compared with healthy controls (P < .001; Fig 2, A).

Ascorbic acid, α-tocopherol, lycopene, and β-carotene were significantly lower in children with asthma compared with healthy controls (P < .001; Fig 2, B).

Enzymatic antioxidants

Both extracellular superoxide dismutase and glutathione peroxidase levels in plasma were significantly decreased in patients with asthma compared with healthy controls (P < .001; Fig 3, A and B).

Amino acids involved in glutathione synthesis

Amino acids involved in γ-glutamyl cycle. Glycine and glutamic acid were significantly lower in children with asthma compared with controls (P < .001 for each). It is noteworthy that the level of glutamic acid level was almost 10 times lower in children with asthma compared with healthy controls (Table II).

Sulfur amino acid precursors. Even though the plasma levels of methionine, cystathionine, and cystine were higher in the asthma group, the difference failed to reach significance between the 2 groups (Table II).

Amino acids susceptible to oxidant injury

Plasma levels of histidine, proline, lysine, threonine, leucine, isoleucine, tyrosine, phenylalanine, tryptophan, and valine are given in Table III. Most of these amino acids displayed lower levels in children with asthma. The difference failed to reach significance for leucine, isoleucine, tryptophane, and valine.

Effect of atopy on the oxidant/antioxidant biomarkers within the asthmatic population

From the panel of amino acids involved in glutathione synthesis, only cysteine was significantly higher in children with atopic asthma (238.3 μmol/L [130.3-371.4]) compared...
with those with nonatopic asthma (139.8 μmol/L [97.0-339.6];\(P = .017\)).

Among the nonenzymatic antioxidants, lycopene was higher in children with atopic asthma (0.46 μmol/L [0.44-0.48]) compared with those with nonatopic asthma (0.45 μmol/L [0.43-0.47];\(P = .027\)). Similarly, of the enzymatic antioxidants, the superoxide dismutase level was significantly higher in children with atopic asthma (984 U/mL [965-1008]) compared with those with nonatopic asthma (969 U/mL [956-999];\(P = .032\)). However, in both cases, the difference was very small.

Factors affecting the oxidant and antioxidant status

For this purpose, we performed a multiple linear regression analysis to determine the factors affecting malondialdehyde and reduced glutathione. In this model, the effect of the disease itself was dominant, and the presence of asthma accounted for 61% of the variability observed in malondialdehyde and 79% of the variability observed in reduced glutathione (\(P < .001\) for both). Addition of glutathione peroxidase into this model increased this figure to 64% for malondialdehyde variability and addition of glutathione peroxidase, lycopene, and glycine to 82% for glutathione variability.

Stability of the biomarkers

All the antioxidant markers including reduced glutathione, malondialdehyde, glutathione peroxidase, superoxide dismutase, ascorbic acid, α-tocopherol, lycopene, and β-carotene were significantly lower in the second measurement in both healthy
Glutathione synthesis is a 2-step reaction that takes place.

The aim of our study was to evaluate comprehensively the various aspects of the antioxidant systems in children with asthma. Our results show that mild asthma in children is associated with significantly decreased levels of both enzymatic and nonenzymatic antioxidant pathways. Enzymatically, levels of both extracellular superoxide dismutase and glutathione peroxidase are decreased. Nonenzymatically, levels decrease in the liver. 32-34 In the initial rate-limiting step, γ-glutamylcysteine is formed from glutamic acid and cysteine. The enzyme acting in this reaction, γ-glutamylcysteine synthetase, is regulated by feedback inhibition by glutathione. In the second step of glutathione synthesis, glutathione synthetase catalyzes the reaction between glycine and γ-glutamylcysteine to form glutathione. Consumption of glutathione as a major antioxidant may lead to an overactivity of the synthetic cycle, ultimately leading to increased consumption and decreased concentrations of the antioxidant systems in children with asthma even though it takes part in glutathione synthesis.

