Allostatic Load Biomarkers and Asthma in Adolescents

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***Rationale*: Allostatic load (AL), a novel measure of the physiologically dysregulated response of the body to stress, represents a biomarker of chronic stress exposure.**

***Objectives*: To determine whether preadolescent children with high AL are more susceptible to asthma as adolescents.**

***Methods*: This was a prospective evaluation of children recruited at 7 to 10 years of age in the nested case-control arm of the Study of Asthma, Genes and Environment and followed until 11 to 14 years of age. AL was measured using eight biomarkers: fasting glucose, total cholesterol, high-density lipoprotein cholesterol, dehydroepiandrosterone sulfate, cortisol, systolic and diastolic blood pressure, and waist-to-hip ratio. AL, created from the sum of biomarkers in a high-risk quartile, was related to prevalence and incidence of asthma using logistic regression.**

***Measurements and Main Results*: Among 352 participants followed until 11 to 14 years of age, prevalent asthma was four times more likely in boys with high (>3) versus low (≤2) AL after adjusting for current asthma/atopy, age, ethnicity, parental history of asthma, and overweight status. Similar results were observed in the analysis of new-onset asthma in boys (adjusted odds ratio, 4.35; 95% confidence interval, 1.19–15.9). In girls, there were no associations between AL and asthma. In the analysis of a subset of biomarkers, combinations of total cholesterol, glucose, and cortisol were associated with similar or greater risk of asthma prevalence or onset in boys.**

***Conclusions*: AL and its biomarkers are associated with an increased likelihood of asthma in adolescent boys. The observed association between AL and asthma may be attributable to a combined subset of AL biomarkers.**

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Clinicians use the Asthma Predictive Index in early life to predict onset of asthma in school-age children. However, there are limited data on which indices are useful for adolescent-onset asthma.

What This Study Adds to the Field

Recognizing the multisystem effects of allostatic load, this research presents evidence for candidate stress and metabolic biomarkers for investigation into their clinical utility as an index for adolescent-onset asthma.

Keywords: psychological stress; allostasis; bronchial asthma; pediatrics; biological markers

New-onset asthma affects up to 10% of youth in the United States(1) and in adolescents imposes an extensive burden of morbidity and disease management onto a period of significant life changes. It is also a condition that persists into adulthood. Risk factors for early childhood asthma have been examined extensively; however, it remains challenging to predict which children will develop asthma as adolescents (2). Strong links have been reported between early life exposure to maternal distress, psychosocial stress, or depressive and anxiety symptoms in children and the development and prognosis of asthma (3–6). Few studies have demonstrated a connection between stressor exposure and asthma in adolescents, and the underlying biological links between these issues remain to be clarified (7).

Allostatic load (AL) is a concept developed to elucidate associations between chronic stress and adverse health outcomes. Allostasis refers to the adaptation of the body, through activation of the sympathetic nervous system, hypothalamus-pituitary-adrenal (HPA) axis, and metabolic system, to maintain physiological balance under stress (8). AL reflects a state of exhaustion after long-term stress exposure, whereby the normal allostasis response becomes dysfunctional in the following sequential manner. First, primary mediators like stress hormones (i.e., epinephrine, norepinephrine, and cortisol) and their antagonist, dehydroepiandrosterone sulfate (DHEA-S), become dysregulated. Second, metabolic (i.e., glucose and lipid metabolism) and cardiovascular (i.e., blood pressure and heart rate) systems adjust themselves in an attempt to compensate for primary mediators. Finally, resultant abnormal organ activities progress from subclinical stages to clinical disease (8). Various studies have measured the primary mediators and

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secondary outcomes of allostasis using a composite AL index to distinguish individuals at relatively high risk for clinical outcomes, including cardiovascular disease, diabetes, mood and cognitive disorders, and all-cause mortality (8–10). Studies have also examined subsets or individual components of the AL index in relation to adverse health outcomes. For example, subgroup analyses in one of the "MacArthur studies of successful aging" demonstrated that epinephrine, waist-to-hip ratio, and cortisol have greater roles in predicting the physical decline in adults, whereas diastolic blood pressure, epinephrine, and glycosylated hemoglobin are strongly associated with cognitive decline (11). Limited research has explored the impact of AL and its biomarkers on diseases in the pediatric years.

The biology that links stress and asthma is complex and requires consideration of multiple interactive pathways of influence. Previous studies have only examined these pathways individually, raising a need for an integrative method for cumulative biological risk assessment (4, 12). The AL index is a composite measure that enables the consideration of compound pathways simultaneously. This biologic measure also addresses the challenges in quantifying stress in epidemiological studies (4), ranging from measures of social circumstances to reports of stressful life events. Such studies have overlooked dramatic interpersonal differences in stress perception, which is influenced by many factors, such as personality and coping mechanisms, and by the effects of multiple environmental stressors (12, 13). The utility of the AL index is that it provides a biological construct to account for interpersonal variations in responses to different stressors (13). AL has been proposed as an explanation for the link between stress and asthma pathogenesis and/or morbidity (14) but has yet to be examined. The objective of the present study was to determine whether preadolescent children with higher levels of AL are more susceptible to asthma as adolescents. The secondary objective was to examine the association of the individual AL biomarkers with asthma and asthma phenotypes. Some of the preliminary results of this study have been previously reported in the form of an abstract (15).

