New Risk Factors for Adult-Onset Incident Asthma

A Nested Case–Control Study of Host Antioxidant Defense

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Abstract

Rationale: Host antioxidant defense, consisting of enzymatic antioxidant activity and nonenzymatic antioxidant micronutrients, is implicated in asthma pathogenesis. Studies of antioxidant defense and adult incident asthma have either used measures of antioxidants estimated from questionnaires or not considered enzymatic aspects of host defense.

Objectives: We conducted the first study designed and powered to investigate the association of antioxidant defenses on adult incident asthma.

Methods: In a nested case–control study, we followed Shanghai women (aged 40–70 years) without prevalent asthma at baseline, over 8 years. Subjects with incident asthma were ascertained prospectively by gold standard testing of symptomatic women and matched to two asymptomatic control subjects.

Measurements and Main Results: Baseline urinary F2-isoprostanes, plasma concentrations of antioxidant micronutrients

(tocopherols, xanthines, carotenes, and lycopene), and antioxidant enzyme activity (platelet-activating factor acetylhydrolase [PAF-AH] and superoxide dismutase) were measured from samples collected before disease onset. Among 65,372 women, 150 (0.24%) developed asthma. F₂-isoprostane levels before asthma onset were not different between cases and control subjects. Doubling of α -tocopherol concentrations and PAF-AH activity was associated with 50 and 37% decreased risk of incident asthma (α -tocopherol: adjusted odds ratio = 0.52; 95% confidence interval, 0.32–0.84; PAF-AH: adjusted odds ratio = 0.63; 95% confidence interval, 0.42–0.93).

Conclusions: In this prospective study, α -tocopherol, within normal reference ranges, and PAF-AH enzymatic activity were associated with decreased asthma development. These modifiable risk factors may be an effective strategy to test for primary asthma prevention.

Keywords: asthma; incidence study; antioxidants; platelet-activating factor acetylhydrolase; α -tocopherol

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At a Glance Commentary

Scientific Knowledge on the

Subject: The antioxidant host defense system is composed of endogenous enzymatic antioxidant activity and exogenous nonenzymatic antioxidants. This is the first study to examine whether quantified plasma biomarkers of both enzymatic and nonenzymatic antioxidant host defense precede adult-onset asthma.

What This Study Adds to the

Field: We demonstrate that before asthma onset, both increased endogenous antioxidant enzymatic activity of platelet-activating factor acetylhydrolase and increased nonenzymatic antioxidant plasma α -tocopherol concentrations are associated with decreased asthma risk. The importance of our findings is that lower measures of antioxidant host defense are risk factors for asthma, preceding both oxidative stress and asthma onset. Targeting enzymatic and nonenzymatic antioxidant defense may be a future strategy for primary asthma prevention.

Adult-onset asthma is a complex disease of unknown etiology with substantial evidence that oxidative stress plays an important role in the immune and inflammatory responses central to asthma (1, 2). Oxidative stress is mediated by the antioxidant host defense system, which can be divided into two components that exert protective effects: (1) nonenzymatic dietary antioxidants, including tocopherols, carotenes, xanthines, and lycopene, that can be measured as micronutrients in plasma; and (2) endogenous antioxidant enzymes also measured in plasma, such as superoxide dismutase (SOD) and platelet-activating factor acetylhydrolase (PAF-AH), among others. Dietary antioxidants, as a part of the nonenzymatic host defense system, have been well studied in prevalent asthma (3). However, enzymes also play an important role in antioxidant defense: SOD facilitates the breakdown of the superoxide anion into oxygen and hydrogen peroxide; PAF-AH, also known as lipoprotein phospholipase-A2, prevents the accumulation of PAF and PAF-like

oxidized phospholipids, potent mediators of inflammation. Both enzymatic and nonenzymatic antioxidants are implicated in the pathogenesis of asthma through mediation of oxidative stress and airway inflammation (4). Moreover, associations of nonenzymatic antioxidants with prevalent disease support the need for additional studies of incident asthma (5–21). Enzymatic and nonenzymatic antioxidant host defense pathways have the potential to be modified, likely contribute to host defense to causal environmental asthma risk factors, and, thus, provide the rationale to investigate their role in asthma inception.

