University of Alberta

DOES CHRONIC STRESS PREDICT ASTHMA IN ADOLESCENTS?

by

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ABSTRACT

Asthma is a common chronic condition in Canadian adolescents. Stress is a proposed risk factor for asthma development. Allostatic load (AL) is a composite measure of chronic stress exposure, and its role in the development of asthma in adolescents was the focus of this thesis. In study 1, we found a significant positive association between high AL and prevalent/incident asthma in adolescent boys, but not girls. Subsequently, in study 2, the effects of individual biomarkers that comprise AL index and their associations with asthma were evaluated. In boys, a combination of total cholesterol and cortisol predicted non-atopic asthma, whereas total cholesterol and blood pressure predicted atopic asthma. In girls, fasting insulin levels predicted non-atopic asthma. In summary, we demonstrated that sub-clinical levels of biomarkers increase the risk of asthma. These findings highlight simple measures of modifiable risk factors can be used by clinicians to predict asthma in adolescents.

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LIST OF SYMBOLS AND ABBREVIATIONS

AL	Allostatic Load
ANS	Autonomous Nervous System
BHR	Bronchial Hyper-Reactivity
BMI	Body Mass Index
CRH	Corticotropin-Releasing Hormone
DBP	Diastolic Blood Pressure
DHEA-S	Dehydroepiandrosterone Sulphate
HbA_{1C}	Hemoglobin A_{1C}
HDL	High-Density Lipoprotein
HPA	Hypothalamus-Pituitary-Adrenal
IHT	Insulin-Hypoglycemia Test
LDL	Low-Density Lipoprotein
PTSD	Post-Traumatic Stress Disorder
SAGE	Study Of Asthma, Genes And Environment
SBP	Systolic Blood Pressure
TC	Total Cholesterol
VLDL	Very Low-Density Lipoprotein
WHR	Waist-To-Hip Ratio

CHAPTER 1 - INTRODUCTION

Outline

This paper-based thesis includes my graduate research to help satisfy the requirements for an M.Sc. degree (Medical Sciences-Pediatrics). My focus has included the physiologic and metabolic responses to chronic stress exposure and its contribution to the development of asthma in adolescents. My study findings are presented as two original manuscripts; both will be prepared to submit for peer-review in Fall 2011. In this introductory chapter, I provide a narrative summary of the literature on stress, allostatic load, and asthma, all of which are fundamental issues that I examined in this thesis.

Background

Asthma is a chronic inflammatory disorder of the airways that is characterized by recurrent episodes of (partially reversible) airway inflammation, persistent bronchial hyper-reactivity (BHR) and airway remodeling (1). Asthma is present in up to 10% of children in developed countries (2), and Canada ranks near the top of the list of countries with high asthma levels. Among Canadian adolescents, 13% have physician-diagnosed asthma (3). Although asthma is one of the most prevalent chronic conditions in adolescents, few studies have investigated asthma epidemiology and risk factors in this age group (4). Considering the multi-factorial and heterogeneous nature of this condition and its poorly-characterized

biological basis, it is challenging to predict which boys and girls will develop asthma in adolescence. However, one of the variables that likely plays a key role is stress. Indeed, from the prenatal period to the adolescent years, stress is believed to be an important risk factor in the initiation and exacerbation of asthma.

A strong relationship between psychosocial stressors and asthma has been documented in previous studies (5). Stressful life events, early life exposure to maternal distress and childhood depression/anxiety are possible risk factors of asthma in children and adolescents (6-8). Exposure to multiple social and environmental stressors can increase the risk of childhood asthma in a synergistic manner. For example, when environmental exposures such as air pollution or poor housing conditions are combined with social issues like domestic violence, these factors collectively increase the risk of asthma in children (9, 10). On a similar note, adolescents living in the inner city, which are often characterized by stressful neighborhoods (i.e., low socioeconomic status, safety concerns) are at increased risk of asthma and present with elevated asthma-related morbidity (6, 11).

Despite these observations, the underlying mechanisms connecting early life stressors to adverse health outcomes such as asthma in childhood and adolescence are not fully understood. Suggested mechanisms linking stress to asthma include adaptation of cardiopulmonary activity (12), hypothalamicpituitary-adrenocortical (HPA) axis (13) and autonomous nervous system (ANS) activity (13, 14).

Alterations in these systems are at the roots of the stress response and are in agreement with the concept of allostasis and allostatic load (AL) (15, 16).

AL is a concept that was developed to elucidate the associations between chronic stress exposure and adverse health outcomes. More specifically, in response to a stressful event, the human body makes a number of physiologic changes to maintain homeostasis in a short-term. The stress response normally includes an increased heart rate and elevated blood pressure, alterations in the metabolism of serum lipids and carbohydrates, and the secretion of stress hormones such as cortisol, epinephrine and norepinephrine. In the short-term, these physiologic responses to stress are called "Allostasis"; however, these changes impose a heavy physical cost over time. With long-term or frequent exposure to stress, the body's systems might fail to respond and adapt appropriately. This state, when the body fails to respond properly under stressful, continuous "wear and tear", is called AL.

Ever since the AL concept was first defined by Sterling and Eyer in 1988 (17), researchers have studied the relationship between AL index and adverse health outcomes in children and adults. Children who have high AL scores generally have more days absent from school due to illness (18). In addition, high AL scores in pregnant African American women may help to explain elevated mortality and morbidity in their offspring (19). AL concept has also been proposed to explain findings regarding depression, post-traumatic stress disorder (PTSD) and neurodevelopmental disruption in stressed children and adolescents

(20-22). However, most of the published literature on AL has been in adult populations with AL index being associated with a variety of adverse health outcomes including ischemic heart disease, peripheral arterial disorders, hypertension, type2 diabetes, depression, PTSD, cognitive decline and general mortality in adults (23-27). AL index may also be a better marker of cumulative biological risk and predictor of all-cause mortality versus the metabolic syndrome (28).

Although the effect of stress on the development of asthma has been studied to some extent, the link between AL and asthma in children and adolescents has not been addressed previously. In addition, stress is a subjective construct and quantifying it can be very complex because an individual's degree of perceived stress is the result of intra-personal factors, previous exposures and coping skills, as well as the stressful event itself. AL index represents an objective measure of the physiologic changes of the body in response to stress. The focus of this thesis was to extend pediatric research in the area of AL and to examine its role in the genesis of asthma in adolescence.

Objectives and Hypotheses

The primary objective of this research was to determine whether the presence of chronic stress, as measured by high AL score in childhood, was associated with an increased risk of having prevalent and incident asthma in adolescence. A secondary objective included comparing the effects of individual AL

components on the development of asthma in order to identify the most parsimonious model that could predict adolescent asthma. We hypothesized that: (a) High levels of AL in children increased the risk of prevalent and incident asthma in adolescents and (b) the contributions of individual components of the AL index were not equal in predicting asthma.

Outline of Thesis

This thesis consists of two manuscripts, which are presented in chapters 2 and 3. The first paper (chapter 2) addresses the primary objective of this research whereby AL, as a composite index, is examined in relation to prevalent and incident asthma in adolescents. The second paper (chapter 3) examines the secondary objective in which each of the individual components of AL index are examined in their relation to predict asthma in adolescents. In chapter 4, the thesis concludes by summarizing study limitations, epidemiological implications, clinical relevance of these findings and future directions. Appendices (1-3) are also included to highlight research methodologies, describe comprehensively all data analyses, and share the research ethics approval documents related to these studies.

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CHAPTER 2

Allostatic Load Predicts Asthma in Adolescent Boys

Bahreinian S, Ball GD, Colman I, McNeil BJ, Becker AB, Kozyrskyj AL.

Introduction

Asthma is present in up to 13% of Canadian adolescents (1). Although asthma is both prevalent and chronic, it remains challenging to predict which children will have persistent asthma during adolescence (2). Previous studies show strong associations between psychosocial stressors with the development and prognosis of atopic diseases including asthma (3). Stressful life events, childhood adversity and early life exposure to maternal distress are possible risk factors for both asthma development and exacerbation in children (4-7). Fewer studies have examined associations between stress exposure and asthma in adolescents (7). Moreover, the underlying mechanisms connecting early life stressors to later development of asthma are not fully understood.

Allostatic overload/load (AL) is a concept developed to elucidate associations between chronic stress exposure and health outcomes later in life (8, 9). In response to stressful conditions, various physiologic responses in the human body such as the hypothalamus-pituitary-adrenal (HPA) axis and metabolic system are activated to maintain the physiological balance of an organism (10). These physiologic reactions to stress are called “allostasis” and are part of the normal “fight or flight” response. However, after repeated exposure to stress, exhaustion occurs and body systems fail to show appropriate allostatic responses to stress. The point at which the body fails to respond properly under continuous “wear and tear” is known as AL. The AL index has been associated with a variety of adverse

health outcomes such as neurodevelopmental delay, heart disease, mental illness and all-cause mortality (11-13).

Although the association between stress and asthma development has been studied, the physiologic pathway that links these two states are not fully elucidated (3). In addition, experiencing stress is a subjective matter and measuring stress can be complex. The degree of stress perceived by an individual is a result of personality, previous exposures and coping skills, as well as the stressful event itself. AL index measures the physiologic changes of the body in response to stress and represents an objective measure of stress effects. It has been proposed as an explanation for the link between stress and asthma pathogenesis and/or morbidity (14), but has not been examined to date. The overall goal of this research was to determine whether the presence of chronic stress (as measured by AL index in childhood) was associated with asthma prevalence and incidence in adolescence.

Methods

This paper reports on a prospective follow-up of children in the Study of Asthma, Genes and Environment (SAGE) (15). SAGE, approved by the University of Manitoba Human Research Ethics Board (H2002:078), is a birth cohort study of 13980 children born in Manitoba in 1995. In 2002, a mail-out survey was sent to the households of all these children still living in the province, asking about their health outcomes and environmental exposures. Subsequently, 7 to 10 year old

children (mean age: 9 years) with and without parent-reported asthma were randomly recruited from both rural and urban areas for a nested case-control study. These children were followed for a total period of 4 years and revisited at two time points at ages 9 to 12 (mean age: 10.5 years) and 11 to 14 years (mean age: 12.5 years). This paper reports on a measure of AL index at 10.5 years (n=439) and its relation to both prevalent and incident asthma at 12.5 years (n=352).