METHODS

This study was a prospective follow-up of the 1995 Study of Asthma, Genes and Environment (SAGE) birth cohort (16), approved by the University of Manitoba Research Ethics Board (H2002:078). From a mail-out survey to SAGE households, children with or without parent-reported asthma were randomly recruited from rural and urban areas into a nested case-control study at 7 to 10 years of age (mean, 9 yr) and were revisited at 9 to 11 years of age (mean, 10.5 yr) and 11 to 14 years of age (mean, 12.5 yr). At 9 and 12.5 years of age, asthma and atopy were confirmed by a pediatric allergist and a positive skin prick test to any of 14 common indoor and outdoor allergens, respectively (16, 17). At 10.5 years of age, an AL index was derived from these eight markers: early morning fasting blood sample determinations of serum glucose, total cholesterol (TC), high-density lipoprotein (HDL), DHEA-S (an HPA axis antagonist), and cortisol, as well as measurements of resting systolic and diastolic blood pressure and the waist-to-hip ratio, an indicator of central adiposity (8, 13, 18–23). Waist circumference was measured half way between the iliac crest and lowest rib, and hip circumference was measured at the maximum circumference over the buttocks.

Similar to other studies (8, 13, 18–23), a value of 1 was added to the AL score for each biomarker value that fell into the highest quartile for glucose, TC, systolic blood pressure, diastolic blood pressure, and waist-to-hip ratio, and the lowest quartile for HDL and DHEA-S. Because low cortisol levels represent a dysregulated HPA axis response to stress (24) and are valid indicators of AL in prepubertal children (25), a value of 1 was added for cortisol levels below or above the 12.5th percentile (13, 19). Sex-specific cut-off points were applied for each biomarker (Table 1). A total AL score of greater than 3 (highest quartile) was defined as high AL in both sexes.

Several confounding factors (age, ethnicity [white vs. other], history of food allergy, atopy or asthma, postsecondary education of parent, and exposure to secondhand smoke) were derived from parent questionnaires (26–30). Using validated Tanner stage schematics (31, 32), puberty stage was self-reported at 10.5 years of age and dichotomized as prepubertal (Tanner < 2) or pubertal (Tanner ≥ 2). Overweight status was defined as a BMI z score greater than 1.04 at 10.5 years of age (33).

Purposeful stepwise logistic regression (34) was conducted using SPSS 18.0 to determine the association between high AL at 10.5 years of age and prevalent and incident asthma at 12.5 years of age. Parent educational level, pubertal stage, and secondhand smoke exposure did not meet our $P < 0.10$ criterion for initial variable selection and were not retained in final models. Separate models with overweight status as a covariate were run to assess AL independent from overweight. Analyses were stratified by sex subsequent to an interaction between AL and sex. To determine the association of AL with new-onset adolescent asthma, prevalence analyses were adjusted for asthma at 9 years of age, and incident analyses were conducted after excluding children with atopic asthma at 9 years of age because childhood atopic asthma is more likely to persist until adolescence (35).

A subset analysis was performed to examine the association of each individual AL biomarker with asthma and asthma phenotypes. Because studies have shown a decrease in glucose levels parallel to increases in insulin secretion and insulin resistance in girls who enter puberty, we also examined the association of high fasting insulin level with asthma in girls (36, 37). To identify the combination of biomarkers that potentially determined the associations between AL and asthma, we tested different combinations of biomarkers in relation to asthma and asthma phenotypes. The candidate set of biomarkers for these analyses was based on the level of significance for the individual associations ($P < 0.10$). The Hosmer and Lemeshow goodness-of-fit test was applied to all models.

RESULTS

A total of 439 children with an AL measurement at 10.5 years of age were included; from this sample, 160 (36.4%) had asthma, and 248 (56.5%) lived in urban areas. After 2 years, 352 children were revisited at 12.5 years of age (80.2%). The sex-specific distribution of the AL index is illustrated in Figure

TABLE 1. CUT-OFF POINTS FOR ALLOSTATIC LOAD COMPONENTS

	Cut-Off Points	
	Boys	Girls
Systolic blood pressure, mm Hg	⩾116	⩾116
Diastolic blood pressure, mmHg	⩾68	⩾68
Waist-to-hip ratio	⩾0.89	⩾0.87
Fasting serum glucose, mmol/L	⩾5.20 (⩾93.6 mg/dl)	⩾5.10 (⩾91.8 mg/dl)
Serum total cholesterol, mmol/L	⩾4.54 (⩾175.6 mg/dl)	⩾4.50 (⩾174.0 mg/dl)
Serum HDL, mmol/L	⩽1.37 (⩽53.0 mg/dl)	⩽1.33 (⩽51.4 mg/dl)
Serum DHEA-S, nmol/L	⩽1.32 (⩽3.6 ng/ml)	⩽1.20 (⩽3.3 ng/ml)
Serum cortisol, nmol/L	⩽62.75 (⩽1,731.3 μg/dl) or ⩾257.31 (⩾7,099.2 μg/dl)	⩽65.92 (⩽1,818.7μg/dl) or ⩾193.11 (⩾5,327.9 μg/dl)
Total AL	>3	>3

Definition of abbreviations: AL = allostatic load; DHEA-S = dehydroepiandrosterone sulfate; HDL = high-density lipoprotein.