Despite the importance of oxidative stress in asthma, studies of dietary antioxidants have shown modest effects on asthma development and control (19, 22). We hypothesized that both the endogenous enzymatic and exogenous nonenzymatic host defense to environmental exposures that induce oxidative stress are dually important in asthma development. Thus, this is the first study designed and powered with the primary objective to investigate the association between measures of both enzymatic and nonenzymatic antioxidant host defense and oxidative stress, measured before disease onset, on incident asthma. Identifying common and modifiable risk factors for asthma inception provides a potential new strategy for primary and possibly secondary asthma prevention by dually targeting the enzymatic and nonenzymatic components of the antioxidant host defense system. Some of the results of these studies have been previously reported in the form of an abstract (23).

Methods

Study Population: The Shanghai Women's Asthma and Allergy Study

The study population included 65,372 women with no previous diagnosis of asthma, followed over 8 years (24), as shown in Figure 1. The baseline population from whom this group was drawn is the 74,942 participants of the Shanghai Women's Health Study. Detailed methods of the Shanghai Women's Asthma and Allergy Study are available in a previous publication (25). Baseline information and biospecimens were collected during in-person interviews from March 1997 to May

2000. Demographic characteristics, medical history, anthropometrics, usual dietary habits, physical activity, and other lifestyle factors were assessed. The major outcome of interest, incident asthma, was ascertained through a two-step process, first by assessment of self-reported asthma symptoms collected on two questionnaires, each administered in person by trained staff approximately each 2 years and up to 8 years after baseline on the entire cohort. Second, those reporting asthma symptoms were contacted by telephone by a pulmonologist to confirm reported symptoms. Individuals with new-onset symptoms underwent confirmatory testing in a hospital setting by methacholine challenge testing, or β_2 -agonist reversibility for those with FEV₁ of 70% or less.

A nested study was conducted including all available confirmed asthma cases ($n = 150$) matched to two control subjects ($n = 294$), who reported no new-onset symptoms of asthma (Figure 1). Matching variables were age, date of baseline biospecimen collection, body mass index (BMI), and self-reported smoking status. All study participants provided in-person informed consent with approval from the institutional review boards of Vanderbilt University, Zhongshan Hospital of Fudan University, and Shanghai Cancer Institute.

Measures of Oxidative Stress and Host Antioxidant Defense

All samples were collected at baseline, before asthma onset. Details about specimen collection, laboratory analytes, and quality control are presented in the online supplement and are described briefly here. Urinary dinor dihydro metabolites of F2-isoprostanes were measured using liquid chromatography/tandem mass spectrometry (26). Plasma concentrations of antioxidants (β -cryptoxanthin, zeaxanthin, α - and β -carotene, lycopene, and α - and γ -tocopherol) were measured by high-performance liquid chromatography (27, 28). Plasma antioxidant activity of PAF-AH was detected by a spectrophotometer-based enzymatic assay and expressed in nmol/ml/min (29). Plasma SOD activity (U/ml) was measured using a Cayman Chemicals kit (Ann Arbor, MI). Sample collection procedures limited which enzyme activity was able to be assayed or reported.

Figure 1. Flow diagram of participants through enrollment, screening, and asthma identification phases of the Shanghai Women's Asthma and Allergy Study (SWAAS), 2003 to 2009, Shanghai, China.

Statistical Analysis

Demographic and other baseline characteristics were described between

subjects with incident asthma and matched control subjects using mean and SD or median and interquartile range for

continuous variables, as appropriate, and frequencies and proportions for categorical variables. Due to skewness, biomarker

variables were analyzed as continuous natural log transformed or log base 2 for ease of interpretation, as well as by quartiles. Conditional logistic regression for matched pairs with available biomarker data was used to assess the association between the level of urinary F₂-isoprostane metabolite, antioxidant concentrations, antioxidant enzyme activity, and asthma risk after adjusting for the following covariates: workplace smoking exposure, exercise, fat intake, and vitamin supplement (defined in the online supplement). Covariates were selected *a priori* for their known relationship with asthma or impact on host antioxidant defense. Results from multivariable regression models are presented with adjusted odds ratios (ORs) for asthma and 95% confidence intervals (95% CIs). Additional interaction and sensitivity analyses (section E4) and power calculations (section E5) are described in the online supplement. Statistical analyses were performed using R version 3.1.0 (30).

