Differences in Associations Between Markers of Antioxidative Defense and Asthma Are Sex Specific

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ABSTRACT

Background: Lungs are exposed to high levels of oxygen, air pollutants, and smoke, all of which stimulate the production of reactive oxygen species (ROS). In addition, inflammatory cells produce ROS, and thus there may be increased demand for antioxidants, including antioxidant enzymes, in inflammatory lung diseases such as asthma. Sex-specific differences have been noted for asthma, which in postpubertal subjects is predominantly found in females. These sex-specific differences may be associated with differences on the molecular level as well.

Objective: The aim of this cross-sectional study was to examine associations between markers of antioxidative defense and asthma, and to investigate whether these associations were different between women and men.

Methods: Based on the European Community Respiratory Health Survey protocol, subjects were enrolled in a study of asthma risk factors. The multicenter study was conducted in 5 west Danish counties between 2003 and 2006, and the subjects were recruited as a case-enriched random sample of 10,000 Danish inhabitants aged 20 to 44 years selected by their civil registration number. Participants were identified by positive answers to asthma questions on a screening questionnaire, random sampling, or both. Serum selenium concentrations and antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase [GPX], glutathione reductase [GR], and glucose-6-phosphate dehydrogenase [G6PD]) in erythrocytes were measured. Asthma was defined as either current asthma symptoms with bronchial hyperresponsiveness (BHR) or a continuous asthma score based on 8 questions.

Results: A total of 1191 mostly white women and men (mean [SD] age, 34.0 [7.1] and 35.1 [7.1] years, respectively) were enrolled in the study. Current asthma symptoms were present in 29.9% (200/670) of women and 22.5% (117/521) of men, with women reporting more positive answers (51.1% vs 40.9%, respectively; P < 0.01) to asthma questions. Serum selenium concentrations were measured in 1151 subjects (640 women, 511 men), and antioxidant enzyme activities were measured in 295 subjects (161 women, 134 men). Women had higher enzyme activities of most antioxidant enzymes (GPX, P = 0.006; GR,
Although the serum selenium concentration was inversely associated with asthma in both sexes, there was a female preponderance, with 3.5% lower serum selenium in subjects with current asthma symptoms with BHR (n = 77) compared with controls (n = 287). GR activity was associated with asthma in men, with 5.7% higher enzyme activity in subjects with current asthma symptoms with BHR (n = 14) compared with controls (n = 77). However, a significant interaction with gender was observed for analyses of GR (P = 0.02), but not for analyses of selenium.

Conclusions: In this study of asthma risk factors, women had higher levels of enzyme activities than did men in a randomly selected Danish population, and sex-specific differences were found in the associations between markers of antioxidative defense and asthma. (Gend Med. 2010;7:115–124) © 2010 Excerpta Medica Inc.

Key words: selenium, superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, asthma.

INTRODUCTION
Oxidative stress plays an important role in the injurious inflammatory responses in asthma as well as other inflammatory lung diseases. Oxidative stress is elucidated by both low activity of antioxidants and end points of oxidative degradation of lipids. When oxidant species are increased, antioxidant enzymes respond by an upregulation of their activity to compensate for the excess of reactive oxygen species (ROS). Because lungs are exposed to high levels of oxygen, inhaled air pollutants, and smoke from active and passive smoking, they manage a vast amount of ROS. The antioxidative defense capacity may be exceeded, with subsequent inflammation of lung tissue. In addition, inflammatory processes induce ROS production, which may further disturb the redox balance and thereby induce oxidative stress. An important effect of oxidative stress in lungs is the upregulation of protective antioxidant genes, thus entailing increased activity. However, many antioxidant enzyme activities are found to be decreased in inflammatory lung diseases. Reducing the negative effects of ROS on tissue requires cascades of enzymes, including those shown in Figure 1. One of these enzymes, glutathione peroxidase (GPX), requires selenium in its active site. The antioxidative effects of selenium are partly determined by its incorporation as selenocysteine in GPX and other selenoproteins with redox properties, and these other enzymes may contribute to the antioxidant properties of selenium independently of GPX.