In human beings, transsulfuration constitutes a significant source of cysteine. 35,36 In a series of reactions, methionine is first converted to homocysteine by transmethylation, which is ultimately converted to cysteine as a result of a transsulfuration reaction by addition of serine. Methionine is an essential amino acid, and human beings rely on exogenous food for its supply. Our results show that all sulfur-containing amino acids are higher in children with asthma even though there was no decrease in the differences between the controls failed to reach significance. In vitro studies on rat hepatocytes have shown that the production of glutathione is increased as cysteine or methionine concentrations in the medium are decreased. 37 Therefore, one would expect higher cysteine levels to lead to higher glutathione levels. In contrast with this, however, our results show that even though there was no decrease in these sulfur-containing amino acids, glutathione levels were decreased in children with asthma. These observations suggest that increased levels of cysteine may be insufficient to balance the consumption of glutathione. One possible explanation for this may be the presence of a defect in the cysteine transport system. 38 or increased levels of thiol-containing compounds may be just another indicator of increased oxidative stress in asthma because antioxidation of homocysteine and cysteine results in the production of H2O2. Alternatively, the synthesis of glutathione is favored at low concentrations of cysteine, and the degradation of cysteine to taurine and sulfate is increased at high concentrations. 37 Therefore, higher levels of cysteine may divert the metabolic pathway to degradation and thus account for the lower levels of glutathione observed in our study. Increased levels of homocysteine and cysteine have been reported to be associated with cardiovascular diseases, cerebrovascular diseases, and renal ischemia and failure. 34,39 In addition to inserting asthma on the list of the diseases that are associated with increased cysteine levels, our results show that cysteine levels are further elevated in children with atopic

### TABLE II. Amino acids involved in glutathione synthesis

<table>
<thead>
<tr>
<th>Amino acid*</th>
<th>Healthy</th>
<th>Asthma</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids involved in γ-glutamyl cycle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>297 (252-368)</td>
<td>262 (213-325)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>587 (346-909)</td>
<td>59 (36-95)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sulfur containing precursors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>19 (15-28)</td>
<td>29 (23-41)</td>
<td>NS</td>
</tr>
<tr>
<td>Cystathionine</td>
<td>471 (205-1453)</td>
<td>663 (370-1063)</td>
<td>NS</td>
</tr>
<tr>
<td>Cysteine</td>
<td>80 (45-205)</td>
<td>203 (117-367)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, Not significant.

*Levels are expressed in μmol/L.

†Mann-Whitney U test.
TABLE III. Plasma levels of amino acids that are susceptible to oxidation

<table>
<thead>
<tr>
<th>Amino acid*</th>
<th>Healthy</th>
<th>Asthma</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>473.7 (160.7-1286.8)</td>
<td>391.6 (210.5-729.4)</td>
<td>.002</td>
</tr>
<tr>
<td>Proline</td>
<td>287.4 (230.5-351.3)</td>
<td>258.7 (176.0-345.4)</td>
<td>.048</td>
</tr>
<tr>
<td>Lysine</td>
<td>158.1 (95.5-243.2)</td>
<td>124.7 (85.5-185.2)</td>
<td>.037</td>
</tr>
<tr>
<td>Threonine</td>
<td>143.6 (96.4-180.4)</td>
<td>43.0 (30.2-59.2)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Leucine</td>
<td>140.4 (117.2-167.9)</td>
<td>129.4 (110.8-166.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>75.9 (62.4-90.1)</td>
<td>66.9 (54.2-88.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>118.8 (84.7-180.3)</td>
<td>89.4 (63.2-129.2)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>114.7 (91.2-159.0)</td>
<td>80.9 (62.4-114.4)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>54.1 (42.5-68.3)</td>
<td>48.0 (38.7-60.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Valine</td>
<td>259.3 (214.1-312.9)</td>
<td>252.2 (199.5-347.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, Not significant.
*Levels are expressed in μmol/L.
†Mann-Whitney U test.

FIG 4. Plasma levels of reduced glutathione, malondialdehyde (A), superoxide dismutase (B), glutathione peroxidase (C), ascorbic acid, α-tocopherol (D), lycopene, and β-carotene (E) measured in the same samples at 2 time points 3 years apart (n = 16 in each group). There was a significant difference between the first and second measurement for each biomarker (P < .001 for each). The difference between the healthy controls and children with asthma remained significant for each biomarker at the second measurement (P < .001 for each).
asthma compared with nonatopic asthma. Even though these results suggest that allergic inflammation may also affect cysteine levels, the exact mechanisms underlying these observations remain to be determined.

In addition to the amino acids taking part in the glutathione synthesis, our study provides a comprehensive analysis of the amino acids that are susceptible to oxidation.29 The majority of the protein amino acid residues that we investigated were lower in children with asthma, suggesting an oxidative modification. Accumulation of oxidized proteins was associated with aging and age-related diseases such as Alzheimer disease, amyotrophic lateral sclerosis, and diabetes.40 There is very little information on the relationship between the amino acids and asthma. Our results suggest that asthma also favors consumption of amino acids, which are susceptible to oxidative modification. In one of the few studies, Fogarty et al16 reported that patients with asthma had higher fasting plasma cystine levels than controls and found a strong inverse relationship between fasting plasma glycine levels and asthma risk. Our study confirms the observations related to higher cysteine levels and lower glycine levels in asthma and suggests that these may be associated with oxidative stress. However, unlike our study, the study by Fogarty et al16 did not show any difference in the glutamic acid and methionine levels between the 2 groups. The observed differences between the 2 studies could be a result of the differences in the age and ethnicity of the study groups. Alternatively, the differences could be a result of the fact that all the patients in the study by Fogarty et al16 were on inhaled steroids, whereas our patients all had mild asthma and were on no controller medication. Another responsible factor could be the dietary differences and the time of sampling (fasting vs random), although this is unlikely.