1. No statistically significant differences were found between participants who did or did not attend the follow-up visit with respect to any of the study covariates, except for age and ethnicity. Children who were not present at the follow-up visit were on average 3.3 months older than their study peers ($P < 0.001$). Attrition at follow-up was lower for whites than for nonwhites (17.2 vs. 28.0%, respectively; $P < 0.01$). Although nonwhite families (primarily First-Nations) were recruited independently to maximize participation in this study (16), they accounted for the majority of the loss to follow-up.

All 352 children who were present at both visits were included in the prevalence analysis. A total of 115 (32.7%) participants had prevalent asthma; of these, 70 (21.6%) and 33 (10.2%) had atopic and nonatopic asthma, respectively. Twelve participants with asthma did not consent to skin prick testing. Regarding physical development, 67.9% of boys and 41.2% of girls were categorized as prepubertal (Tanner stage < 2). In general, total asthma and both asthma phenotypes tended to be more prevalent in boys with high AL scores and girls with low AL scores, but differences were not statistically significant before adjustment for covariates (Figure 2). Table 2 shows the distribution of AL index and prevalent asthma among different covariates. High AL was less prevalent in white and normal-weight children ($P < 0.05$). Asthma, on the other hand, was more prevalent among children who were younger or atopic, who had a positive history of food allergy, or who had parental history of asthma. Similar associations were observed between AL scores and ethnicity or overweight among children in the incidence analyses. Incident asthma was more prevalent among children with a history of food allergy or parental history of asthma ($P < 0.05$; data not shown).

Prevalence Study: Asthma

In boys, asthma at 12.5 years of age was more prevalent among those with high AL when they were 10.5 years of age, although this association was not statistically significant without adjustment (Table 3). Existing asthma or atopy and parental history of asthma at 9 years of age were also statistically significant predictors of asthma at 12.5 years of age. After adjusting for the covariates in model 1, there was a 33% increased likelihood of asthma for each single unit increase in the AL score (Table 3; continuous AL score). Also, boys who had high AL scores were 2.7 times more likely to have asthma in adolescence compared with those in the low-AL group. These associations were statistically significant and independent of age, ethnicity, parental history of asthma, and existing asthma or atopy at 9 years of age. Existing asthma and atopy, as well as parental history of asthma, remained significant predictors for asthma at 12.5 years of age (data not shown). After additional adjustment for overweight status (model 2), we observed a 63% increased chance of having asthma for each unit AL score increase (Table 3; continuous AL score). Additionally, the association between high AL and asthma increased to an odds ratio of 4. These associations were independent of all covariates, including overweight status. In girls, high AL was not associated with asthma at 12.5 years of age, and this finding was consistent at baseline and after the addition of potential covariates in regression models.

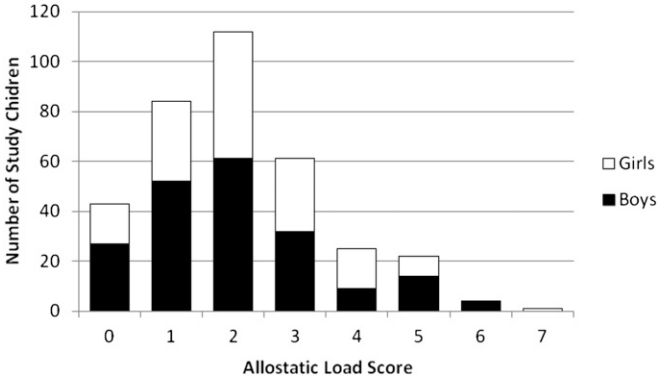

Figure 1. Sex-specific distribution of the allostatic load score among study children.

Prevalence Study: Asthma Phenotypes

Boys who had high AL scores were 4.6 times more likely to have atopic asthma at 12.5 years of age after controlling for covariates in model 2 (Table 3). Nonatopic asthma was significantly more prevalent among boys with high AL in model 1, independent of asthma and atopy status at 9 years of age. Nevertheless, after overweight status was added in model 2, this association became less significant (Table 3). Atopic asthma at 9 years of age, history of food allergy, and parental asthma were statistically associated with atopic asthma at 12.5 years of age, whereas nonatopic asthma at 9 years of age was the only predictor for nonatopic asthma in adolescent years (data not shown). In contrast, high AL scores among girls did not show a significant association with atopic or nonatopic asthma in either of the models (Table 3).

Incidence Study: Asthma

Excluding children with atopic asthma at 9 years of age, the incidence analysis included 258 children with 49 new cases of asthma (Table 3). In boys, high AL score increased the likelihood of asthma development in adolescence more than 4-fold after adjusting for the covariates in model 2. History of food allergy was another significant predictor of asthma development in model 2 (data not shown).