Asthma diagnosis was confirmed clinically both at 9 and 12.5 years by a pediatric allergist according to the 2003 Canadian Pediatric Asthma Consensus Guidelines (16). In addition, children underwent skin prick testing to 14 common allergens (i.e., tree pollen, weed pollen, ragweed, grass pollen, *Alternaria*, *Cladosporium*, *Penicillium*, house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, cockroach, cat, dog, feathers and peanut) at both visits to define atopic versus non-atopic asthma. Children with a positive skin prick test to at least one allergen were labeled atopic. Over 90% of non-atopic children who have repeated wheezing symptoms at school age will have no wheezing symptoms or impaired lung function by puberty (17). Therefore, we excluded children who had atopic asthma at 9 years for the purpose of the incidence analysis at age 12.5.

AL has been measured using different markers and scoring systems in children and adolescents to measure the cardiovascular system, body fat distribution, glucose and lipid metabolism, HPA axis and the autonomic nervous system (18-

22). We applied a comprehensive AL scoring system, including the following eight markers: Resting systolic and diastolic blood pressure (SBP and DBP, respectively), as indicators of the cardiovascular system activity; waist-to-hip ratio (WHR) as an indicator of central body fat distribution; fasting glucose for assessing glucose metabolism; serum total cholesterol (TC) and high-density lipoprotein (HDL) as indicators of lipid metabolism; serum dehydroepiandrosterone sulphate (DHEA-S) as a functional HPA axis antagonist; and fasting serum cortisol to indicate the HPA axis activity. An early morning fasting blood sample was obtained at age 10.5 for measures of glucose, TC, HDL, DHEA-S and cortisol. SBP and DBP were calculated as the average of three measurements using a mercury manometer while the individual was seated, waist circumference was measured (to the nearest 0.1cm) as half way between the iliac crest and the lowest rib, and hip circumference was measured (to the nearest 0.1cm) at the maximum circumference over the buttocks. WHR was subsequently calculated.

For each biomarker, a value of one was added to the AL score when a child's biomarker value fell in the high risk quartile (in relation to the study population). The high risk quartile was comprised of children with the highest quartile for SBP, DBP, WHR, glucose and TC; and the lowest quartile for HDL and DHEA-S. Cortisol was an exception; a value of one was added if a child's cortisol level fell below or above the 12.5^{th} percentile (23), which is consistent with research suggesting that both low and high levels of cortisol represent the physiological

dysregulated response of the HPA axis to stressors (24, 25). The cut-off points for AL components were calculated separately for boys and girls (Table 2-1). Subsequently, children who were in the highest quartile for the total AL score (>3) were characterized as having high AL. Similar scoring systems have been used in other pediatric studies (18-21, 26).

A variety of risk factors for asthma (i.e., age, ethnicity, overweight status, positive history of food allergy in the child, parental history of atopy or asthma, parental education level, child pubertal stage and exposure to second hand smoke) were treated as potential confounders in our models (27-31). Information on age, ethnicity (Caucasian *versus* First-Nation/Métis/Asian/other), parental history of asthma/allergy and parental level of education (less than or equal to post secondary education in either parent) were derived from parent questionnaires. Height and weight were measured to the nearest 0.1cm and 0.1kg, respectively, and sex- and age-specific body mass index (BMI) was calculated. Overweight was defined as BMI-z > 1.04 (34). History of food allergy was derived from the physician visit questionnaire at age 9. Puberty stage was self-reported by children at 10.5 years using previously validated Tanner stage schematics (32, 33) and data were dichotomized as either early (Tanner scores < 2) or late (Tanner scores ≥2) puberty. Exposure to second hand smoke was extracted from home environment questionnaires.

Data were analyzed using purposeful stepwise logistic regression modeling with *SPSS Statistics 18.0* software to determine the association between high AL and

both prevalent and incident asthma expressed as odds ratios. Based on the baseline associations with either high AL or asthma, different covariates were selected for final models at p-values < 0.10. Since parental level of education, pubertal stage and exposure to secondhand smoke did not meet this criterion, none of these covariates were included in the final models. As a number of the AL components might be affected by weight status, we ran separate models that included overweight as a covariate to assess the independent effect of AL from overweight status (model 2). There was a statistically significant interaction between sex and AL score in all models; therefore, we stratified our analysis by sex. To study the independent association of the AL with new-onset adolescent asthma, we adjusted our prevalence analysis for existing asthma at age 9. The goodness of model fit was assessed using Hosmer and Lemeshow Test at $p > 0.05$.

Results

A total of 439 children with an AL measure at 10.5 years were included; from this sample, 160 (36.4%) had asthma. After two years, 352 children were re-visited at 12.5 years (80.2%). There were no statistically significant differences between participants who did / 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 compared to their peers who attended the follow up visit ($p<0.001$). Attrition at follow-up was lower for Caucasians versus non-Caucasians (17.2% vs. 28.0%, respectively; $p = 0.01$).

Although non-Caucasian families (primarily First-Nations) were recruited independently to maximize participation in this study (15), they accounted for the majority of the loss to follow-up. The sex-specific distribution of the AL index is illustrated in Figure 1.

All 352 children who were present at both visits were included in the prevalence analysis. One hundred and fifteen (32.7%) participants had prevalent asthma; of these, 70 (21.6%) and 33 (10.2%) had atopic and non-atopic asthma, respectively, and 12 asthmatic participants did not consent to skin prick testing. Tanner stages of 2 or higher were self-reported at 10.5 years by 58.8% girls and 32.1% of boys. In general, total asthma and both asthma phenotypes (atopic and non-atopic) tended to be more prevalent in boys with high AL scores and girls with low AL scores, but differences were not statistically significant at baseline (Figure 2). High AL was less prevalent in Caucasian and normal weight children ($p<0.05$). Asthma, on the other hand, was more prevalent among children who were at younger age, were atopic, had a positive history of food allergy, or parental history of asthma. Table 2-2 shows the prevalence of AL and prevalent asthma among different covariates. Regarding the incidence analysis, similar associations were observed regarding AL scores and ethnicity or overweight. Incident asthma was more prevalent among children with a history of food allergy or parental history of asthma ($p<0.05$).

Prevalent Study- Asthma

Existing asthma or atopy and parental history of asthma at 9 years were statistically significant predictors of asthma at 12.5 years. For each one year increase in the age, there was a 50% decreased likelihood of having asthma as an adolescent. In boys, AL increased the risk for having asthma, although this association was not statistically significant at baseline (Table 2-3). After adjusting for the covariates in model 1, there was a 33% increased likelihood of having asthma for each one additional AL score. Also, boys who had high AL scores were 2.7 times more likely to have asthma in adolescence compared to those in low AL group. These associations were statistically significant and independent of age, ethnicity, parental history of asthma and existing asthma or atopy at age 9. Existing asthma and atopy, as well as parental history of asthma remained significant predictors for asthma at 12.5 years after adjustments (data not shown). In the secondary analysis (model 2), we observed a 63% increased chance of having asthma for each single unit AL score increase. Additionally, the odds of having asthma in boys with high AL scores increased to more than four. These associations were independent of all covariates.

In girls, there was no significant association between high AL score at 10.5 years and asthma at 12.5 years neither at baseline nor after adjustments in models.

Prevalent Study- Asthma Phenotypes

Boys who had high AL scores were 4.6 times more likely to have atopic asthma at 12.5 years, after controlling for covariates in model 2. Atopic asthma at age 9,

history of food allergy and positive parental history of asthma were statistically associated with atopic asthma at 12.5 years age in both models (data not shown).

Non-atopic asthma was significantly more prevalent among boys with high AL in model 1 independent of their asthma/atopy status at 9 years and age. Nevertheless, after we added overweight status to our analysis in model 2, this association became less significant (Table 2-3). Having non-atopic asthma at age 9 was the only significant predictor for non-atopic asthma in adolescent years in both models (data not shown).

In girls, in contrast, high AL score was not significantly associated with either atopic or non-atopic asthma in either of the models (Table 2-3).

Incident Study- Asthma

Excluding children with atopic asthma at 9 years, 258 children were included in our incident analysis with 49 new cases of asthma diagnosed at follow-up. In boys, high AL score increased the likelihood of asthma development in adolescence. For each one year increase in age of boys, there was a 65% decreased likelihood of developing asthma in adolescence. None of the other covariates had a statistically significant association with incident asthma at baseline (data not shown). After adjusting for the covariates in model 2, high AL score significantly increased the risk of incident asthma by 4.4 times. History of food allergy was also a significant predictor of asthma development in model 2

(data not shown).

In girls, as shown in table 2-3, high AL scores at 10.5 years did not have a significant association with asthma at 12.5 years. History of food allergy significantly increased the likelihood of asthma development, while older ages significantly decreased the chance of asthma in adolescent girls (data not shown). We were unable to perform the incident analysis for each asthma phenotype due to smaller numbers of children in the incidence study.

Discussion

In this cohort study, we found a direct association between high AL score and asthma development in adolescent boys. Boys who had high AL at age 10.5 were 4 times more likely to have asthma at age 12.5, compared to those with low AL scores. Moreover, each one additional AL score increased the likelihood of having asthma by 60% in boys. These associations were independent of age, ethnicity, existing asthma or atopy at 9 years, parental history of asthma and overweight status. At the same time, high AL was associated with both atopic and non-atopic asthma in boys, but the association with atopic asthma became statistically significant only after adjustment for overweight status. In contrast, statistical significance between high AL and non-atopic asthma was lost after overweight was accounted for. Similar associations were observed for incident asthma in adolescent boys. On the other hand, high AL scores in girls were not associated with prevalent (atopic or non-atopic) and incident asthma. This is the

first study to document an association of AL, an established measure of collective effects of long-term stress, with asthma in adolescents.

AL is a relatively new concept, first introduced in 1988 by Sterling and Eyer (8) and operationalized later in the 1990s and early 2000 (9, 35). The majority of studies on AL have been in adult populations and included cardiovascular, metabolic, psychological and senile cognitive outcomes (12, 13, 36-38). AL has also been measured in a limited number of pediatric studies to explore its use as a construct of cumulative stress in children (18, 20 , 26). High AL in childhood has been correlated with school days missed due to illness (39). Other studies have merely proposed AL concept as a possible explanation for psychological and neuro-developmental disorders in stressed children and adolescents. (11, 40, 41). Thus, our study builds on the literature to show that AL index represents incremental effects of long-term stress. Moreover, it advances the field by examining this index as a predictor of an adverse health outcome (i.e. asthma) in adolescents.

AL is a composite biological measure of chronic stress on different body systems. Some of the individual AL components such as low cortisol levels (42) and high body adiposity (43) have shown positive associations with childhood asthma. More recently, insulin resistance (44) and abnormal lipid metabolism (45) have also been linked to asthma in children. This paper advances the link between stress and asthma three important ways. First, it reports a 4-fold association with AL scores in the highest quartile, showing that multi-body system biomarkers

of stress are powerful predictors of asthma. Second, the results of the incident asthma analysis suggest a causal role for AL in the pathway to asthma in adolescence. Third, we tested AL as a continuous measure and found an association with successive increases to the AL score. This last observation leads us to propose that a composite measure for AL is an improvement over single biological markers since it provides evidence for an additive risk for asthma.