In healthy young adults, markers of oxidative stress have been found to be lower in women than in men, indicating a possible sex-specific difference in the regulation of the redox homeostasis. Postpubertal females have a higher prevalence of asthma than do males, and when exposed to low dust concentrations, females are more susceptible to developing respiratory symptoms. We hypothesized that a possible association between serum selenium concentration and activity of antioxidant enzymes in blood and asthma in subjects aged 20 to 44 years would be different in women and men.

Conclusions: In this study of asthma risk factors, women had higher levels of enzyme activities than did men in a randomly selected Danish population, and sex-specific differences were found in the associations between markers of antioxidative defense and asthma. (Gend Med. 2010;7:115–124) © 2010 Excerpta Medica Inc.

Key words: selenium, superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, asthma.

**Figure 1.** Schematic view of selected functions of antioxidant enzymes, involving inactivation of oxidants and toxins. SOD = superoxide dismutase; $\text{O}_2^-\text{=} =$ superoxide; $\text{H}_2\text{O}_2 =$ hydrogen peroxide; GST = glutathione S-transferase; $\text{R =}$ different toxins; GPX = glutathione peroxidase; GSR = toxins conjugated with glutathione; GSH = reduced glutathione; GSSG = oxidized glutathione; GR = glutathione reductase; NADP$^+$ = oxidized form of nicotinamide adenine dinucleotide phosphate; NADPH = reduced NADP; G6PD = glucose-6-phosphate dehydrogenase.
SUBJECTS AND METHODS

Study Population

The study population consisted of subjects recruited as a case-enriched random sample (Figure 2) and has been described elsewhere. In brief, the study was based on the European Community Respiratory Health Survey (ECRHS) protocol. Ten thousand Danish, mostly white women and men, aged 20 to 44 years, received a screening questionnaire in 2002–2003, and 7271 responded. Based on the sampling procedure, in which participants were identified by either positive answers to asthma questions on a screening questionnaire, random sampling, or both (Figure 2), 2303 subjects were invited to participate in the clinical investigation during 2003–2006. The study was approved by the Danish National Committee for Biomedical Research Ethics.

Clinical Investigations

Of the 2303 invited, 1191 subjects agreed to participate in the study and gave written informed consent. The clinical investigation (consisting of an interviewer-administered questionnaire based on the ECRHS protocol, and skin-prick, blood sample, lung function, and bronchial challenge testing) was conducted at 5 clinics in 5 Danish counties: Fune, South Jutland, Vejle, Ribe, and North Jutland.

We used 2 definitions of asthma to determine the presence of asthma or a marker for the severity of asthma: (1) current asthma with bronchial hyperresponsiveness (BHR)—defined as an answer of “yes” to ≥1 of 3 questions (Appendix I) about current asthma with BHR (>20% decline in forced expiratory volume in the first second of expiration when challenged with a cumulative dose of up to 2.46 mg of methacholine); and (2) asthma score—a continuous score ranging from 0 to 8, defined as the sum of positive answers to 8 main symptom questions (Appendix II), as described by Pekkanen et al. This approach has been used because symptoms of asthma can be considered as a continuum in the general population. A higher score (greater number of “yes” answers) reflects a higher probability of having asthma.

Atopy was defined as having a positive skin-prick test (mean wheal size, ≥3 mm) to any of 13 common allergens.

Blood Samples

Venous blood samples were collected, centrifuged, separated, and stored at −80°C until analyzed. The
Association of Official Analytical Chemists modified fluorometric method, validated for investigations of selenium in organic material,\(^\text{13}\) was used to analyze serum selenium concentration (μg/L). Enzyme activities in erythrocytes were analyzed in subjects stratified for \textit{GPX1} genotypes (Pro198Leu, rs1050450) to associate genotypes to activity,\(^\text{9}\) but otherwise the subjects were randomly selected. Principles of the analyses of enzyme activities and selenium have previously been described. In brief, enzyme activities were determined in erythrocyte hemolysates by using a Cobas Mira autoanalyzer (F. Hoffmann-La Roche, Diagnostic Systems, Basel, Switzerland), as described by Andersen et al.\(^\text{14}\) The unit of activity (U) for GPX, glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PD) was defined as μmol of nicotinamide adenine dinucleotide phosphate oxidized per minute, and the results were expressed as U/g protein. The U for superoxide dismutase (SOD) was defined as inhibited formation of μmol of formazan dye per minute, and the results were expressed as U/g of protein.