In girls, a high AL score was not predictive of asthma in both models (Table 3); however, as in boys, history of food allergy was associated with asthma development (data not shown). We were unable to perform the incidence analysis for each asthma phenotype due to small sample sizes.

AL Components and Asthma

After adjustment for model 2 covariates (Table 4), abnormal cortisol response, as a single biomarker, was more likely to be present in boys with prevalent nonatopic asthma. High TC was significantly associated with new-onset asthma in boys. In girls, on the other hand, high serum glucose had a statistically significant inverse association with prevalent nonatopic asthma (Table 5). Table 6 shows the final results of the subset analyses for combined AL biomarkers. In summary, boys who possessed high levels of at least two biomarkers (elevated TC, elevated glucose, and abnormal cortisol levels) were 3.0 and 3.3 times more likely to have prevalent and new-onset asthma as adolescents, respectively. In the analysis of asthma phenotypes, having elevated levels of TC and glucose increased the likelihood of having atopic asthma by approximately five times in boys. As with the AL index, elevations in TC (clinical cut-off $\geq$ 5.2 mmol/ml) (38) and glucose (clinical cut-off $\geq$ 5.6–6.9 mmol/l) (39, 40) were at subclinical levels. Nonatopic asthma, on the other hand, was 8.2 times more prevalent among boys who had elevated levels of glucose and cortisol. In girls, no biomarker combination predicted asthma in adolescence.

DISCUSSION

In this follow-up study of 352 children, we for the first time report a link between AL and asthma susceptibility in adolescence. Boys who had high AL at 10.5 years of age were four times more likely to have prevalent or incident asthma at 12.5 years of age. Each

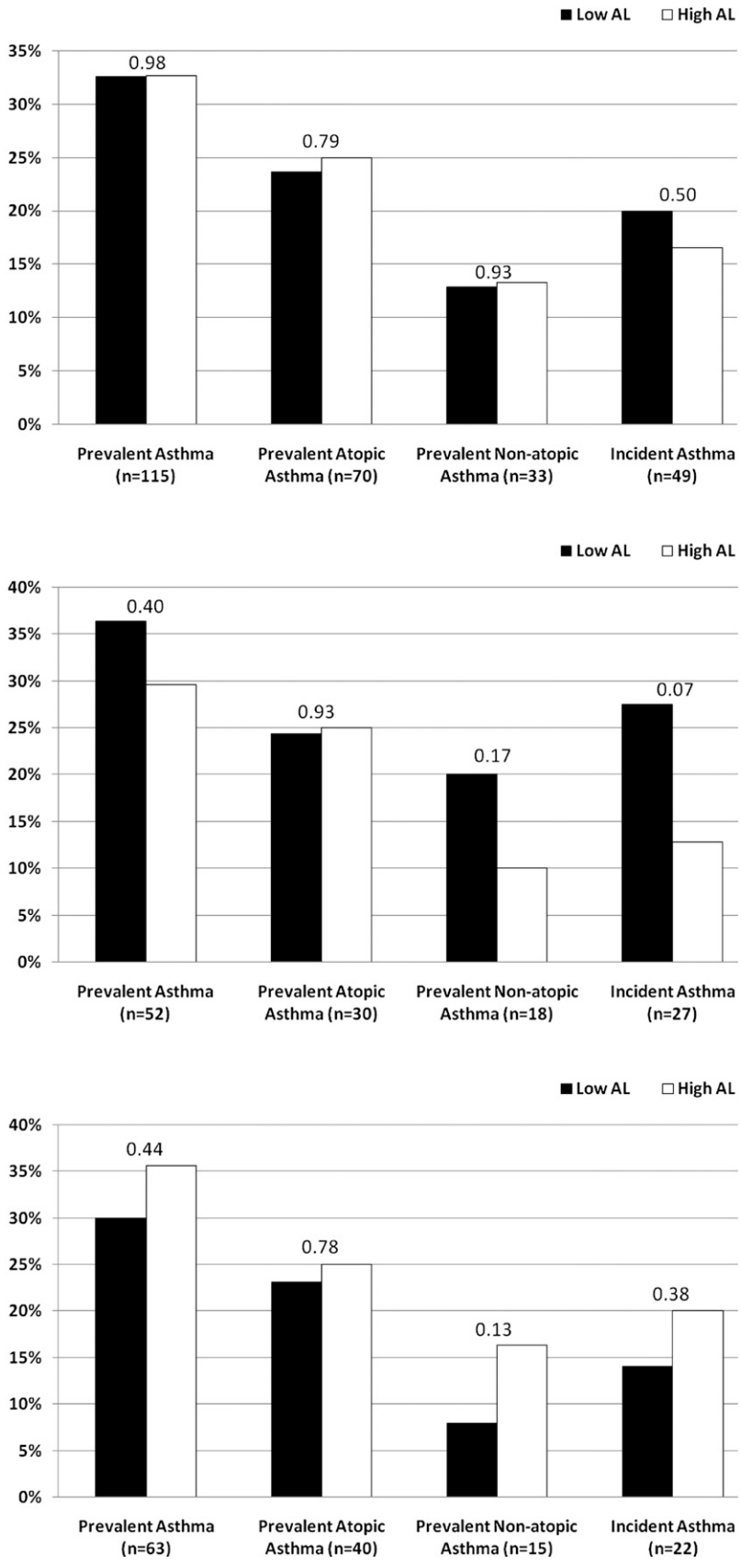

Figure 2. Prevalence and incidence of asthma among participants with high versus low allostatic score. *Top*, All children. *Middle*, Girls. *Bottom*, Boys. AL = allostatic load.