Several of the individual AL components (including blood pressure, lipid profile, glucose metabolism and WHR) can be affected by total body adiposity (46). Since overweight has been linked to stress, stress-induced unhealthy eating behaviors, and asthma in adolescents, (47, 48) overweight status may confound the AL/asthma association. To examine this, we added the overweight measure to multivariate models. The association between AL and atopic asthma was unmasked following adjustment for overweight, revealing an effect of AL on atopic asthma that did not appear to be related to body adiposity. However, adjustment for overweight diminished the association between AL and non-atopic asthma to non-significance. This loss of significance suggests that the adiposity component of the AL score may be driving the association with non-atopic asthma. Indeed, overweight has been found to be associated with non-atopic asthma but not atopic asthma, in children, adolescents and adults (43, 49).

Sex differences were evident in our findings. Consistent with a greater effect of parent stress on the development of wheezy symptoms in boys but not girls (50), AL was associated with prevalent and incident asthma in boys only. In girls,

crude odds ratios indicated a null association between AL and prevalent asthma, but after adjusting for current asthma, an inverse association between AL and non-atopic asthma was uncovered. Since girls in our study were undergoing sexual maturation, we speculate that fluctuating sex hormones may offer an explanation. DHEA levels are increased by post-pubertal estradiol (51). Thus, girls with lower DHEA levels and higher AL scores would have been less likely to have early-onset menarche, representing 40% of girls in Tanner stage less than 2. Early-onset menarche has shown to both increase and reduce onset of asthma in adolescence (30, 52). Although adding Tanner stage to our models did not change the findings, further studies in older girls are needed to determine the validity of the AL score in measuring chronic stress in menstruating girls, if indeed sex hormones affect the onset of non-atopic asthma in adolescence.

There are a number of strengths in our research. The total number of study children was quite large for the prevalent study in relation to existing studies. Asthma and asthma phenotypes were diagnosed by a pediatric allergist based on established guidelines by Canadian Pediatric Asthma Consensus Guidelines (16). AL was measured using biological components similar to previous studies in adults and children (18-23). However, we did not have a direct measure of ANS activity (epinephrine or norepinephrine secretions), which is the main limitation of our study. Although this was a longitudinal study, children were followed for a 2-year period, which might be considered insufficient for an evaluation of chronic stress. However, our analyses were adjusted for existing asthma at age 9. In

addition, we also performed an incident analysis where only new cases of asthma were studied. We could not perform the incident analysis for asthma phenotypes due to the limited number of new asthma cases during the follow-up period. Future studies that follow a larger number of children for a longer period of time are recommended to investigate the causal association between AL and the onset of atopic and non-atopic asthma.

Asthma is one of the most common chronic diseases in adolescents (53); however, risk factors that contribute to asthma development in this age group have not been thoroughly studied. Since asthma impacts the lives of both children and their families (54), it is important to identify whether any modifiable risk factors that are included in the AL index that could be potentially targeted to prevent its development. Future research should investigate the contribution of individual components of the AL score in order to develop a simple measure (or measures) that can be used by clinicians in their practice to predict asthma in adolescence.

Tables

Table 2-1 Cut-off points for AL components

	Cut-off Points	
	Boys	**Girls**
Systolic Blood Pressure (mm-Hg)	≥116	≥116
Diastolic Blood Pressure (mm-Hg)	≥68	≥68
Waist-to-Hip Ratio	≥0.89	≥0.87
Fasting Serum Glucose (mmol/L)	≥5.20	≥5.10
Serum Total Cholesterol (mmol/L)	≥4.54	≥4.50
Serum HDL (mmol/L)	≤1.37	≤1.33
Serum DHEA-S (μmol/L)	≤1.32	≤1.20
Serum Cortisol (μg/dl)	≤62.75 or ≥257.31	≤65.92 or ≥193.11
Total AL	≥3	≥3

HDL: high-density lipoprotein; DHEA-S: dehydroepiandrosterone sulphate;
AL: allostatic load

Table 2-2 Prevalence of high allostatic load and asthma among different covariates

	All Children		High AL Score		P value	Prevalent Asthma (age 12.5)								
						All Asthma			Atopic Asthma			Non-atopic Asthma		
	n	%	n	Prevalence		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														
Caucasian	274	78.1%	80	29.2%	0.04*	88	32.1%	0.63	56	24.3%	0.87	23	11.7%	0.22
non-Caucasian	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 (Post Secondary)														
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 $p<0.05$

Table 2-3 Risk of asthma subsequent to exposure to high AL and other risk factors (crude and adjusted odds ratios)

		Boys			Girls		
		Crude OR	**Adjusted OR Model 1**	**Adjusted OR Model 2**	**Crude OR**	**Adjusted OR Model 1**	**Adjusted OR Model 2**
All Asthma[1] (n=352)	**AL Score Continuous**	1.11 (0.90 to 1.36)	1.33 (1.00 to 1.83)*	1.63 (1.12 to 2.37)*	0.88 (0.68 to 1.13)	0.72 (0.50 to 1.03)	0.70 (0.48 to 1.03)
All Asthma[1] (n=352)	**AL** Low High	1 1.29 (0.68 to 2.46)	1 2.70 (1.01 to 7.23)*	1 4.01 (1.37 to 11.80)*	1 0.74 (0.36 to 1.50)	1 0.33 (0.11 to 1.04)	1 0.32 (0.10 to 1.02)
Atopic Asthma[2] (n=319)	**AL** Low High	1 1.11 (0.51 to 2.41)	1 2.30 (0.62 to 8.56)	1 4.58 (1.04 to 20.16)*	1 1.04 (0.45 to 2.41)	1 0.63 (0.17 to 2.36)	1 0.55 (0.14 to 2.13)
Non-atopic Asthma[3] (n=282)	**AL** Low High	1 2.26 (0.76 to 6.69)	1 3.72 (1.03 to 13.48)*	1 3.82 (0.91 to 16.08)	1 0.44 (0.14 to 1.46)	1 0.45 (0.11 to 1.78)	1 0.33 (0.07 to 1.52)
Incident Asthma[4] (n=258)	**AL** Low High	1 1.54 (0.59 to 4.01)	1 2.06 (0.73 to 5.83)	1 4.35 (1.19 to 15.93)*	1 0.39 (0.13 to 1.12)	1 0.48 (0.16 to 1.48)	1 0.44 (0.14 to 1.39)

* Statistically significant at $p<0.05$

1 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

2 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

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

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

Figures

Figure 2-1 The sex-specific distribution of the AL index among study participants

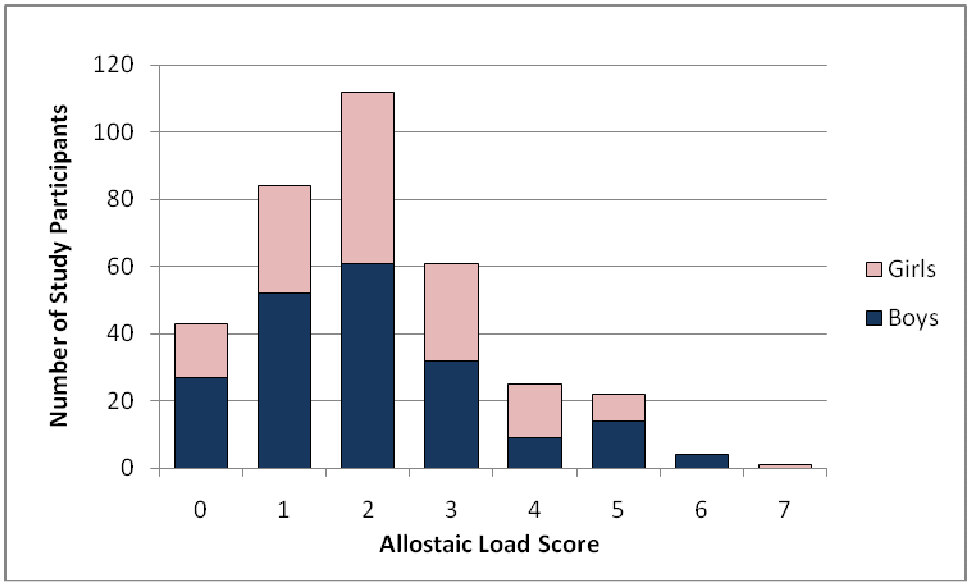
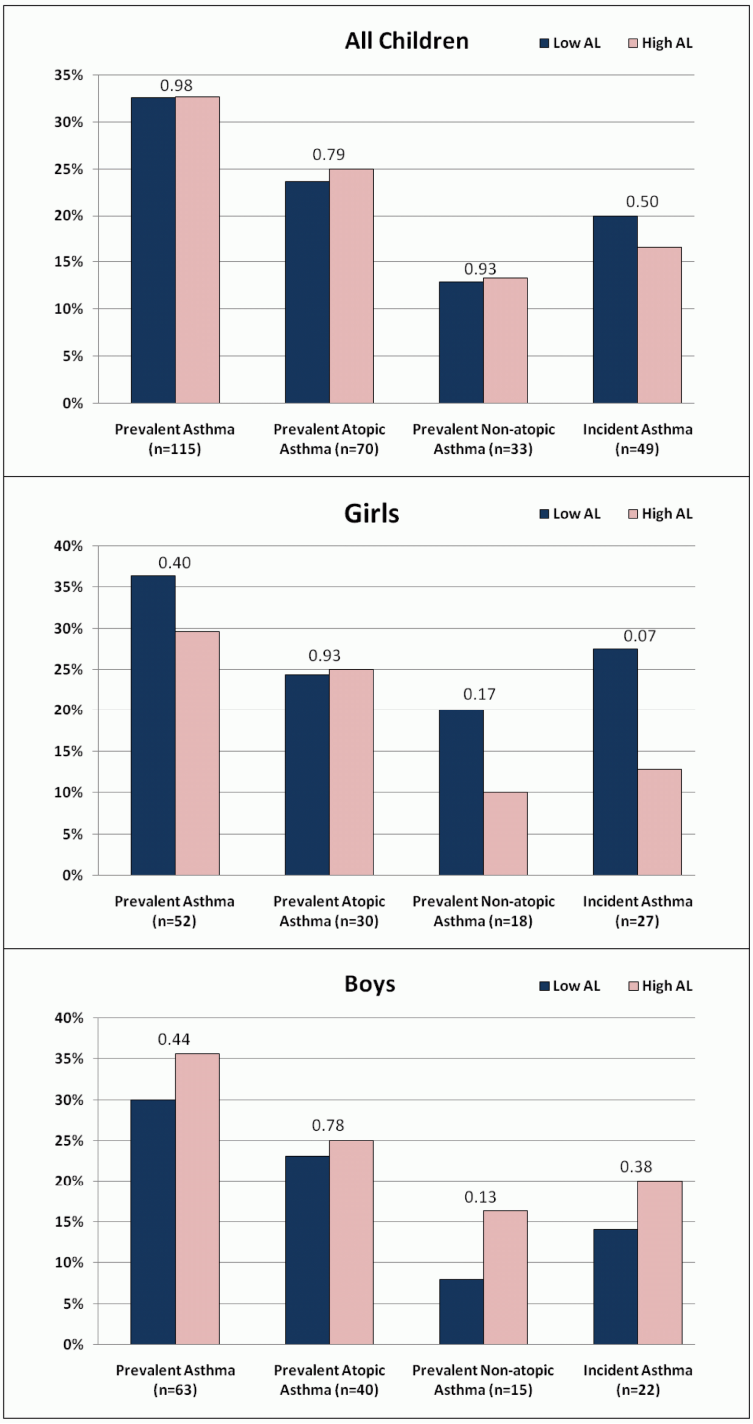


Figure 2-2 Prevalence and incidence of asthma among participants with high *versus* low allostatic index



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CHAPTER 3

Exploring The Modifiable Bological Risk Factors Of Asthma In Adolescents

Bahreinian S, Ball GD, Vander Leek TK, Becker AB, Kozyrskyj AL.