Results

Baseline Population Characteristics

Among 65,372 women followed for a mean of 7.5 years, 585 met criteria for probable asthma, among whom 449 underwent methacholine challenge testing or test of reversibility; and 136 refused testing or could not be recontacted for scheduling. One hundred fifty (0.24%) had a positive methacholine challenge test or test of reversibility for an asthma incidence rate of 0.028 per 100 person-years. Characteristics of the 6,481 women who did not respond to the first initial survey and the 136 women who were not available for pulmonary function testing are provided in section E6 of the online supplement. Baseline demographic factors for subjects with incident asthma, control subjects, and the entire cohort are provided in Table 1. Subjects with incident asthma and matched control subjects were similar on husband smoking and any exercise in the past 5 years, occupational category, and educational achievement. Subjects with incident asthma and control subjects differed on workplace smoking (55 vs. 39%, respectively) and report of any supplemental vitamin use (27 vs. 18%).

The median concentrations and interquartile ranges of urinary measures

of oxidative stress, antioxidant nutrients, and antioxidant enzyme activity are presented in Table 2 in subjects with incident asthma and control subjects from a matched analysis, unadjusted for additional covariates. Boxplots of all measurements are presented in the online supplement (Figure E7). The median plasma micronutrient values are qualitatively comparable to a U.S. population (31) of women aged 40 to 59 years for combined case-control samples for β -cryptoxanthin (8.5 vs. 7.3 $\mu\text{g/dl}$), α -carotene (2.1 vs. 2.6 $\mu\text{g/dl}$), and γ -tocopherol (0.25 vs. 0.20 mg/dl), but substantially lower for lycopene (6.7 vs. 41.1 $\mu\text{g/dl}$); other micronutrient values were not available for comparison. Four percent of the Shanghai women met the deficiency threshold for α -tocopherol at 0.5 mg/dl (31), and 1.1% were above the upper reference range of normal values at 2.0 mg/dl, with the remaining 95% falling within established ranges (32). α -Tocopherol concentrations observed in this study were comparable to another Chinese population (33, 34) and slightly lower than a U.S. adult population (35).

Baseline Measures of Oxidative Stress and Asthma

A urinary F₂-isoprostane metabolite corrected for urine creatinine concentrations at baseline, before asthma development, was measured. There was no difference in the urinary F₂-isoprostane metabolite between those who developed incident asthma and control subjects (median baseline concentrations, 36.3 vs. 37.2 ng/mg creatinine; $P = 0.96$) in matched analysis (Figure E7).

Baseline Antioxidant Concentrations and Asthma

Figure 2 shows the risk of asthma by plasma concentrations of antioxidants based on a unit increase on the log scale. Using conditional logistic models that adjusted for dietary fat intake, exercise, workplace smoking exposure, and vitamin use, we observed no associations for risk of asthma with increasing concentrations of β -cryptoxanthin (twofold increase; OR = 1.05; 95% CI, 0.86–1.29), β -carotene (OR = 0.85; 95% CI, 0.64–1.13), α -carotene (OR = 1.13; 95% CI, 0.97–1.31), lycopene (OR = 0.96; 95%

Table 1. Baseline Characteristics of Incident Cases, Control Subjects, and the Total Cohort at Enrollment in the Shanghai Women's Asthma and Allergy Study, 1997 to 2000