additional unit increase in AL score increased the likelihood of prevalent asthma by 60% in boys. High AL was associated with atopic and nonatopic asthma in boys. All associations were independent of genetic risk and observed at subclinical elevations of several biomarkers. Upon further testing of the AL biomarker subsets, elevations in the TC and glucose combination were found to increase the likelihood of atopic asthma, whereas the glucose and cortisol subset was associated with nonatopic asthma

TABLE 2. PREVALENCE OF HIGH ALLOSTATIC LOAD AND PREVALENT ASTHMA AMONG DIFFERENT COVARIATES

	All Children n (%)	High Allostatic Load Score			Prevalent Asthma (age 12.5 yr)								
					All Asthma			Atopic Asthma			Nonatopic Asthma		
		n	Prevalence (%)	*P* Value	n	Prevalence (%)	*P* Value	n	Prevalence (%)	*P* Value	n	Prevalence (%)	*P* Value
Atopy (age 9)													
No	175 (49.9)	60	34.3	0.40	33	18.9	<0.001*	4	2.9	<0.001*	26	16.1	0.05*
Yes	176 (50.1)	53	30.1		81	46.0		65	43.0		7	7.5	
Food allergy													
No	310 (88.1)	103	33.2	0.22	88	28.4	<0.001*	47	18.4	<0.001*	32	13.3	0.5
Yes	42 (11.9)	10	29.3		27	64.3		23	63.9		1	7.1	
Sex													
Female	153 (43.5)	54	29.6	0.26	52	34.0	0.64	30	24.6	0.86	15	10.4	0.16
Male	199 (56.5)	59	35.3		63	31.7		40	23.7		18	16.4	
Parental history of asthma													
No	172 (55.8)	62	36.0	0.17	48	27.9	0.03*	28	19.4	0.05*	18	13.4	0.98
Yes	136 (44.2)	39	28.7		54	39.7		33	29.7		12	13.3	
Ethnicity													
White	274 (78.1)	80	29.2	0.04*	88	32.1	0.63	56	24.3	0.87	23	11.7	0.22
Nonwhite	77 (21.9)	32	41.6		27	35.1		14	23.3		10	17.9	
Overweight													
No	241 (68.7)	53	22.0	<0.001*	81	33.6	0.62	51	25.5	0.42	21	12.4	0.64
Yes	110 (31.3)	59	53.6		34	30.9		19	21.1		12	14.5	
Tobacco smoke exposure at home													
No	260 (73.9)	81	31.2	0.52	89	34.2	0.29	54	25.2	0.43	27	14.4	0.25
Yes	92 (26.1)	32	34.8		26	28.3		16	20.8		6	9.0	
Pubertal stage													
Early	196 (56.5)	57	29.1	0.23	63	32.1	0.65	40	23.8	0.78	18	12.3	0.63
Late	151 (43.5)	53	35.1		52	34.4		30	25.2		15	14.4	
Parental education (postsecondary)													
No	17 (5.2)	7	41.2	0.39	7	41.2	0.47	4	28.6	0.74	2	16.7	0.67
Yes	311 (94.8)	97	31.2		102	32.8		64	24.6		28	12.5	

* Statistically significant at $P < 0.05$.

in adolescent boys. These subset associations were comparable to, if not of a greater magnitude than, those observed with the total AL score. In girls, high AL scores were not associated with prevalent (atopic or nonatopic) or incident asthma.

First introduced by Sterling and Eyer (41), the concept of allostasis was operationalized in the 1990s and established as a measure of the collective impacts of long-term stress exposure (42). The majority of studies on AL have been conducted in

TABLE 3. ASSOCIATION OF ALLOSTATIC LOAD AND ASTHMA

	Boys			Girls		
	Crude OR	Adjusted OR Model 1	Adjusted OR Model 2	Crude OR	Adjusted OR Model 1	Adjusted OR Model 2
All asthma* (n = 352)						
AL score continuous	1.11 (0.90–1.36)	1.33 (1.00–1.83)†	1.63 (1.12–2.37)†	0.88 (0.68–1.13)	0.72 (0.50–1.03)	0.70 (0.48–1.03)
AL low	1	1	1	1	1	1
AL high	1.29 (0.68–2.46)	2.70 (1.01–7.23)†	4.01 (1.37–11.8)†	0.74 (0.36–1.50)	0.33 (0.11–1.04)	0.32 (0.10–1.02)
Atopic asthma‡ (n = 319)						
AL low	1	1	1	1	1	1
AL high	1.11 (0.51–2.41)	2.30 (0.62–8.56)	4.58 (1.04–20.2)†	1.04 (0.45–2.41)	0.63 (0.17–2.36)	0.55 (0.14–2.13)
Nonatopic asthma§ (n = 282)						
AL low	1	1	1	1	1	1
AL high	2.26 (0.76–6.69)	3.72 (1.03–13.5)†	3.82 (0.91–16.1)	0.44 (0.14–1.46)	0.45 (0.11–1.78)	0.33 (0.07–1.52)
Incident asthma‖ (n = 258)						
AL low	1	1	1	1	1	1
AL high	1.54 (0.59–4.01)	2.06 (0.73–5.83)	4.35 (1.19–15.9)†	0.39 (0.13–1.12)	0.48 (0.16–1.48)	0.44 (0.14–1.39)