Introduction

Asthma affects a large population of children and adolescents in western countries including Canada (1). Some environmental and physiological variables such as lifestyle behaviors (2) and overweight (3) have been postulated as possible risk factors leading to high asthma prevalence in these countries. With recent parallel escalations in the prevalence of both pediatric overweight and asthma (4), a link between these two conditions has been suggested. In addition to increased weight, stress is believed to be another risk factor for asthma development (5, 6). Early life exposure to maternal distress (7), childhood depression/ anxiety (8) and stressful life events (9) increase the chance of developing asthma in children. A recent meta-analysis by Chida et al. demonstrated a bidirectional association between psychosocial stress and allergic diseases (5). It is clear that exposure to stress increases the chance of developing an atopic condition; conversely, having an allergic disease (i.e. asthma, food allergy, allergic rhinitis and atopic dermatitis) can increase stress in individuals (5). Chronic exposure to stress is a foundation to cardiovascular, metabolic and physical changes, some of which are similar to those in overweight and obese individuals.

Stress-induced metabolic changes have been described according to the concept of allostasis and allostatic overload/load (AL) (10). Allostasis refers to the process of maintaining physiological balance of the organism by dynamically changing and matching organ activities in response to stress (11). AL, alternatively, is the

state of exhaustion after long-term stress exposure when the organism is no longer capable of keeping the appropriate dynamic biological balance (12). In the state of AL, the normal allostasis response becomes dysfunctional in a sequential manner. First, stress hormones (i.e., epinephrine, norepinephrine and cortisol) become over-activated while their antagonist (Dehydroepiandrosterone Sulphate or DHEA-S) is dysregulated (primary mediators). Second, the metabolic (i.e., glucose and lipid metabolism, body fat) and cardiovascular (i.e., blood pressure, heart rate) systems adjust in an attempt to compensate for the overreaction of primary mediators (secondary outcomes). Finally, the abnormal organ activities progress from sub-clinical stages to clinical disease or tertiary outcomes (10).

By identifying the sub-clinical levels of abnormal organ activities, we might be able to predict risk of developing the tertiary health outcomes. Various studies have measured the primary mediators and secondary outcomes using a composite measure, usually called the AL index or AL score, in order to detect groups of individuals who are at high risk for tertiary health outcomes, including ischemic heart disease (13), hypertension (13), diabetes (13), depression (14), memory or cognitive decline (14) and all-cause mortality (15). While AL index is a combination of multiple system activities, some studies have also examined a sub-set or the individual components of the AL index in relation to adverse health outcomes. For example, sub-group analyses in one of the "MacArthur studies of successful aging" demonstrated that epinephrine, waist-to-hip ratio (WHR) and cortisol had greater roles in predicting the physical decline, while diastolic blood

pressure (DBP), epinephrine and glycosylated hemoglobin (HbA_{1C}) were strongly associated with cognitive decline (16).

In a previous study of AL index and asthma in adolescents, we illustrated a positive association between high AL index and risk of having both prevalent and new-onset asthma in boys (17). As the total AL index involves multiple physical and biological markers, it may be difficult to integrate it into routine clinical practice of health care providers. Moreover, we speculate individual AL components may have unequal contributions in predicting asthma. Therefore, the purpose of this study was to determine a parsimonious subset of AL index markers that predicted the development of asthma in adolescents. To achieve this objective, we examined the association of AL components with both prevalent and incident asthma in adolescents while accounting for asthma phenotypes.

Methods

This study was performed as a part of a birth cohort study named the Study of Asthma, Genes and Environment (SAGE). The SAGE study has been approved by the University of Manitoba Human Research Ethics Board (H2002:078). As described elsewhere (18), it is a birth cohort of all children (n=13980) born in Manitoba, Canada in 1995, who were still living at the same province when the SAGE study initiated at 2002. In addition, a nested case-control study was performed on a sub-group of SAGE participants (n=723) where children with and without parent-reported asthma were recruited at the age of 7 to 10 years (wave

1, mean age=9, random selection in rural and urban areas). SAGE children involved in the nested case-control study were followed for a total period of 4 years and visited biennially at 9 to 12 years (wave 2, mean age= 10.5) and 11 to 14 years (wave 3, mean age=12.5). This paper is a report on the physiological measures of the SAGE participants at 10.5 years old (wave 2) and prevalence and incidence of asthma at 12.5 years old (wave 3).

Study participants, originally recruited based on parental report of asthma, were visited by a pediatric allergist to confirm their asthma status at 9 and 12.5 years old. Asthma diagnosis was made clinically according to the 2003 Canadian Pediatric Asthma Consensus Guidelines (19). All study participants also undertook skin prick tests to 14 common allergens, which included tree pollen, weed pollen, ragweed, grass pollen, *Alternaria*, *Cladosporium*, *Penicillium*, house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, cockroach, cat, dog, feathers and peanut. Participants were considered atopic if they had a positive reaction to at least one allergen in the skin prick test. Children who had asthma were categorized as atopic or non-atopic asthma based on their skin prick test results. Since non-atopic asthma at school age is transient in many cases and disappears by puberty (20), we included children who had non-atopic asthma at age 9 for the purpose of the incidence analysis at 12.5 years old.

As discussed earlier, we have studied the impact of AL index as a composite measure of physiological stress response system on prevalent and incident asthma in adolescents (17). In the current study, we selected the following eight physical

and biological markers, which were similar to the individual AL components of our previous study: Resting systolic and diastolic blood pressure (SBP and DBP, respectively), as markers of the cardiovascular system activity; waist-to-hip ratio (WHR) representing the central body fat distribution; serum total cholesterol (TC) and high-density lipoprotein (HDL) to take lipid metabolism into account; serum dehydroepiandrosterone sulphate (DHEA-S), which is a functional antagonist to the hypothalamus-pituitary-adrenal (HPA) axis; and fasting serum cortisol to directly include the HPA axis activity. The only exception was the physiological parameter representing glucose metabolism; we replaced fasting serum glucose from our previous AL index with fasting serum insulin in this study. This decision was based on the observation that most children maintain very tight control of glucose levels within the normal range by secreting high quantities of insulin in response to insulin resistance (21, 22). In other words, most children are able to compensate for some degree of insulin resistance and prevent hyperglycemia via hyperinsulinemia. Thus, we believe that fasting serum insulin may better represent insulin resistance and risk for chronic disease (i.e., type 2 diabetes) in children, although some studies of AL in adolescents (23) have included a measure of glycemia (i.e., fasting glucose).

An early morning fasting blood sample was drawn from all participants who consented at age 10.5 years for measures of insulin, TC, HDL, DHEA-S and cortisol. Blood pressure was assessed using a mercury manometer with participants seated and the average of the three measurements was reported. Waist

and hip circumferences were measured (to the nearest 0.1 cm) over light clothing at the mid-line between the lowest rib and the iliac crest and the greatest circumference over the buttocks, respectively.

Consistent with our previous study, we dichotomized each of eight physical or biological markers as high or low risk groups based on the distribution of the biomarkers within the population under the study. The high risk group was the highest quartile (compared to other study participants) for the measures of SBP, DBP, WHR, HOMA-IR, TC and cortisol and the lowest quartile for HDL and DHEAS. The cutoff points for high versus low risk groups were calculated for each sex independently (Table 1).

Information on the covariates including age, ethnicity, overweight status, positive history of food allergy in the child and parental history of atopy or asthma were also collected and considered in our analysis as potential confounders. Ethnicity (Caucasian versus non-Caucasian [First Nations, Métis, Asian and other]) and parental history of asthma and allergy were reported by parents in the parent questionnaire at age 9 years. History of food allergy was derived from the physician visit questionnaire at age 9. At age 10.5 years, height and weight of study participants were measured to the nearest 0.1cm and 0.1kg, respectively; body mass index (BMI) was calculated with overweight defined as BMI Z-score > 1.04 (24).

Data analyses were executed by purposeful stepwise logistic regression modeling

using *SPSS Statistics 18.0* software. Independent sample student t-tests and Chi square tests were applied in the exploratory data analysis to test for differences in asthma-related covariates at baseline and to examine possible differences between participants who did or did not complete measurements at the follow-up time point. We examined the association between biological markers of AL at 10.5 years and both prevalent and incident asthma at 12.5 years. A sub-group analysis was performed on asthma phenotypes. Covariates were included in the final models, which was similar to the final models in our previous study. Although information on parental level of education, exposure to secondhand smoke at home, and puberty stage were also available, these variables were not included in the final models because they were not associated with high AL score or asthma in our past study. As there was a statistically significant interaction between sex and AL score in our previous study (17), analyses were stratified by sex. The goodness of model fit was assessed using the Hosmer and Lemeshow Test at $p > 0.05$.

Results

Among 407 SAGE case-control participants at wave 2 (mean age of 10.5 years), 123 had asthma (87 atopic and 35 non-atopic asthma). There were 230 male and 177 female participants. Eighty percent of these participants (n=327) were followed until wave 3 (mean age of 12.5 years) and were included in our analyses. Children who did not attend the follow-up visit were, on average, 3 months older

than other participants (p<0.001). Higher attrition was observed among non-Caucasian participants (27.7% non-Caucasian versus 17% Caucasians, p = 0.02), participants with high DBP (27.6% high risk versus 16.2% low risk DBP, p = 0.008), and those with low HDL values (22.4% versus 11.7%, p = 0.02). No statistically significant differences were noted regarding other biological markers or covariates.