Statistical Analysis

In this study population, GPX activity, G6PD activity, and serum selenium concentration were distributed normally (histogram and quantile-quantile plots), while SOD and GR activity both showed normal distribution after logarithmic transformation. In a random sample of subjects, an assessment of differences between the sexes in these markers of antioxidative defense was performed using a \textit{t} test and multiple linear regression adjusted for age, body mass index (BMI), study center, and smoking habits (current or former [1 month prior to examination] or never smokers).

Various methods were used to examine associations between markers of antioxidative defense and asthma models. The dichotomized variable “current asthma with BHR” was assessed separately in women and men by simple logistic regression for each of the variables, serum selenium and enzyme activities. Subjects with current asthma symptoms without BHR were excluded from this analysis. The association of serum selenium and enzyme activities with current asthma and BHR was analyzed in both sexes separately in multiple logistic regression models adjusted for age, BMI, atopy, study center, and smoking habits. The asthma score was analyzed by negative binomial regression, reporting relative change in asthma score for serum selenium and enzyme activities, again both unadjusted and adjusted for the same variables as was current asthma with BHR.

To assess true differences in risk between the sexes, we used interaction terms in the analysis of associations between selenium and GR and asthma outcomes.

Statistical analyses were performed with Stata version 9.2 (StataCorp LP, College Station, Texas). For all analyses, \(P < 0.05\) was considered the level of significance.

RESULTS

Subjects

A total of 1191 women and men (mean [SD] age, 34.0 [7.1] and 35.1 [7.1] years, respectively) were enrolled in the study. Only subjects meeting all relevant criteria were included in the analyses. To assess sex-specific differences in serum selenium concentration, a random sample of 709 subjects was asked to provide blood samples; in the adjusted analyses, 6 had incomplete information. In the unadjusted analyses of serum selenium and asthma scores, 1140 subjects were eligible for analysis and 11 subjects were excluded, owing to incomplete information for the asthma score. In the adjusted analyses, incomplete information for other variables resulted in the exclusion of another 43 subjects. In the analyses of selenium and current asthma with BHR, 146 subjects did not perform the methacholine challenge, and another 286 subjects did not fulfill the inclusion criteria as a case or a control. Therefore, 719 subjects were eligible for analysis, 7 of whom had incomplete information in the adjusted analyses.

For analyses concerning enzyme activities, a random sample of 179 subjects was selected; in the adjusted analyses, 1 had incomplete information on smoking habits. In the unadjusted analyses of enzyme activities and asthma score, 291 of 295 subjects were eligible; 4 subjects had incomplete information on the asthma score. Another
5 subjects were excluded from the adjusted analyses, owing to incomplete information for the other variables. In the analyses of enzyme activity and current asthma with BHR, 105 subjects were excluded either because they did not perform the methacholine challenge or they did not fulfill the inclusion criteria as a case or a control. Four of the 190 subjects had incomplete information in adjusted analyses.

**Symptoms and Bronchial Hyperresponsiveness**

In this cohort of relatively young Danes, more women than men reported asthma symptoms, and BHR was more prevalent among women (Table I). We found marked sex-specific differences in symptoms: although women reported more symptoms than did men, the prevalence of atopy was higher among men.

**Serum Selenium Concentration**

No significant sex-specific differences were seen in serum selenium concentration; in the 709 subjects randomly recruited, mean (SD) concentration was 82.6 (12.1) μg/L in women and 83.8 (11.7) μg/L in men. Women had an inverse association between current asthma with BHR and serum selenium (odds ratio = 0.74; 95% CI, 0.56–0.97) (Table II). Serum selenium was 3.5% lower for the 77 symptomatic subjects compared with the controls (P < 0.05). In men, no association was observed between current asthma with BHR and serum selenium.

Using the asthma score, a weak inverse association was found with serum selenium in both sexes. In an analysis adjusted for age, BMI, atopy, study center, and smoking habits, the relative changes in asthma score were 0.85 (95% CI, 0.74–0.98) and 0.92 (95% CI, 0.84–1.00) per 10 μg/L of change in serum selenium concentration in men and women, respectively (Table II).