Definition of abbreviations: AL = allostatic load; OR = odds ratio.

* Model 1 is adjusted for existing asthma and atopy, age, ethnicity, and parental history of asthma; model 2 is adjusted for same covariates plus overweight status.

† Statistically significant at $P < 0.05$.

‡ Model 1 is adjusted for existing asthma/atopy, age, positive history of food allergy, and parental history of asthma; Model 2 is adjusted for same covariates plus overweight status.

§ Model 1 is adjusted for existing asthma/atopy and age; model 2 is adjusted for same covariates plus overweight status.

‖ Model 1 is adjusted for atopy, age, and positive history of food allergy; model 2 is adjusted for same covariates plus overweight status.

TABLE 4. ASSOCIATION OF INDIVIDUAL ALLOSTATIC LOAD BIOMARKERS AND ASTHMA IN BOYS

	Prevalent Asthma			
	Asthma (*n = 199*)*	Atopic Asthma (*n = 169*)†	Nonatopic Asthma (*n = 144*)‡	Incident Asthma (*n = 140*)§
Systolic blood pressure	1.48 (0.49–4.46)	1.05 (0.24–4.53)	0.98 (0.22–4.25)	1.73 (0.46–6.54)
Diastolic blood pressure	1.38 (0.49–3.86)	3.22 (0.79–13.2)	0.62 (0.11–3.32)	1.13 (0.35–3.64)
Waist-to-hip ratio	1.78 (0.48–6.44)	0.44 (0.06–3.29)	2.71 (0.62–11.9)	2.49 (0.61–10.1)
Glucose	2.18 (0.86–5.50)	3.07 (0.83–11.3)	3.35 (0.95–11.9)	1.86 (0.65–5.28)
Total cholesterol	2.26 (0.88–5.78)	2.30 (0.64–8.22)	1.54 (0.41–5.79)	2.03 (1.03–8.92)**
HDL	1.22 (0.44–3.42)	0.64 (0.15–2.79)	2.16 (0.55–8.42)	1.28 (0.37–4.41)
DHEA-S	0.83 (0.32–2.19)	0.93 (0.26–3.31)	0.61 (0.13–2.92)	0.88 (0.27–2.88)
Cortisol	2.25 (0.83–6.10)	1.92 (0.19–4.34)	3.89 (1.00–15.5)**	1.58 (0.52–4.83)

Definition of abbreviations: DHEA-S = dehydroepiandrosterone sulfate; HDL = high-density lipoprotein. Values are adjusted odds ratios with 95% confidence intervals in parentheses.

* Final model is adjusted for existing asthma and atopy, age, ethnicity, overweight status, and parental history of asthma.

† Final model is adjusted for existing asthma/atopy, age, positive history of food allergy, overweight status, and parental history of asthma.

‡ Final model is adjusted for existing asthma/atopy, age and overweight status.

§ Final model is adjusted for atopy, age, positive history of food allergy, and overweight status.

** Statistically significant at $P < 0.05$.

adult populations and report on cardiovascular, metabolic, psychological, and senile cognitive outcomes (8). Studies considering AL in pediatric populations are limited and fall under three themes. In one grouping of publications, the AL theory is merely proposed as an explanation for psychological and neuro-developmental outcomes after stressful life circumstances in children and adolescents (43, 44). Comprising a second theme, the AL index is applied as a tool to assess cumulative stress in children (8, 22, 23, 45), demonstrating long-lasting effects of childhood stressors on AL markers continuing into adulthood (46). Finally, a few pediatric studies have examined the AL index in childhood in relation to adverse outcomes, such as school days missed due to illness (47) or impaired working memory in early adulthood (9). Our study builds on this literature to demonstrate that AL, as an index representing incremental effects of long-term stress, is linked to asthma in adolescents.

Isolated AL components, such as impaired cortisol responses (48) and high body adiposity (49), have shown positive associations with asthma in youth. More recently, insulin resistance (50) and abnormal lipid metabolism (51) have been linked to childhood asthma. The metabolic syndrome, a proinflammatory state characterized by central obesity, high blood pressure, dyslipidemia, and insulin resistance (39), has recently been reported in children and adults with asthma (39, 52). The present findings enhance our understanding of the roles played by these biomarkers in adolescent boys with asthma. Initially, we found a 4-fold association with AL scores in the highest quartile, showing that multibody system biomarkers are significantly associated with adolescent asthma. By also testing the AL index as a continuous measure (Table 3), we discovered evidence for a linear association between incremental increases in the AL score and asthma likelihood. Next, results from the incident asthma analysis confirmed that change in the biomarkers preceded asthma onset.