Prevalent Study- Asthma

In total, 327 participants, which included 108 boys and girls with asthma (65 cases were atopic and 32 cases were non-atopic; 11 additional cases did not consent for skin prick testing), were involved in the analysis of prevalent asthma at 12.5 years old. Positive history of asthma in parents (crude OR 2.14, p=0.03) and existing asthma (crude OR 19.75, p<0.001) or atopy (crude OR 2.56, p=0.006) at 9 years were statistically significant predictors of asthma at 12.5 years old. We found that for each one year increase in age, the chance of having asthma in adolescence decreased by 50% (p=0.003). Boys who had low DHEA-S levels at 10.5 years were significantly more likely to have asthma at 12.5 years. Having high TC and cortisol at 10.5 years also increased risk of asthma at 12.5 years, but these associations were not statistically significant (Table 3-2). After adjusting the analysis for the covariates of age, ethnicity, overweight, positive parental history of asthma and existing asthma or atopy at age 9, the association between low DHEA-S and asthma disappeared; however, the positive association between high TC and cortisol with asthma became stronger (Table 3-3). Combined, boys who

had both high TC and cortisol levels were 2.5 times more likely to have asthma compared to their peers with low levels (adjusted OR 2.52, 95% CI 1.01 to 6.17). This association was statistically significant and independent of age, ethnicity, overweight, positive parental history of asthma and existing asthma or atopy at age 9 (Table 3-4). Positive parental history of asthma (adjusted OR 2.42, $p=0.05$) and existing asthma at 9 years (adjusted OR 15.63, $p<0.001$) continued to be significant predictors of asthma at 12.5 years after adjustments.

In girls, on the other hand, there was no statistically significant association between the biological risk factors at 10.5 years and asthma at 12.5 years old, either at baseline or after controlling for the covariates. (Tables 3-5 and 3-6).

Prevalent Study- Asthma Phenotypes

At baseline, low DHEA-S at 10.5 years significantly increased the chances of having atopic asthma in boys at 12.5 years. Also, having a high WHR had a negative association with prevalent atopic asthma at baseline (Table 3-2). Both of these associations were no longer significant after adjustment for age, overweight, positive history of food allergy in child, parental history of asthma and existing asthma or atopy at age 9. Conversely, the initially weaker associations between high TC and DBP with atopic asthma became stronger after these adjustments (Tables 3-2 and 3-3). After combining TC and DBP, we observed a 5 times increased likelihood of having atopic asthma in boys who had both high TC and DBP compared to their peers with low levels (Table 3-4). Having a positive

history of food allergy (adjusted OR 4.28, p=0.04), parental history of asthma (adjusted OR 4.94, p=0.03) and atopic asthma at age 9 (adjusted OR 76.96, p<0.001) were also statistically associated with atopic asthma at age 12.5 years in the final models.

Except for DBP, being in the high risk group for all the other biological markers increased the risk of having prevalent non-atopic asthma in boys at baseline (Table 3-2). After controlling for age, overweight and existing asthma or atopy at age 9, the associations of high SBP and low DHEA-S with non-atopic asthma at age 12.5 years disappeared (Table 3-3). Similar to prevalent asthma, grouping the measures of TC and cortisol generated a predictive index for non-atopic asthma in boys. Specifically, boys who had high levels of TC and cortisol at 10.5 years were more than 3 times more likely to have non-atopic asthma at 12.5 years, after controlling for the covariates (Table 3-4).

In girls, high levels of TC significantly increased risk of having atopic asthma at baseline (Table 3-5). This association became non-significant after adjustment for the covariates of age, overweight, positive history of food allergy in child, parental history of asthma or atopy and history of asthma/atopy at 9 years (Table 3-6). No single or combined biological measure was significantly associated with atopic asthma in girls in the final models.

Non-atopic asthma was significantly more prevalent among girls who had high insulin levels in the final model (Table 3-6). Similar to boys, atopic and non-

atopic asthma at childhood increased the likelihood of having the same asthma phenotype in adolescent years (all $p<0.001$). Other covariates (e.g., age, overweight, positive parental history of asthma) did not show a statistically significant association with asthma phenotypes in girls.

Incident Study- Asthma

After excluding participants who had atopic asthma at the age of 9 years, 240 adolescents were included in the analysis of incident asthma (46 cases of asthma). In boys, age had a significant negative association with incident asthma at 12.5 years at baseline (crude OR 0.24, $p=0.006$). None of the individual biomarkers or other covariates had a statistically significant association with incident asthma at baseline (Table 3-2). After adjusting for age, overweight, positive history of food allergy or atopy at 9 years, high TC significantly increased the risk of incident asthma by more than 3 times (Table 3-3). In addition, risk of developing asthma at adolescence was 4.6 times higher in boys having high levels of both TC and cortisol at 10.5 years. This association was statistically significant and independent of the other covariates (Table 3-4). In girls, none of the biomarkers had a significant association with incident asthma, either at baseline or after controlling for other covariates (Tables 3-5 and 3-6).

Discussion

The objective of this community-based follow-up study of over 320 adolescents

was to find a parsimonious subset of AL index variables that predicted asthma and asthma phenotypes. Our main finding was that we found a positive association between having high levels of TC and cortisol at 10.5 years with both prevalent and incident asthma (specifically, non-atopic asthma) in adolescent boys. In addition, high TC combined with high blood pressure at 10.5 years old significantly increased risk of having atopic asthma in adolescent boys. In girls, conversely, neither of these combinations predicted asthma development in adolescence; however, the likelihood of having non-atopic asthma at 12.5 years old was significantly higher among girls who had high insulin levels at age 10.5.

Epidemiological studies have long reported a link between overweight and asthma both in children and adults (3, 25). More recent studies have revealed a stronger association between central obesity, compared to high BMI, with asthma (26, 27). Central obesity, as one of the key features of the metabolic syndrome, is associated with a disturbed metabolic profile, which typically includes high blood pressure, dyslipidemia, impaired glucose metabolism and insulin resistance (28). Metabolic syndrome is a pro-inflammatory state and is common among patients with asthma (29). An abnormal metabolic profile in children who have asthma has attracted a lot of research interest recently. Two recent studies demonstrated high levels of triglycerides (30) and TC (31) in asthmatic children. These links, similar to the findings from the current study, were independent of overweight status (30, 31). It is clear that hypercholesterolemia also has pro-inflammatory characteristics (32, 33). Further, Del-Rio-Navarro et al. found a higher prevalence of metabolic

syndrome and higher blood pressure among obese asthmatic boys compared to obese non-asthmatic boys; however, this association was not apparent in asthmatic girls (28). These results are comparable to our findings of a link between high TC and blood pressure with atopic asthma in adolescent boys, but not in girls.

Stress is a known risk factor of asthma development (5, 6). High cortisol levels, a physiologic response of the HPA axis to stress, have been reported in children who experience long-term stress exposure (34). Children at high risk for allergic conditions also exhibit higher cortisol levels in response to stress (35, 36). In this study, a combination of high TC (as a measure of lipid metabolism) with high cortisol (as an indicator of stress) was a strong predictor of both prevalent and incident asthma in adolescent boys independent of their overweight status. This finding is in agreement with the notion of allostasis and stress response, as opposed to metabolic syndrome and obesity-related metabolic changes, as a risk factor for asthma.

Insulin resistance is a metabolic abnormality that is often reported in children and adults who have asthma (37, 38). It is not clear whether insulin resistance is a causal factor for asthma or they simply share a common pathogenesis such as high pro-inflammatory state. Nevertheless, a recent population-based prospective study supported the causal pathway by showing that insulin resistance precedes incident asthma-like symptoms in adults (39). We also demonstrated that high levels of fasting insulin, as an indicator of insulin resistance, are associated with

an increased risk of having non-atopic asthma in adolescent girls. Insulin resistance has been shown to increase during the puberty both in boys and girls (40, 41). As puberty starts at earlier ages in girls, the changes in peripheral insulin resistance (indicated by higher insulin levels) become noticeable at earlier ages in adolescent girls compared to boys (41), which might explain the sex difference observed in this study. Hypothetically, the link between insulin resistance and asthma would change in boys as they progress with their pubertal changes in an older age group compared to our study. Indeed, future studies following boys for a longer period of time as they progress through their puberty are required to test this hypothesis.

There are meaningful metabolic and hormonal differences in adolescents with and without asthma. Specifically, there is a tendency for adolescents with asthma to have a systemic pro-inflammatory state, which is characterized by high levels of TC and cortisol in boys, as well as increased insulin resistance in girls. Comparing the magnitude of the odds ratios, we demonstrated that the relative contribution of these biological risk factors in asthma development were comparable with the risk of having well-known and established asthma risk factors, such as positive history of atopy or food allergy in the child or positive parental history of allergic disease. Sex-dependent differences in the biomarkers of the inflammatory state are evident at this age group, some of which also reported in other studies (28). The underlying mechanisms for these differences are not fully explained and need further investigation. However, we hypothesized

that the sex differences may at least partially reflect pubertal development, which can impact insulin resistance (40, 41), blood pressure (42), and TC (43) in boys and girls, and these differences may be less dramatic once sexual maturation is fully achieved.

This study adds to the literature in four important ways. First, this is a community based follow-up study of asthma that enables an exploration of causal relationships. Although the follow-up period was somewhat limited to evaluate a chronic condition, we adjusted the prevalence analysis for existing asthma at age 9 to indicate the independent associations of studied biomarkers with new-onset adolescent asthma. Previous reports of asthma in childhood and adolescence are mainly cross-sectional studies where no sequential association could be established. Some of these studies were performed in high risk populations (i.e. obese or morbidly obese children), thereby limiting the generalizability of the results (28, 31, 38). Second, we performed a sub-analysis for the asthma phenotypes and detected different biological risk factors for prevalent atopic versus non-atopic asthma. Unfortunately, we could not perform the sub-analysis for new-onset asthma phenotypes due to the small number of new asthma cases. Future longitudinal studies with larger number of participants are recommended to investigate the causal association between abnormal biological markers and the onset of atopic and non-atopic asthma. Third, we investigated different risk factors among boys and girls by completing stratified analysis according to sex. The sex differences, possibly more apparent at this age group when pubertal

changes are ongoing, have been overlooked in many studies. Finally, we offer a single or combined index of two biological markers that have the potential to predict asthma development in adolescents. By reducing the number of biomarkers from the total AL index (reported in chapter 2), we sought to generate a convenient, parsimonious index that can easily be applied in clinical practice. More importantly, our results demonstrated that even sub-clinical, high levels of biomarkers such as cholesterol, blood pressure, cortisol and insulin increase the risk of developing asthma. Moreover, the biomarkers that predict asthma are different among adolescent boys versus girls, as well as atopic versus non-atopic asthma. These findings show that modifiable risk factors can be used by clinicians to predict asthma in adolescence. Future studies are recommended to test the potential protective effect of modifying these biological risk factors on asthma in the adolescents with and without well-known and established asthma risk factors.