**Enzyme Activities**

In randomly recruited subjects, marked sex-specific differences in enzyme activities were seen. Enzyme activities were highest in women (GPX, P = 0.006; GR, P < 0.001; and G6PD, P = 0.009), although SOD results were nonsignificant (Table III). We found an association between GR activity and current asthma with BHR: the logarithm of activity in the 14 symptomatic men was 5.7% higher than in the male controls (P = 0.01) (Table IV). After adjusting for age, BMI, atopy, study center, and smoking habits, the association did not reach significance. A significant interaction was observed between sex and GR (P = 0.02). There were no associations between SOD, GPX, G6PD, and current asthma with BHR in either men or women.

Analysis of the associations between asthma score and enzyme activities indicated a significant association with GR activity in men only: P = 0.004, unadjusted; and P = 0.02, adjusted for age, BMI, atopy, study center, and smoking habits (Table IV). However, the interaction term GR × gender regarding the asthma score was nonsignificant. Asthma score was not associated with any of the other enzyme activities.

**DISCUSSION**

The present study investigated whether the serum concentration of selenium and the activities of key antioxidant enzymes in erythrocytes differed between women and men, and whether they were altered in individuals with asthma. Enzyme activities were higher in women compared with men for GR, GPX, and G6PD, but not for SOD. Other studies with a broader age distribution have not consistently shown higher enzyme activities in females.14–16

**Selenium and Asthma**

We found serum selenium concentrations to be lower in subjects with current asthma with BHR in the combined analysis but with a nonsignificant interaction term, indicating no true difference in risk between the sexes, although the association seemed strongest in women. Our findings are, however, contradictory to a recent European case–control study of 1145 subjects in which no association between asthma and selenium was reported.17 In that study, recruitment was based on questionnaires, with cases defined as having both self-reported asthma and, in addition, either wheezing, shortness of breath, or waking at night with breathlessness in the previous 12 months. Similar to the findings of the present study, Omland et al18 found, in a case–control study of 154 young
**Table I.** Demographic and clinical characteristics of the study population consisting of a case-enriched random sample of Danish women and men (N = 1191). Values are presented as n/N (%), unless otherwise specified.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age, y</td>
<td>34.0 (7.1) (n = 670)</td>
<td>35.1 (7.1) (n = 521)</td>
</tr>
<tr>
<td>Mean (SD) body mass index, kg/m²</td>
<td>25.6 (5.7) (n = 666)</td>
<td>25.7 (3.6) (n = 516)</td>
</tr>
<tr>
<td>Atopy*</td>
<td>227/641 (35.4)</td>
<td>226/516 (43.8)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>356/668 (53.3)</td>
<td>290/521 (55.7)</td>
</tr>
<tr>
<td>Current</td>
<td>197/668 (29.5)</td>
<td>151/521 (29.0)</td>
</tr>
<tr>
<td>Former</td>
<td>115/668 (17.2)</td>
<td>80/521 (15.4)</td>
</tr>
<tr>
<td>Current asthma symptoms*</td>
<td>200/670 (29.9)</td>
<td>117/521 (22.5)</td>
</tr>
<tr>
<td>Bronchial hyperresponsiveness*</td>
<td>164/569 (28.8)</td>
<td>88/476 (18.5)</td>
</tr>
<tr>
<td>Asthma score*†‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/8</td>
<td>325/665 (48.9)</td>
<td>305/516 (59.1)</td>
</tr>
<tr>
<td>1/8</td>
<td>88/665 (13.2)</td>
<td>64/516 (12.4)</td>
</tr>
<tr>
<td>2/8</td>
<td>63/665 (9.5)</td>
<td>31/516 (6.0)</td>
</tr>
<tr>
<td>3/8</td>
<td>51/665 (7.7)</td>
<td>33/516 (6.4)</td>
</tr>
<tr>
<td>4/8</td>
<td>43/665 (6.5)</td>
<td>26/516 (5.0)</td>
</tr>
<tr>
<td>5/8</td>
<td>34/665 (5.1)</td>
<td>24/516 (4.7)</td>
</tr>
<tr>
<td>6/8</td>
<td>26/665 (3.9)</td>
<td>16/516 (3.1)</td>
</tr>
<tr>
<td>7/8</td>
<td>20/665 (3.0)</td>
<td>9/516 (1.7)</td>
</tr>
<tr>
<td>8/8</td>
<td>15/665 (2.3)</td>
<td>8/516 (1.6)</td>
</tr>
</tbody>
</table>

*P < 0.01, women versus men.