	Cohort (N = 65,372)	Subjects with Incident Asthma (N = 150)	Control Subjects (N = 294)
Mean age (SD)*	51.9 (9.0)	52.5 (8.7)	52.3 (8.6)
Mean BMI (SD)*	24.0 (3.4)	24.7 (3.7)	24.5 (3.4)
Smoking (ever)*	3	5	3
Education			
College educated	13	16	10
High school	28	25	27
Middle school	38	39	39
<Middle school	21	19	24
Any exercise in past 5 yr	35	33	34
Family history of asthma	10	27	12 [†]
Husband smoking	62	61	63
Workplace smoking	38	55	39 [‡]
Occupation			
Professional	28	26	26
Clerical	21	25	19
Manual laborer	51	49	55
Mean total fat intake (SD), g	29 (13)	30 (11)	29 (12)
Mean total kcal intake (SD)	1,677 (404)	1,715 (358)	1,678 (387)
Any supplemental vitamin use	20	27	18 [§]

Definition of abbreviation: BMI = body mass index.

Data are presented as % unless otherwise noted.

*Indicates matching variable.

[†] $P < 0.001$; comparing cases to control subjects.

[‡] $P < 0.01$; comparing cases to control subjects.

[§] $P < 0.05$; comparing cases to control subjects.

Table 2. Baseline Median (Interquartile Range) Concentrations of Measures of Oxidative Stress, Antioxidant Enzyme Activity, and Plasma Antioxidants in the Shanghai Women's Asthma and Allergy Study, 1997 to 2000

Baseline Measure	Subjects with Incident Asthma (N = 150)	Control Subjects (N = 294)	P Value*
Oxidative stress [†]			
Urinary F2- isoprostane, ng/mg creatinine	36.3 (25.3–51.7)	37.2 (26.2–54.4)	0.96
Antioxidant concentrations (plasma) [‡]			
β-Cryptoxanthin, μg/dl	9.1 (5.4–16.6)	8.3 (5.0–15.0)	0.35
Zeaxanthin, μg/dl	39.3 (30.9–54.7)	42.3 (34.1–54.2)	0.43
β-Carotene, μg/dl	22.3 (14.4–29.9)	21.4 (15.3–31.6)	0.28
α-Carotene, μg/dl	2.1 (1.6–3.0)	2.1 (1.5–3.1)	0.08
Lycopene, μg/dl	6.8 (3.9–12.6)	6.7 (4.0–12.4)	0.79
γ-Tocopherol, mg/dl	0.25 (0.17–0.33)	0.24 (0.18–0.33)	0.97
α-Tocopherol, mg/dl	0.78 (0.66–0.95)	0.82 (0.69–1.01)	0.06
Antioxidant enzyme activity (plasma) [§]			
SOD, U/ml	7.9 (6.8–10.3)	8.4 (7.1–10.3)	0.12
PAF-AH, nmol/ml/min	66.2 (52.7–83.1)	70.9 (57.3–84.0)	0.02

Definition of abbreviations: PAF-AH = platelet-activating factor acetylhydrolase; SOD = superoxide dismutase.

*P value, unadjusted conditional logistic regression.

[†]Urine for isoprostane measurements was available on 416 individuals.

[‡]Plasma for antioxidant concentrations was available on 345 individuals.

[§]Plasma for enzymatic activity was available on 386 individuals.

CI, 0.84–1.11), γ-tocopherol (OR = 1.00; 95% CI, 0.73–1.37), and zeaxanthin (OR = 1.03; 95% CI, 0.69–1.54). Zeaxanthin demonstrated a curvilinear association with asthma risk, which is depicted graphically in the online supplement (Figure E8). We found a protective relationship between higher α-tocopherol concentrations at baseline with asthma (for a twofold α-tocopherol increase, OR = 0.52; 95% CI, 0.32–0.84; *P* = 0.007).

In a quartile analysis, the association between the top quartile and bottom quartile (reference category) of α-tocopherol was protective (OR = 0.37; 95% CI, 0.19–0.70; *P* = 0.002) and consistent with the above finding. Table 3 converts log-transformed α-tocopherol concentrations back to the untransformed scale (36) and shows that for each baseline value observed at each 5th, 10th, 25th, 50th, 75th, and 90th percentile, an increase in 0.1 mg/dl over the baseline value is associated with an approximately 5% decrease in risk of asthma.