Tables

Table 3-1 Cut-off points for the individual biomarkers

	Cut-off Points	
	Boys	**Girls**
Systolic Blood Pressure (mm-Hg)	≥116	≥116
Diastolic Blood Pressure (mm-Hg)	≥68	≥68
Waist-to-Hip Ratio	≥0.89	≥0.87
Fasting Serum Insulin (pmol/L)	≥56.00	≥71.25
Serum Total Cholesterol (mmol/L)	≥4.54	≥4.50
Serum HDL (mmol/L)	≤1.37	≤1.33
Serum DHEA-S (μmol/L)	≤1.32	≤1.20
Serum Cortisol (μg/dl)	≥193.40	≥154.85

HDL: high-density lipoprotein; DHEA-S: dehydroepiandrosterone sulphate;
AL: allostatic load

Table 3-2 Risk of asthma subsequent to exposure to high levels of physiologic biomarkers in boys (crude odds ratios)

	Prevalent Asthma			Incident Asthma (n=130)
	Asthma (n=185)	**Atopic Asthma (n=155)**	**Non-atopic Asthma (n=133)**	
SBP	1.01 (0.50 to 2.03)	1.09 (0.47 to 2.51)	1.07 (0.32 to 3.60)	0.88 (0.30 to 2.62)
DBP	0.88 (0.43 to 1.82)	1.36 (0.60 to 3.10)	0.49 (0.10 to 2.32)	0.80 (0.27 to 2.37)
WHR	0.58 (0.26 to 1.33)	0.28 (0.08 to 0.99)*	1.61 (0.51 to 5.10)	1.03 (0.34 to 3.08)
Insulin	1.05 (0.51 to 2.19)	0.79 (0.31 to 1.99)	1.68 (0.53 to 5.36)	1.03 (0.34 to 3.08)
TC	1.58 (0.81 to 3.11)	1.66 (0.75 to 3.68)	1.53 (0.48 to 4.86)	2.30 (0.88 to 6.00)
HDL	0.93 (0.46 to 1.88)	0.81 (0.33 to 1.96)	1.96 (0.64 to 5.95)	0.84 (0.28 to 2.49)
DHEA-S	2.27 (1.13 to 4.53)*	2.51 (1.12 to 5.63)*	1.50 (0.44 to 5.15)	1.30 (0.43 to 3.92)
Cortisol	1.06 (0.54 to 2.08)	0.79 (0.34 to 1.86)	2.25 (0.76 to 6.71)	1.36 (0.52 to 3.55)

* Statistically significant at $p<0.05$

Table 3-3 Risk of asthma subsequent to exposure to high levels of physiologic biomarkers in boys (adjusted odds ratios)

	Prevalent Asthma			Incident Asthma (n=130)[4]
	Asthma (n=185)[1]	Atopic Asthma (n=155)[2]	Non-atopic Asthma (n=133)[3]	
SBP	1.41 (0.46 to 4.28)	1.13 (0.27 to 4.73)	0.91 (0.19 to 4.27)	1.60 (0.43 to 6.01)
DBP	1.30 (0.45 to 3.78)	3.14 (0.75 to 13.21)	0.48 (0.08 to 2.79)	0.99 (0.31 to 3.21)
WHR	1.57 (0.44 to 5.60)	0.42 (0.06 to 3.09)	3.09 (0.63 to 15.11)	2.31 (0.57 to 9.30)
Insulin	0.92 (0.29 to 2.94)	0.33 (0.06 to 1.73)	1.46 (0.33 to 6.44)	1.40 (0.38 to 5.23)
TC	2.49 (0.93 to 6.65)	2.30 (0.63 to 8.37)	1.79 (0.43 to 7.50)	3.39 (1.10 to 10.45)*
HDL	1.29 (0.45 to 3.72)	0.75 (0.17 to 3.30)	2.37 (0.57 to 9.85)	1.34 (0.38 to 4.81)
DHEA-S	0.96 (0.35 to 2.67)	1.22 (0.33 to 4.45)	0.67 (0.13 to 3.50)	1.09 (0.32 to 3.68)
Cortisol	1.81 (0.66 to 4.99)	0.51 (0.12 to 2.21)	2.95 (0.77 to 11.29)	2.28 (0.77 to 6.78)

* Statistically significant at $p<0.05$

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

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

3 Final model is adjusted for existing asthma/ atopy, age and overweight status;

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

Table 3-4 Association of combined biomarkers and asthma in boys (crude and adjusted odds ratios)

		Crude OR	Adjusted OR
Prevalent Asthma[1]	**High TC and cortisol**	1.41 (0.76 to 2.62)	2.51 (1.01 to 6.17)* [1]
Prevalent Atopic Asthma[2]	**High TC and DBP**	1.78 (0.85 to 3.76)	5.12 (1.23 to 21.22)* [2]
Prevalent non-atopic Asthma[3]	**High TC and cortisol**	1.95 (0.86 to 4.46)	3.25 (1.07 to 10.03)* [3]
Incident Asthma[4]	**High TC and cortisol**	2.49 (0.94 to 6.58)	4.58 (1.42 to 14.73)* [4]

* Statistically significant at $p<0.05$

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

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

3 Final model is adjusted for existing asthma/ atopy, age and overweight status;

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

Table 3-5 Risk of asthma subsequent to exposure to high levels of physiologic biomarkers in girls (crude odds ratios)

	Prevalent Asthma			**Incident Asthma (n=110)**
	Asthma (n=142)	**Atopic Asthma (n=113)**	**Non-atopic Asthma (n=102)**	
SBP	0.79 (0.36 to 1.73)	0.80 (0.30 to 2.12)	0.74 (0.22 to 2.49)	0.95 (0.35 to 2.57)
DBP	0.68 (0.31 to 1.48)	0.53 (0.19 to 1.44)	0.80 (0.26 to 2.50)	0.81 (0.30 to 3.17)
WHR	0.58 (0.24 to 1.42)	0.55 (0.19 to 1.62)	0.54 (0.14 to 2.07)	0.52 (0.16 to 1.67)
Insulin	1.09 (0.49 to 2.45)	0.89 (0.32 to 2.49)	1.77 (0.58 to 5.40)	1.03 (0.36 to 2.94)
TC	1.74 (0.81 to 3.76)	2.79 (1.12 to 6.95)*	1.14 (0.33 to 3.94)	1.55 (0.56 to 4.32)
HDL	1.07 (0.50 to 2.28)	1.14 (0.45 to 2.85)	-	0.61 (0.21 to 1.80)
DHEA-S	1.96 (0.92 to 4.18)	2.44 (0.99 to 6.00)	1.77 (0.58 to 5.40)	1.03 (0.36 to 2.94)
Cortisol	0.91 (0.38 to 2.02)	1.01 (0.36 to 2.88)	0.53 (0.11 to 2.56)	0.58 (0.15 to 2.17)

* Statistically significant at $p<0.05$

Table 3-6 Risk of asthma subsequent to exposure to high levels of physiologic biomarkers in girls (adjusted odds ratios)

	Prevalent Asthma			Incident Asthma (n=110)[4]
	Asthma (n=142)[1]	Atopic Asthma (n=113)[2]	Non-atopic Asthma (n=102)[3]	
SBP	0.58 (0.17 to 1.93)	1.50 (0.31 to 7.22)	0.30 (0.04 to 2.01)	1.04 (0.34 to 3.18)
DBP	0.70 (0.23 to 2.11)	0.72 (0.15 to 3.38)	0.92 (0.19 to 4.50)	0.97 (0.34 to 2.81)
WHR	0.71 (0.10 to 2.88)	0.89 (0.16 to 5.04)	0.21 (0.02 to 2.04)	0.48 (0.13 to 1.75)
Insulin	2.57 (0.65 to 10.25)	0.67 (0.10 to 4.49)	13.72 (1.27 to 148.48)*	1.64 (0.49 to 5.44)
TC	1.07 (0.33 to 3.44)	3.02 (0.76 to 11.92)	1.16 (0.18 to 7.62)	1.78 (0.59 to 5.39)
HDL	0.95 (0.27 to 3.30)	0.60 (0.13 to 2.88)	2.84 (0.47 to 17.07)	0.68 (0.20 to 2.35)
DHEA-S	1.40 (0.42 to 4.59)	1.06 (0.26 to 4.27)	2.12 (0.42 to 10.60)	1.10 (0.35 to 3.49)
Cortisol	0.63 (0.18 to 2.16)	0.60 (0.07 to 4.85)	2.22 (0.29 to 17.15)	0.62 (0.16 to 2.39)

* Statistically significant at $p<0.05$

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

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

3 Final model is adjusted for existing asthma/ atopy, age and overweight status;

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

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CHAPTER 4 - CONCLUSION

The bidirectional association between asthma and chronic stress has increasingly attracted researchers' attention in recent years (1). Stress, from the prenatal period to the adolescent years, is a risk factor both for asthma initiation and exacerbation. Prenatal maternal stress can modulate the development of the fetal innate and adaptive immune system, which can enhance susceptibility to asthma or other atopic disorders early in life (2). The effect of maternal prenatal anxiety extends beyond the infancy period into childhood and adolescence (3). Growing from childhood to adolescence, the social lives of boys and girls expand outside the family setting and become increasingly influenced by their local environments, which can impact their health. For example, adolescents living in neighborhoods with high domestic violence are at increased risk of asthma and asthma-related morbidity (4).

A number of underlying mechanisms may link stress and asthma in childhood including over or under reactivity of the HPA, autonomic and the immune system (5). Prenatal and early life stress result in chronic dysregulation of the HPA axis and ANS function, which in turn alters the immune system regulation and increases risk of asthma development (5). These shifts in multiple organ activities in response to prolonged stress exposure are consistent with the concept of allostasis and AL (6). With this notion in mind, we believed that a study of AL

and its relation to asthma development in children and adolescents was both timely and important. The objectives of this thesis were to: (a) Examine whether high AL in childhood increased the likelihood of asthma prevalence and incidence in adolescence (chapter 2); (b) Examine whether individual or a subset of AL measures predicted the development of prevalent or incident asthma in adolescents (chapter 3).