†A higher score (greater number of positive answers on 8 asthma questions [Appendix II]) reflects a higher probability of having asthma.

‡Percentages may not equal 100 due to rounding.

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**Table II.** Association between asthma and serum selenium concentration in a case-enriched random sample of Danish women and men.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Analysis</th>
<th>Current Asthma With BHR*</th>
<th>Asthma Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n/N</td>
<td>Odds Ratio (95% CI)</td>
</tr>
<tr>
<td>Women</td>
<td>Unadjusted</td>
<td>77/364 (0.79 (0.62–1.00)</td>
<td>634</td>
</tr>
<tr>
<td></td>
<td>Adjusted‡</td>
<td>76/362 (0.74 (0.56–0.97)</td>
<td>597</td>
</tr>
<tr>
<td>Men</td>
<td>Unadjusted</td>
<td>54/355 (0.96 (0.75–1.24)</td>
<td>506</td>
</tr>
<tr>
<td></td>
<td>Adjusted‡</td>
<td>53/350 (0.96 (0.71–1.31)</td>
<td>500</td>
</tr>
</tbody>
</table>

BHR = bronchial hyperresponsiveness.

*Current asthma with BHR was defined as a positive answer to ≥1 of 3 questions (Appendix I) about asthma with concurrent BHR. Analyzed in a logistic regression model; odds ratios are given per 10 μg/L of change in serum selenium concentration.

†Asthma score was the number of positive answers to 8 questions pertaining to asthma (Appendix II). Analyzed by negative binomial regression; relative change in asthma score is given per 10 μg/L of change in serum selenium concentration.

‡Adjusted for age, body mass index, atopy, study center, and smoking habits.
men recruited from farming schools and conscripts to the army, that serum selenium was significantly inversely associated with mild asthma ($P = 0.04$). These findings may partly be due to differences in smoking habits between individuals with asthma and nonasthmatics, because several studies have observed that serum selenium is inversely associated with smoking.$^{18-20}$ In a Cochrane review on selenium supplementation for asthma, Allam and Lucane$^{21}$ reported that, although there was limited evidence for the benefits of dietary supplements, the accumulated data indicated that asthma was associated with reduced circulatory selenium status. Further studies are needed in other settings and with other designs, especially clinical trials, before any clinical implications might be extrapolated.

**Enzyme Activities and Asthma**

We found an inverse association between GR activity and asthma, but in males only. Concordant with the present study, male rats have been found to have increased GR when exposed to mild chronic stress ($P = 0.007$), whereas no significant difference has been observed in female rats.$^2$ These findings of an association between GR and asthma may be contradictory to those of a recent case–control study (65 children with asthma vs 6 non-

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### Table III. Erythrocyte antioxidant enzyme activities in Danish women and men recruited as a random sample. Values are presented as mean (SD) U/g protein.

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Women</th>
<th>Men</th>
<th>$p^*$</th>
<th>$p^†$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD log</td>
<td>6.31 (0.15)</td>
<td>6.29 (0.17)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>GPX</td>
<td>56.1 (8.07)</td>
<td>52.4 (8.21)</td>
<td>0.003</td>
<td>0.006</td>
</tr>
<tr>
<td>GR log</td>
<td>1.67 (0.14)</td>
<td>1.58 (0.12)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G6PD</td>
<td>3.4 (0.76)</td>
<td>3.2 (0.77)</td>
<td>0.040</td>
<td>0.009</td>
</tr>
</tbody>
</table>

SOD = superoxide dismutase; GPX = glutathione peroxidase; GR = glutathione reductase; G6PD = glucose-6-phosphate dehydrogenase.

$^*$Determined by t test ($n = 179$; 90 women, 89 men).

$^†$Determined by linear regression adjusted for age, body mass index, study center, and smoking habits ($n = 178$; 90 women, 89 men).