Baseline Antioxidant Enzyme Activity and Asthma

SOD activity was not significantly associated with asthma development (Figure 2), where the OR is based on a twofold biomarker concentration increase (adjusted OR = 0.69; 95% CI, 0.41–1.2; *P* = 0.18). Increased

PAF-AH activity was inversely associated with risk of asthma development: for a twofold increase in concentration, adjusted OR = 0.63; 95% CI, 0.42–0.93; *P* = 0.02. In a quartile analysis, the top quartile compared with the bottom quartile of PAF-AH was associated with a decreased risk of asthma (OR = 0.60; 95% CI, 0.35–1.01; *P* = 0.054). Table 3 shows that for each 10 nmol/ml/min increase in PAF-AH activity over each baseline percentile value, there is a 6 to 16% decrease in asthma risk, depending on the baseline level.

Effect Modification

Effect modification was assessed for both enzymatic and nonenzymatic antioxidants by workplace smoking exposure and family history of asthma. The only significant interaction was between SOD activity and workplace smoking exposure on the risk of incident asthma (*P* = 0.05). Among those exposed to workplace smoking, higher SOD activity was associated with a decreased risk of asthma (OR = 0.62; 95% CI, 0.39–0.98) compared with those who were not exposed to workplace smoking (OR = 1.10; 95% CI, 0.75–1.61), based on a unit increase in the log scale. There were no significant interactions (*P* > 0.05) between markers of host antioxidant defense and asthma by family history of asthma.

To explore the joint effects of PAF-AH activity and α-tocopherol, we performed interaction analyses, which are graphically depicted in the online supplement (Figure E9). Higher PAF-AH activity appears to decrease asthma risk, particularly when α-tocopherol levels are low or moderate, and this protective association attenuates with increasing and protective α-tocopherol concentrations (*P* value for interaction = 0.09).

Discussion

This is the first study specifically designed and powered to measure the protective association of both nonenzymatic antioxidants and enzymatic antioxidant activity in asthma inception. The population was chosen because of the low prevalence of known contributors to asthma development: largely never-smokers and normal BMI. All participants experienced similar exposure to air pollution in a single Chinese city. Despite a strong body of literature that prevalent asthma is associated with oxidative stress (37–41), in this large incidence study, oxidative stress as measured by F2-isoprostane did not predate asthma development. This is an important finding, first suggesting that the chronic inflammation that is the hallmark of asthma may not be present before clinical disease recognition or that the host is able to resolve oxidative stress before disease onset. Second, both nonenzymatic and enzymatic indices of antioxidant defense, specifically higher antioxidant α-tocopherol concentrations and PAF-AH enzyme activity, were associated with decreased asthma risk. This supports our hypothesis that both lower baseline concentrations of antioxidants, as well as antioxidant enzyme activity, predate asthma inception and are likely important in asthma pathogenesis.

Higher concentrations of α-tocopherol were associated with decreased risk of asthma among subjects with incident asthma compared with matched control subjects, with a twofold increase in α-tocopherol concentrations associated with an approximately 48% decrease in asthma risk. These measured concentrations of α-tocopherol fall well within normal reference ranges, suggesting that the decreasing risk of asthma could be achieved through dietary changes and not require high-dose supplements.

Figure 2. Adjusted odds ratios (ORs) for association between asthma and antioxidant concentrations and antioxidant enzyme activity in the Shanghai Women's Asthma and Allergy Study (2003–2009), matched case–control study. Conditional multivariable logistic regression was used including covariates: any exercise in past 5 years, workplace smoking exposure (yes/no), and vitamin supplement use (yes/no). *Zeaxanthin demonstrates a nonlinear association that is presented in the online supplement (Figure E8). ORs are based on a twofold biomarker increase. PAF-AH = platelet-activating factor acetylhydrolase; SOD = superoxide dismutase.