However, examining subset combinations of the AL biomarkers, which included metabolic and cardiovascular risk factors (TC and glucose) and a stress hormone (cortisol) in relation to asthma (Table 6), we documented associations with asthma that were improved over the corresponding biomarkers in isolation (Tables 4 and 5) and that were equally comparable to those of the total AL index (Table 3). These findings suggest that the reported links between total AL index and asthma are attributable to the subset combinations of biomarkers. These results provide evidence for multisystem biomarkers in adolescent-onset asthma that are not solely derived from metabolic, cardiovascular, or HPA axis activity and confer that all AL biomarkers are not uniformly involved in this process.

Multiple interactive biomarkers were linked to asthma in this study. High cholesterol (39, 51) and insulin resistance (39, 50) can promote a proinflammatory state. Lipid and glucose

TABLE 5. ASSOCIATION OF INDIVIDUAL ALLOSTATIC LOAD BIOMARKERS AND ASTHMA IN GIRLS

	Prevalent Asthma			
	Asthma (*n = 153*)*	Atopic Asthma (*n = 122*)†	Nonatopic Asthma (*n = 110*)‡	Incident Asthma (*n = 119*)‖
Systolic blood pressure	0.60 (0.20–1.85)	1.58 (0.32–7.68)	0.49 (0.11–2.27)	1.02 (0.48–4.44)
Diastolic blood pressure	0.74 (0.26–2.13)	0.75 (0.16–3.52)	0.65 (0.18–2.41)	0.89 (0.32–2.48)
Waist-to-hip ratio	0.63 (0.17–2.33)	0.95 (0.16–5.56)	0.53 (0.10–2.72)	0.49 (0.14–1.75)
Glucose	0.44 (0.15–1.23)	1.32 (0.34–5.20)	0.10 (0.02–0.65)**	0.41 (0.13–1.21)
Total cholesterol	1.21 (0.43–3.39)	2.24 (0.60–8.36)	0.82 (0.20–3.46)	1.19 (0.51–3.37)
HDL	1.01 (0.32–3.19)	0.61 (0.12–2.99)	0.72 (0.16–3.20)	0.69 (0.21–2.34)
DHEA-S	1.08 (0.38–3.05)	0.88 (0.22–3.49)	1.58 (0.43–5.80)	0.80 (0.26–2.41)
Cortisol	0.63 (0.18–2.16)	0.60 (0.07–4.85)	2.22 (0.29–17.1)	0.62 (0.16–2.39)
Insulin	2.57 (0.65–10.3)	0.67 (0.10–4.49)	13.7 (1.27–14.8)**	1.64 (0.49–5.44)

Definition of abbreviations: DHEA-S = dehydroepiandrosterone sulfate; HDL = high-density lipoprotein. Values are adjusted odds ratios with 95% confidence intervals in parentheses.

* Final model is adjusted for existing asthma and atopy, age, ethnicity, overweight status, and parental history of asthma.

† Final model is adjusted for existing asthma/atopy, age, positive history of food allergy, overweight status, and parental history of asthma.

‡ Final model is adjusted for existing asthma/atopy, age, and overweight status.

‖ Final model is adjusted for atopy, age, positive history of food allergy, and overweight status.

** Statistically significant at $P < 0.05$.

TABLE 6. ASSOCIATION OF COMBINED BIOMARKERS AND ASTHMA IN BOYS

		Crude OR	Adjusted OR
Prevalent asthma*	Total cholesterol, glucose, and cortisol	1.67 (0.81–3.45)	3.02 (1.04–8.78)†
Prevalent atopic asthma‡	Total cholesterol and glucose	1.58 (0.65–3.82)	4.76 (1.10–20.5)†
Prevalent non-atopic asthma§	Glucose and cortisol	3.37 (1.02–11.1)†	8.23 (1.75–38.7)†
Incident asthma‖	Total cholesterol, glucose, and cortisol	1.84 (0.64–5.27)	3.36 (1.02–11.2)†

Definition of abbreviation: OR = odds ratio.

* Final model is adjusted for existing asthma and atopy, age, ethnicity, overweight status, and parental history of asthma.

† Statistically significant at $P < 0.05$.

‡ Final model is adjusted for existing asthma/atopy, age, positive history of food allergy, overweight status, and parental history of asthma.

§ Final model is adjusted for existing asthma/atopy, age, and overweight status.

‖ Final model is adjusted for atopy, age, positive history of food allergy, and overweight status.

metabolism are also affected by stress. Stress-induced elevations in TC levels have been observed for as long as 3 years after exposure (53). Also, hyperresponsiveness to stress has been prospectively linked with elevated fasting glucose and insulin resistance (54). Chronic sympathetic nervous system overactivity is posited to explain these observations, likely through lipolytic and glycogenolytic effects of epinephrine and norepinephrine and their inhibitory effect on glucose uptake (53, 54). It is equally plausible that elevated levels of TC and glucose in adolescent boys with atopic asthma are the result of stress-induced sympathetic nervous system overactivity. We did not have a direct measure of environmental stressors or the sympathetic nervous system activity to test this explanation. We were able to measure cortisol levels, and our findings point to a direct role for HPA activity and insulin resistance in nonatopic asthma among boys.