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### Table IV. Association between asthma and glutathione reductase activity in a case-enriched random sample of Danish women and men.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Analysis</th>
<th>Current Asthma With BHR*</th>
<th>Asthma Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Odds Ratio (95% CI)</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>n/N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>Unadjusted</td>
<td>23/99</td>
<td>0.92 (0.65–1.31)</td>
</tr>
<tr>
<td></td>
<td>Adjusted$^‡$</td>
<td>23/98</td>
<td>0.96 (0.64–1.43)</td>
</tr>
<tr>
<td>Men</td>
<td>Unadjusted</td>
<td>14/91</td>
<td>1.97 (1.14–3.40)</td>
</tr>
<tr>
<td></td>
<td>Adjusted$^‡$</td>
<td>14/91</td>
<td>2.03 (0.97–4.24)</td>
</tr>
<tr>
<td>Combined analysis, adjusted$^†$</td>
<td></td>
<td>37/189</td>
<td>0.92 (0.61–1.38)</td>
</tr>
<tr>
<td></td>
<td>Gender (female = 0)</td>
<td></td>
<td>0.00 (0.00–0.28)</td>
</tr>
<tr>
<td></td>
<td>Interaction (gender × GR)</td>
<td></td>
<td>2.2 (1.03–4.80)</td>
</tr>
</tbody>
</table>

BHR = bronchial hyperresponsiveness; GR = glutathione reductase.

$^*$Current asthma with BHR was defined as a positive answer to ≥1 of 3 questions (Appendix I) about current asthma. Analyzed with a logistic regression model; odds ratios are given as the change in GR activity of 0.1 log (U/g protein).

$^†$Asthma score was the number of positive answers to 8 questions pertaining to asthma (Appendix II). Analyzed by negative binomial regression; relative change in asthma score is given as the change in GR activity of 0.1 log (U/g protein).

$^‡$Adjusted for age, body mass index, atopy, study center, and smoking habits.
asthmatic children and 35 healthy adults) in which no association was found between GR activity and asthma; however, the results were not analyzed separately for each sex. On the other hand, two different asthma outcomes were employed in our study, and we found consistent GR associations with asthma, both in diagnosis and severity, which strengthens our confidence in our findings. Published data have been conflicting regarding the effect of SOD or GPX activity on asthma. To our knowledge, no English-language studies have been published describing an association between G6PD activity and asthma.

The present study is one of the largest to analyze associations between enzyme activities and asthma. However, the subjects included were not experiencing acute asthma attacks, and thus the associations described herein can only be applied to stable asthma. In addition, the findings are limited by our sample size, especially because we reduced the number of subjects eligible for analysis by differentiating between the sexes. We therefore cannot exclude misinterpretation due to multiple comparisons.

Asthma is a diagnosis with fluctuations in symptoms, and no gold standard exists for defining asthma in epidemiologic studies. A differential bias might have been introduced by wrongly diagnosing a case as asthma instead of chronic obstructive pulmonary disease (COPD), with this error being more likely in smokers than in non-smokers. However, the present cohort was relatively young and primarily had short smoking exposure, indicating that the risk of COPD among these smokers should be low. The observed sex-specific differences in antioxidative defense and the association with asthma might be influenced by sex hormones and oral contraceptive use, by dietary factors other than BMI, and by other unmeasured confounders.

CONCLUSIONS
The present study highlights the need to consider sex-specific differences when analyzing antioxidant enzymes and their association with asthma. In this Danish population, we found that women had higher levels of enzyme activities than did men, and that asthma was associated with GR activity in men only, whereas serum selenium was inversely associated with asthma in both sexes. Future studies assessing an association of risk in a longitudinal design may elucidate whether the observations are causal.

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Appendix I. Questions defining current asthma.

1. “Have you been woken by an attack of shortness of breath at any time in the last 12 months?”
2. “Have you had an attack of asthma in the last 12 months?”
3. “Are you currently taking any medicine (including inhalers, aerosols, or tablets) for asthma?”

Appendix II. Questions used to generate a continuous asthma score.

1. “Have you had wheezing or whistling in your chest at any time in the last 12 months? If yes: Have you been at all breathless when the wheezing noise was present?”
2. “Have you woken up with a feeling of chest tightness at any time in the last 12 months?”
3. “Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months?”
4. “Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months?”
5. “Have you been woken by an attack of shortness of breath at any time in the last 12 months?”
6. “Have you ever had asthma?”
7. “If yes: Have you had an attack of asthma in the last 12 months?”
8. “If yes: Are you currently taking any medicines including inhalers, aerosols, or tablets for asthma?”