Main Findings

In chapter 2, we investigated the association between AL with asthma and asthma phenotypes in a sample of Canadian adolescents of 11 to 14 years old. A direct association between high AL index and asthma development in adolescent boys was found. Specifically, with high AL at age 10.5 years, boys were four times more likely to have asthma at age 12.5 years compared to those with low AL index. In addition, for each one point increase in AL score, there was a 60% increased likelihood of boys having prevalent asthma at 12.5 years old. These associations were independent of age, ethnicity, overweight status, positive parental history of asthma and existing asthma/atopy at 9 years old. High AL was also associated with an increased risk of prevalent atopic and non-atopic asthma as well as incident asthma in adolescent boys. Conversely, high AL index did not show a statistically significant association with either prevalent (atopic or non-atopic) or incident asthma in adolescent girls. This was the first study to

investigate AL index, as an indicator of chronic stress exposure and asthma in adolescents both cross-sectionally and longitudinally.

In chapter 3, we examined the association between the individual biomarkers of AL and prevalent and incident asthma in order to identify a parsimonious model to predict asthma in adolescents. A positive association was found between high TC and cortisol levels at 10.5 years old and both incident and prevalent asthma (non-atopic asthma) in adolescent boys of 12.5 years old. Moreover, with both high TC and diastolic blood pressure at 10.5 years old, boys were five times more likely to have atopic asthma in adolescence than their peers with low levels of these markers. In girls, on the other hand, the chance of having non-atopic asthma at 12.5 years old was considerably higher (~14 times) among those with high insulin levels at age 10.5 compared to their peers with low insulin.

Asthma Phenotypes

Although AL index was a risk factor for both atopic and non-atopic asthma in adolescent boys (chapter 2), when we investigated the individual components of the AL index, different biological risk factors emerged for each asthma phenotype (chapter 3). It has long been appreciated that childhood asthma is highly variable both in its clinical presentation and natural history (7). Diverse genetic and environmental exposures have been recognized for different asthma phenotypes (8). For example, genetic predisposition is associated with higher risk of atopic

conditions (9), whereas obesity has shown stronger associations with non-atopic asthma (10).

To date, few studies have investigated the differences in biological measures (i.e., lipid profile, insulin resistance) that may account for asthma phenotype presentations in children; findings from those studies have been inconsistent (11-13). For example, in a cross-sectional report of 10-year old children, higher concentrations of apolipoprotein Al (the primary lipoprotein affiliated with HDL cholesterol) were associated with a higher prevalence of non-atopic wheeze; on the other hand, apolipoprotein B (the primary lipoprotein associated with VLDL and LDL cholesterol) was not associated with symptoms of asthma or allergy (11). Conversely, a cohort study of 5-year old children found similar concentrations of HDL in children with and without atopic sensitizations (13). Another report showed that high levels of TC during infancy and throughout childhood have a protective effect against allergic sensitization in early adulthood (13).

The contradictory findings regarding biological markers and asthma phenotypes are not limited to lipid profiles; studies of glucose metabolism, hyperglycemia and insulin resistance and asthma phenotypes have also demonstrated conflicting results. For example, studies have shown a lower prevalence of atopy and asthma-like symptoms among children who have type 1 diabetes compared to their non-diabetic siblings (14), which suggests an inverse association between

hyperglycemia and atopy/asthma. However, recent studies have focused on asthma and risk factors that are traditionally linked to type 2 diabetes (i.e., obesity and insulin resistance) (12, 15-17). For example, higher levels of insulin resistance have been reported in children and adults who have asthma (especially atopic asthma) compared to their peers without asthma (12, 15-17).

The reason for these conflicting results is not entirely clear. Despite our knowledge of their diverse origins and natural course of different asthma phenotypes, they have not been thoroughly investigated in epidemiological and clinical research. As discussed in a recent review by Spycher et al., the number of publications on pediatric asthma phenotypes has increased only recently from less than five publications annually in the mid-1990s to about 25 in 2005 and above 40 in recent years (7). This explains part of the ongoing uncertainty and conflicting body of literature about asthma phenotypes since the field is still emerging. Moreover, these different findings may also be a function of the complex etiology of the disease (i.e., genetics, environment gene-environment interactions, and intra-personal factors) (9, 18). Additional longitudinal research with larger populations of children and adolescents will help to explain the underlying mechanisms for these differences between studies.

We observed an increased risk of having both asthma phenotypes in adolescent boys who had high TC levels. In girls, a high degree of insulin resistance was associated with enhanced risk of non-atopic asthma. These results tend to

contradict some of the findings reported in the aforementioned studies (12, 13, 17). Considering the distinct origins of atopic versus non-atopic asthma, it is pivotal to account for phenotype-specific risk factors in the analysis by adjusting for a number of covariates that are related to each asthma phenotype. Unlike past studies, we performed independent explanatory data analysis for each asthma phenotype and the final models were tailored for different covariates specific to atopic versus non-atopic asthma, which we believe help to explain our findings.

Sex Differences

Sex-related differences in asthma risk factors have been reported (19-21). For example, the link between family or environmental stress and childhood asthma has been reported to be equal in both sexes (2), stronger in boys (19), and stronger in girls (20). In chapter 2, AL was associated with asthma in boys only. We speculated that the main explanation for the observed sex differences is related to pubertal changes and differences in sex hormones between boys and girls. Pubertal changes start at earlier ages in girls compared to boys and sex hormones (i.e., estradiol) have been shown to increase the DHEA/cortisol ratio, both of which are the components of the AL index (22). Early onset menarche, on the other hand, has been speculated to both increase (23) and decrease (24) risk of developing asthma in adolescent girls. In summary, post-menarcheal girls have lower total AL scores secondary to the effect of estradiol on DHEA and cortisol,

and menarche has an undetermined effect on the development of new-onset asthma in girls. Based on these observations, we recommend future studies be conducted with older girls to determine the utility of the AL index in measuring chronic stress in menstruating girls and examine its relation to asthma development in these individuals.

In addition, sex-specific biological risk factors were identified in chapter 3. Our findings in boys were similar to another study that reported a higher prevalence of metabolic syndrome in male adolescents with asthma compared to their peers without asthma (21). This association was not evident in girls either in the above-mentioned study or in our study. Pubertal development also affects the TC (25), blood pressure (26) and insulin resistance (27, 28) in adolescents. For example, boys and girls have higher peripheral insulin resistance as they enter puberty (28). Girls tend to experience puberty-related insulin resistance at earlier ages compared to boys (28), which may explain the larger magnitude of the association observed in girls in this study. Further studies investigating adolescents at older age groups are required to test this hypothesis.

Validity of Study Measures

Outcome Variable- Asthma

Asthma diagnosis was confirmed both at age 9 years (wave 1) and the follow-up visit at age 12.5 years (wave 3) using the following two measures: (a) All children were examined by a pediatric allergist for clinical diagnosis according to previously-validated standards (29); (b) Participants underwent skin prick tests to the 14 common aforementioned allergens to define their asthma phenotype. Children with a positive skin prick test to at least one allergen were categorized as atopic.

Exposure Variable- Allostatic Load

AL index has been measured in previous studies using different markers and scoring systems (30-35). However, a similarity of these scoring systems is the inclusion of a number of organ activities including the cardiovascular system, body fat distribution, glucose and lipid metabolism, and the HPA axis. To test the impact of chronic stress on asthma development, we used a comprehensive and extensive AL scoring system that included the following eight markers:

1-2. Resting SBP and DBP, as indicators of the cardiovascular system activity;

3. WHR to represent body fat distribution;

4. Fasting serum glucose (chapter 2) and fasting insulin (chapter 3) levels to assess the glucose metabolism;

5-6. Fasting serum TC and HDL as indicators of lipid metabolism;

7. Serum DHEA-S which acts as a functional antagonist of HPA axis;

8. Fasting serum cortisol as a marker of HPA axis activity.

Measurement methods and the scoring system are described in chapter 2. Collectively, these variables provide a composite index of AL as a function of chronic stress and metabolic health. This scoring system has been used extensively in other studies both in children and adults with different combinations of AL components (30-35). However, this was the first study to examine its utility in the context of pediatric asthma.

Based on existing literature, the following measures of different organ activities were included in the allostatic response (30-35):

Cardiovascular system: Similar to most studies, resting systolic and diastolic blood pressure were included (30, 32-35).

Body fat distribution: Available and feasible measures of body fat distribution used for AL scoring in previous studies are BMI, waist circumference and WHR.

As BMI is not always the best measure of overweight in children (36), WHR was selected as an indicator of central adiposity for this project (32, 34, 35).

Lipid metabolism: Previous studies have included TC (34, 35) in addition to HDL (31-35) in their AL scoring. The same measures were used in this project to be consistent with existing literature.

Glucose metabolism: As per other studies, fasting serum glucose was included to represent the carbohydrate metabolism in chapter 2 (31, 32, 34). However, we administered fasting insulin level as an indicator of insulin resistance in chapter 3 (37, 38).

HPA axis: Fasting serum cortisol level is not the best measure for assessing the HPA axis function. The provocative testing such as the Corticotropin-Releasing Hormone (CRH) test or the insulin-hypoglycemia test (IHT) are more specific tests for the function of the entire HPA axis (39), but they are less commonly used for the research purposes. Therefore, the overnight urinary excretion of cortisol, salivary cortisol or fasting serum level of cortisol has been employed as substitutes in a variety of studies (31-34, 40, 41). DHEA-S, which acts as a functional antagonist to cortisol, has also been incorporated in previous studies (32, 35).

ANS: The urinary excretions of epinephrine and norepinephrin have been considered in some studies as indicators of ANS activity for AL assessment (30,

32, 33); however, similar to some other studies (31, 34, 35), we have not included these measures.

Measure of Cortisol

Elevated, stress-provoked cortisol levels have been reported in infants and children at risk for allergic diseases due to a positive family history (42, 43). On the other hand, low cortisol levels (in response to stress) have been observed in children who have asthma (44). Therefore, we considered both high and low levels of cortisol as components of the AL index in chapter 2. However, in chapter 3, we examined the association of high cortisol levels with asthma in adolescents, which is a simpler parsimonious approach that is likely to be more useful in clinical practice. Indeed, the main findings for the analysis of individual biomarkers (chapter 3) were comparable when we examined either high or low cortisol levels (data not shown).

Study Novelty

AL is a relatively new concept that was first introduced in 1988 by Sterling and Eyer (45) and operationalized later in the 1990s and early 2000s (6, 46). Most studies of AL index have been performed in adults (47-51), nevertheless, the index has been also measured in a few pediatric studies (52-54). High AL scores in childhood has been correlated with the total numbers of days absent from

school due to any kind of illnesses (55) and impaired memory later in early adulthood (30). Another group of studies have merely mentioned the AL concept to explain their findings, such as psychological and neurodevelopmental disorders in stressed children and adolescents, but these hypotheses have not yet been examined empirically (56-58). Therefore, we believe that the novel findings presented in this thesis advance our understanding of the link between the AL index and asthma in children and adolescents.