Prior studies of vitamin E, however, have not all demonstrated a protective association with asthma. Two longitudinal dietary intake studies have demonstrated inconsistent results with incident asthma: the Nurses' Health Study, in which decreased dietary intake of vitamin E was similarly associated with higher risk of asthma development (42), and the E3N study, in which no association was found between measures of dietary pattern and asthma phenotypes (43). However, these two studies did not distinguish between vitamin E isoforms and used self-reported dietary intake instead of plasma concentrations. The differentiation of vitamin E isoforms is an important distinction, as they were associated with different effects in this study and, second, vitamin E dietary intake does not necessarily reflect plasma concentrations of the constituent tocopherols. A possible mechanism to explain the differential influence of vitamin E isoforms in asthma inception has been demonstrated in animal models, where the α -tocopherol isoform decreases lung inflammation, whereas γ -tocopherol promotes allergic airway

inflammation (44, 45). In a children's study of serum vitamin E and longitudinal asthma, a suggestive relationship was observed between higher serum α -tocopherol concentrations at 1 year of age with decreased risk of wheezing symptoms by age 6 years that was not observed with other self-reported asthma and atopy outcomes (46). Because of the vast differences in age and the use of self-reported outcomes, it is difficult to compare this study with ours. In prevalent asthma, supplementation with α -tocopherol has also been shown to decrease measures of oxidative stress and decrease inflammation (47). The lack of association observed with other micronutrients on incident disease in this study may reflect an association of these other micronutrients only with prevalent asthma or may reflect limitations in how to interpret the protective effect of these micronutrients from these prior studies' cross-sectional study designs (5, 18, 39, 48, 49).

This is the first incidence study to assess the role of antioxidant enzyme activity in asthma inception and report of decreased PAF-AH activity predating asthma onset.

PAF-AH is an enzyme that breaks down PAF and PAF-like phospholipids, which are potent mediators of inflammation. Because animal and human studies provide evidence that the administration of PAF can induce bronchoconstriction, bronchial hyperreactivity, mucus secretion, or higher vascular permeability (50–56), PAF degradation would be hypothesized to have a beneficial effect. For prevalent asthma, PAF receptor antagonists have been studied and, for the most part, have not been efficacious (15, 57–67). These data do not preclude that PAF-AH activity is not important in asthma inception, as the limited success of receptor antagonists may be related to their study in prevalent disease, PAF receptor heterogeneity (57), or the fact that PAF-AH reacts with multiple substrates besides PAF, and thus PAF antagonists may target another inflammatory mediator. In support of a potential mechanism of PAF-AH activity in incident disease, the administration of rupatadine, a PAF antagonist, demonstrated reversal of the airway remodeling process and a decrease in inflammation in a murine model of asthma (68).

We also explored the association between SOD activity and incident asthma and found no significant differences between subjects with incident asthma and control subjects, although SOD activity trended toward a protective association. Other studies have demonstrated decreased SOD activity in prevalent asthma not only localized in airways and bronchial lavage fluid but also systemically (10, 69). The lack of significant association of this antioxidant enzyme may be due to inadequate power, or it may suggest that increased burden of oxidative stress coupled with decreased ability to manage such burden, or inactivation, occurs in prevalent disease but may not be important in disease development.

Despite the strengths of our population-based study designed specifically to address the role of antioxidant defense in asthma inception, several limitations deserve mention. This study focuses on a large population of women living in urban Shanghai and may have limited generalizability to other populations on the magnitude of observed effect sizes due to different exposure profiles, including the high levels of pollution in Shanghai. Nevertheless the underlying biologic mechanisms of their effect on asthma should be similar, although the effect size could

Table 3. Odds Ratios (95% Confidence Intervals) for Asthma Based on an Increase in Antioxidant Host Defense Measurements from Baseline Percentiles on the Untransformed Scale for Each 0.1 mg/dl Increase in α -Tocopherol and 10 nmol/ml/min in Platelet-Activating Factor Acetylhydrolase