In addition to this detailed analysis of the amino acids and glutathione metabolism, we show that the levels of the antioxidant vitamins are significantly decreased in children with asthma. Ascorbic acid, α-tocopherol, and carotenoids are potent dietary antioxidants. Our study confirms the previous observations that all are lower in asthma41-48 and contribute to the decreased capacity of the antioxidant defenses.

To complement our analysis of the nonenzymatic antioxidant system, we determined the levels of the enzymes that act at the extracellular level to decrease the oxidative burden, namely ECSOD and glutathione peroxidase. ECSOD is the major superoxide dismutase of pulmonary fluids and interstitial spaces of the lungs. Glutathione peroxidases are active both intracellularly and extracellularly. Glutathione peroxidase, in conjunction with ECSOD, constitutes a major first-line defense against the inhaled oxidants.4 The significantly lower levels of the extracellular antioxidant enzymes that we observed in children with asthma can be both the cause and the result of the existing oxidative burden. Because these are not mutually exclusive, both mechanisms may be operative. Whatever the underlying mechanism may be, because transgenic mice overexpressing ECSOD have been found to be resistant to the effects of hyperoxia, there is reason to believe that there is a strong association between asthma and decreased levels of antioxidant enzymes.49

Our study has some weaknesses. First of all, we have no recorded information on the diet of the children, which might have influenced the results. In addition, the blood samples were drawn not after fasting but randomly when the patients presented to the clinic. Even though it is possible that these factors may have influenced the results, we think that this is unlikely for a couple of reasons. First, our analysis failed to show any difference in the body mass index between the asthmatic and healthy populations, and all the results were adjusted for body mass index. Second, and more importantly, the levels of methionine, which is an essential amino acid, were actually higher in the asthmatic population, although they would be expected to be lower under circumstances in which nutrition is impaired. Third, the levels of cysteine, which, similar to glutamic acid and glycine, is involved in the γ-glutamyl cycle, are increased in subjects with asthma, suggesting that factors are operative other than diet that specifically affect the levels of these amino acids.

Another weakness of our study concerns the study population. The populations from which that cases and controls were selected are different, and comparisons may be prone to selection bias even though they were from the same city. In one of our previous studies,15 we have shown that the results from this control population did not differ significantly from controls who belonged to the same population as cases. In addition, we adjusted for every possible confounding factor in our analysis. However, a selection bias cannot be fully excluded.

Another factor that might have influenced our results was the time that elapsed between collection and analysis of the samples. In this sense, samples from healthy controls were kept at room temperature longer (as long as 8 hours) compared with children with asthma (as long as 2 hours). In addition, samples from healthy children were stored at –80°C for more than 5 years, whereas samples from children with asthma were stored for less than 3 years. Because our analyses apparently showed that time increases oxidants and decreases antioxidants in stored samples, the time effect would have been expected to have decreased the gap between the patients with asthma and controls in our populations. Thus, there is reason to believe that the difference could have in fact been greater if the control samples had not been stored longer than asthma samples. In addition, we were able to show that 3 years after the analysis, all differences continued to exist at the same significance level in 16 randomly selected patients for each group. Therefore, even though our results are not likely to be influenced by the storage of samples, these observations definitely indicate that one should extremely be careful about handling and storage of the samples while investigating the oxidant/antioxidant systems.

Trials of various treatment approaches to fight against the oxidant attack have produced highly inconsistent results,3 and current guidelines do not include any anti-oxidant recommendations in the treatment of asthma.50 In addition to the extremely complex nature of the oxidant insult,1 our study show that the antioxidant machinery stress is multifaceted, involving a variety of biochemical mechanisms. Many of these pathways are significantly impaired in asthma, and the presence of the disease itself is the most dominant factor determining the extent of the oxidant/antioxidant balance. Therefore, a single antioxidant measure is unlikely to have enough strength to counteract the existing oxidant/antioxidant imbalance in asthma. This observation should be taken into account in all attempts to fight the oxidant injury in asthma.

Clinical implications: Antioxidant treatment approaches targeting a single component of the oxidant/antioxidant system are unlikely to be successful in asthma.
REFERENCES