Several of the studied biomarkers, including blood pressure, lipid profile, glucose metabolism, and waist-to-hip ratio, can be affected by total body adiposity (40). Because overweight status has been linked to stress-induced unhealthy eating behavior (55) and to asthma in adolescents (49), it may confound the association between AL or a subset of its biomarkers with asthma. When overweight was added as a separate measure to models, the association between AL and atopic asthma was unmasked in boys, revealing an effect of AL on atopic asthma that was independent of body adiposity. In nonatopic asthma, adjustment for overweight also increased the odds ratio for AL but widened the confidence interval to include the value of 1. In contrast, the association between the cortisol-glucose subset of AL and nonatopic asthma remained independent of total body adiposity. Because being overweight is common in adolescents with nonatopic asthma (49), the last finding suggests that overweight confounded the association between nonatopic asthma and components of AL other than cortisol and glucose.

Sex differences were clearly evident in our findings. Consistent with a greater effect of parent stress on the development of wheezy symptoms in boys but not girls (56), AL and its biomarkers were associated with prevalent and incident asthma in boys only. In girls, crude odds ratios indicated a null association between AL and prevalent asthma, but an inverse association was uncovered after adjusting for current asthma. Because pubertal changes start at an earlier age in girls compared with boys, we speculate that fluctuating hormones, such as elevations in the DHEA/cortisol ratio (57) or insulin resistance observed during midpuberty in girls (36, 37, 57), would affect the AL score. Early-onset menarche is speculated to both increase (29) and decrease (58) the risk of developing asthma in adolescent girls. Adjustment for self-reported pubertal stage did not change model results in our study. Thus, puberty, which was present in 60% of study girls, has unclear effects on the AL and asthma association in our study. Because almost 70% of study boys were considered prepubertal, it is unlikely that our findings were influenced by pubertal-induced hormonal changes in boys.

Of the eight individual components of AL, few biomarkers were associated with asthma phenotype; elevated cortisol in boys and insulin levels in girls increased the likelihood for nonatopic asthma. Similar to the overall AL score, a significant inverse association was observed between relatively high glucose levels and prevalent nonatopic asthma in adolescent girls. Initially, this finding appeared to be incongruent with our insulin results and with literature documenting a higher prevalence of insulin resistance among children and adults who have asthma (50, 59). However, in contrast to the parallel rise in glucose and insulin seen in adults before the development of type 2 diabetes, recent studies are consistently showing a slight drop in glucose that is concurrent with insulin resistance in pubertal girls (36, 37). This new literature supports our observations of lower glucose and higher insulin levels with nonatopic asthma in adolescent girls in puberty.

There are a number of strengths to our research: *1*) the total number of participants was quite large for the prevalence study, *2*) asthma and asthma phenotypes were diagnosed by a pediatric allergist based on established guidelines (17), and *3*) allostatic load was measured using biomarkers similar to previous studies in children and adolescents.

A few limitations were also apparent in this study. No direct measure of sympathetic nervous system activity (epinephrine or norepinephrine) was available for the composition of AL score. Also, we did not use a stress questionnaire to identify specific environmental (either psychological or physical) stressors. Evidence behind the theory that the composite AL score and its components are a reflection of long-term stress exposure formed the basis of our study. Without inquiring into stressful life events, we can only speculate that a high AL score and elevated biomarker levels were the outcome of chronic stress. Future study is required to compare and contrast AL biomarkers versus paper and pencil stress measures to predict the development of asthma. Furthermore, the 2-year follow-up time period may be insufficient to evaluate the impact of AL biomarkers. However, our study was conducted over the critical age period when adolescent asthma first develops, and our analyses were adjusted for preexisting asthma. In addition, we also performed an incidence analysis where only new cases of asthma were studied. Larger cohorts of children followed over a longer period of time are needed to investigate the causal association between AL and its biomarkers with new-onset atopic and nonatopic asthma.

Asthma is one of the most common chronic diseases in adolescents and affects the lives of youth and their families (60). The AL concept enabled us to assess a set of multisystem, stress-responsive biomarkers that had not been considered collectively in relation to asthma in this age group. Our results show that even subclinical, elevated levels of stress and metabolic biomarkers increase the likelihood of adolescent asthma and differ by gender and asthma

phenotype. Clinicians currently use the Asthma Predictive Index (61) to predict asthma in young schoolchildren, an index not validated in older children and composed of risk factors that are nonmodifiable. AL and its components are amendable to change in later childhood despite originating from stressors that may occur in early life and go undetected. We reported associations with the AL composite measure and a subset of its components. It is possible that the association between AL index and asthma was predominantly derived from a smaller subset of biomarkers. Although this finding would promote convenience and cost savings in the clinical setting, our findings warrant replication in other studies before the AL composite measure of multisystem biomarkers is dismissed.

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