	Baseline Percentile					
	5th	10th	25th	50th	75th	90th
Baseline value α -tocopherol at different percentiles,* mg/dl	0.53	0.57	0.67	0.80	0.99	1.21
OR for asthma based on a 0.1-mg/dl increase (95% CI) from each baseline percentile value	0.94 (0.90–0.98)	0.94 (0.90–0.98)	0.95 (0.91–0.99)	0.95 (0.92–0.99)	0.95 (0.92–0.99)	0.96 (0.93–0.99)
Baseline value PAF-AH at different percentiles, nmol/ml/min	33	41	56	70	84	101
OR for asthma based on 10-nmol/ml/min increase (95% CI) from each baseline percentile value	0.84 (0.73–0.93)	0.87 (0.77–0.98)	0.90 (0.82–0.98)	0.91 (0.85–0.99)	0.93 (0.87–0.99)	0.94 (0.89–0.99)

Definition of abbreviations: CI = confidence interval; OR = odds ratio; PAF-AH = platelet-activating factor acetylhydrolase.

*Normal range for α -tocopherol: 0.5–1.6 mg/dl.

vary. In this study, the baseline samples were only collected at one time point in the 3 years between 1997 and 2000, precluding trajectory analysis of repeated measurements of biomarkers. Asthma cases were identified through two follow-up surveys with confirmation through gold standard testing. We used stringent research criteria for a diagnosis of asthma, and thus many of the probable subjects with asthma may meet clinical criteria for a doctor diagnosis of asthma in a population setting; however, this should result in conservative bias and underestimation of incident asthma. Self-reported symptoms of asthma are also general and nonspecific, which may also explain why a small proportion of women reporting symptoms met the criteria for an asthma diagnosis. Because asthma is an episodic disease, it was not possible to determine the date of onset of disease with precision. We additionally did not exclude asthma in the control subjects by pulmonary testing, only the absence of any asthma symptoms. This study relies on women to self-report previous history of asthma, and it is possible that our incident cases or control subjects may have had asthma diagnoses as children. We expect that the inability to recall diagnoses would be nondifferential with respect to case-control status or more likely to impact control subjects, who do not have asthma, and not bias our findings away from the null.

Next, we could not determine from our data whether effects of antioxidants are from food sources as well as supplements. However, when we restricted analyses to subjects without vitamin supplement use, our results remained unchanged. Although the women in this study are from seven districts of Shanghai with very similar exposures, there may have been additional unmeasured environmental confounders, such as living in close proximity to a busy road or other high-pollution sources that may have impacted the results. Although we cannot rule out potential selection bias due to differential follow-up on subjects' exposure characteristics (*see* section E6), we believe that we have used a proper control with our a nested case-control design that matched on age, BMI, smoking, and specimen collection date in addition to our multivariable analyses that controlled for confounding covariates. Last, our dichotomous measurement of exercise may not capture the full spectrum of physical activity as a covariate.

Although our analyses are not adjusted for multiple comparisons, these measurements were specified *a priori* based on known associations with prevalent asthma, and we present both statistically significant and nonsignificant results. Of the nine hypothesized host defense biomarkers studied, two reached statistical significance at a conventional $P < 0.05$; however, the magnitude of the effect sizes

of biomarkers PAF-AH and α -tocopherol are in the expected direction and have the biological relevance to suggest that these results are plausible and of scientific importance for additional follow-up.

This is the first study to demonstrate the potential protective effect of nonenzymatic antioxidants and enzymatic antioxidant activity in adult asthma inception. Our results demonstrate that lower PAF-AH activity and α -tocopherol concentrations are risk factors for incident asthma, representing possible targets for primary asthma prevention. To date, available data support only a modest effect of dietary modification using stoichiometric antioxidants such as vitamin E on prevalent asthma. However, the competing effects of the vitamin E isoforms, α -tocopherol and γ -tocopherol, may in part contribute to conflicting results in prior studies (70). Furthermore, given that those who develop asthma likely have lower concentrations of α -tocopherol before disease development, supplementation requirements may be substantially greater for persons at risk for asthma. Our findings that lower antioxidant concentrations and enzyme activity precede oxidative stress and asthma onset support the idea that decreased host antioxidant defense may be important in asthma inception. Alternative approaches to primary and secondary disease prevention for asthma might be dually targeting increasing host

nutrient antioxidants and antioxidant enzyme activity. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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