Clinical Relevance

Asthma is one of the most common chronic diseases in adolescents (59), yet risk factors that contribute to asthma development in this age group have not been thoroughly studied. This chronic condition has a considerable impact on the lives of both affected children and their families (60). Therefore, it is extremely important to investigate whether the development of asthma can be predicted based on modifiable risk factors, which will help to inform prevention approaches. We demonstrated that sub-clinical levels of biomarkers such as cholesterol, blood pressure, cortisol and insulin can increase the risk of developing asthma. Moreover, by comparing the magnitude of the odds ratios, we demonstrated that the relative contribution of these biological risk factors in asthma development were equivalent to the risk of having well-known and established asthma risk factors, such as positive history of atopy or food allergy in

the child or positive parental history of allergic disease. This research provides a measure of simple physical and biomedical components that can be routinely measured in everyday practice, as markers to identify children at risk for developing asthma in adolescence and provide clinicians with an opportunity to develop and apply interventions aimed at asthma prevention.

Methodological Considerations

The following issues highlight several methodological considerations that are relevant to the current thesis:

Utilizing Secondary Data

This study was performed using secondary data from the SAGE project, which was specifically designed as a birth cohort study and a nested case-control sub-section to investigate the early life origins of childhood asthma. The follow up data used to achieve the research objectives of this thesis were not gathered as part of the cohort design, but were collected from participants from the case-control study. As a result, the prevalence of asthma was higher among the participants of this project compared to the general population (secondary to the case-control design), while the asthma incidence was closer to general population (result of the prospective nature of the follow-up data). Therefore, we reported the results of our analysis in two different categories: prevalence analysis which is

close to the case-control design and incidence analysis which represents prospective section of our project.

In addition, as AL was not one of the main focuses of the SAGE study, the urinary excretion of epinephrine and norepinephrine (indicators of ANS activity) were not measured in the study participants. Thus, we could not include a measure of ANS activity in our AL index. This is an important factor to consider because ANS response is one of the organ activities involved both in acute and chronic response of the organism to stress and. several studies have included these measures as components of the AL index.

Direct Measure of Stress

AL index has been used as a measure of long-term stress exposure in previous studies both in adult and pediatric populations. As discussed earlier, a number of studies have explored the use of AL index as a tool to assess cumulative effects of stress in children (52-54, 61). Thus, in the present project, no direct measure was employed to report on acute or chronic stress experienced by the study participants or their families. In other words, our study builds on the literature to suggest that the AL index represents incremental effects of long-term stress. Moreover, it advances the field by examining this index as a predictor of an adverse health outcome (i.e. asthma) in adolescents.

Sample Size

Although there was an adequate number of participants in the prevalent analysis (Appendix 1), the number of children with atopic or non-atopic asthma was smaller in the analysis of asthma phenotypes, which decreased the power of our analysis. In addition, the number of new asthma cases was small, making the sub-group study of asthma phenotypes unfeasible for the incidence analysis. Knowing that most individuals with non-atopic asthma will outgrow their asthma by adolescence (62), we treated those children with non-atopic asthma at 9 years as healthy children and did not exclude them in the analysis of new-onset asthma at 12.5 years old. Thus, we increased the number of participants in the analysis of new-onset asthma; at the same time, we introduced a source of error in the definition of incident asthma cases in our study. This is considered a reasonable compromise because less than 45% of children who had non-atopic asthma at age 9 in this project continued to have the same condition by age 12.5 years (data not shown).

Association *versus* Causation

We investigated the association of AL index, as well as its biological components with both prevalent and incident asthma in adolescents. Considering the sample size limitation for the analysis of incident asthma, our major findings were reported as prevalent asthma and asthma phenotypes. Hence, we could report on associations rather than causations regarding most of our findings. Although we

reported similar findings for the incidence analysis, in addition to adjusting our prevalent analysis for existing asthma in order overcome this limitation, we can only speculate on "cause and effect" associations.

Future Directions

The present project was the first study to examine the association of total AL index and its biological components in childhood with prevalent and incident asthma in adolescence. Considering the limitations of this project, future studies involving larger numbers of children in different age groups are recommended to further investigate these associations.

Sex differences were evident in our findings, although the underlying mechanisms for these differences are not clear and need to be explored. Nevertheless, we speculated that pubertal development may be at least partially responsible for these differences. Future studies are needed to follow children for a longer period of time until the completion of puberty in order to examine pubertal effects on asthma.

We included modifiable biological risk factors (i.e., total cholesterol, blood pressure, cortisol and insulin) that increase the chances of having asthma in adolescents. In order for these findings to be implemented by clinicians to predict asthma in adolescents, future studies are recommended. To determine

generalizability and clinical relevance, these studies should include testing the potential protective effects of modifying these biological risk factors on asthma in adolescents with and without established risk factors for asthma.

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APPENDIX 1- METHODS

Data Source

This thesis reports on a prospective follow-up of children enrolled in the SAGE study. The detailed methodology of the SAGE is described elsewhere (1). In summary, SAGE started as a birth cohort study created from the provincial health care registry of 13980 children born in Manitoba in 1995 (Figure A1-1). In 2002, a mail-out survey was sent to the households of all of these children who were still living in the province, asking about the health outcomes and home environment exposures. First-Nations communities were approached separately. A total number of 3598 of the surveys were returned. Subsequently, a nested case-control study was designed with the intent of recruiting children with (n=400) and without parent-reported asthma (n=400, random selection of respondents in rural and urban areas) at the age 7 to 10 years (Wave 1; mean age: 9 years). Following consent from parents, children were visited by a pediatric allergist to confirm their asthma diagnosis based on the 2003 Canadian Pediatric Asthma Consensus Guidelines (2).

Consequently, 723 children (246 with physician-diagnosed asthma and 477 without asthma) were enrolled. These children underwent skin prick testing to common allergens including tree pollen, weed pollen, ragweed, grass pollen, *Alternaria*, *Cladosporium*, *Penicillium*, house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, cockroach, cat, dog, feathers and

peanut to define their asthma phenotype as atopic versus non-atopic asthma. A blood sample was obtained from all children for the measures of biological markers. Parents were asked to fill out a detailed survey on their current and past home environment as well as their medical history.

Children involved in the SAGE nested case-control study were followed for a total period of four years and revisited at two time points at ages 9 to 12 (Wave 2; mean age: 10.5 years) and 11 to 14 years (Wave 3; mean age: 12.5 years) (Figure A1-2). At wave 2 (n= 569), blood samples were collected to measure the five biomarkers that were used as biological components of AL index. Blood pressure and anthropometrics were also measured and used as physical components of the AL index. A total number of 439 children had valid measures on all eight components of the AL index and were included in this thesis. However, the total number of participants in the analysis of individual biological markers in chapter 3 was slightly lower (n=407) because there were a number of missing values on the measure of insulin. Children were asked to report their puberty stage at 10.5 years (wave 2) using previously-validated Tanner stage images (3, 4). After the follow-up period, study participants (n=352, chapter 2; n=327, chapter 3) were revisited by the same pediatric allergist at the age of 11 to 14 years (Wave 3; mean age: 12.5 years). At this visit, asthma diagnosis and phenotypes were confirmed following the same criteria and procedures as described at age 9 years (wave 1).

Sample Size Calculation

In order to calculate the sample size, α error was fixed at 0.05 and power was set at 80%. Using the following equation (5):

$$n = \frac{r+1}{r(\lambda-1)^2\,\pi^2}\left[z_\alpha\sqrt{(r+1)\,p_c\,(1-p_c)} + z_\beta\sqrt{\lambda\pi\,(1-\lambda\pi)+r\pi(1-\pi)}\,\right]^2$$

Where:

$\alpha = 0.05$ (2.5% each side for 2-sided analysis); $Z_{\alpha=}$ 1.96

Power = 80%; $Z_{\beta=}$ 0.84

$\lambda = \pi_1/\pi_2$, where π_1 is the prevalence of asthma in exposed group and π_2 is the prevalence of asthma in reference group. In this study, we used $\lambda=2$ based on a previous study that indicated a 2-fold increase in the chances of having asthma in children who have chronically been exposed to psychological stress (6).

$r = n_1/n_2$ where n_1 is the total number of individuals in the exposure group. In this study, children who fell under the highest quartile of the total AL scores within the population under the study were considered the high AL group or the exposure group. Therefore, we had a proportion of 1/3 or 0.67 between the number of children in the exposed and unexposed groups.

π= 0.13, the prevalence of asthma in the general population of the same age group as participants of this study (7).

P_c= 0.182 as per this equation

$$p_c = \frac{\pi(r\lambda + 1)}{r + 1}$$

Based on these calculations, a minimum of 290 participants were required for this project.

Reducing Bias

Measurement Bias

Outcome variable: Several definitions have been employed for asthma diagnosis in other studies, including physician diagnosis, medication use on health records, BHR and parental report (8). In this project, asthma diagnosis was confirmed at two visits (9 and 12.5 years old) using three measures: (a) All the children involved in the study were examined by a pediatric allergist for clinical diagnosis (2), (b) All children undertook a Methacholine challenge test, which gives a valid measure of presence of BHR, and (c) Children underwent skin prick tests to 14 common allergens as mentioned above. Children with a positive skin prick test to at least one allergen were labeled atopic. Consequently, we developed a robust measure for asthma diagnosis in this study.

Explanatory Variable: As discussed earlier, AL index has been measured in various studies using different combinations of variables. To have a valid measure of AL index, we used similar biological components as measured in other studies (10-12, 17-19). However, we did not have a marker of ANS activity in our AL components, which was a limitation of this study (chapter 4).

Confounding Bias

In order to have a comprehensive model for asthma prediction, we accounted for possible confounding factors that might influence the association between AL index and asthma development. We considered well-characterized risk factors for asthma such as age, sex, ethnicity, overweight, positive history of food allergy, and positive parental history of atopy or asthma as possible confounding factors in our models (20-23). Table A1-1 shows all the study measures and source of data collection for each measurement.

Tables

Table A1-1 Variable measures

Measure	Operational Definition	Data Source
Explanatory Variables		
AL Score	SBP (mm-Hg) DBP (mm-Hg) WHR Fasting Glucose (mmol/L) Fasting Insulin (pmol/L) TC (mmol/L) — Upper quartile HDL (mmol/L) DHEA-S (μmol/L) — Lower quartile Fasting cortisol (µg/dl) — Upper and lower 12.5 percentile	Fasting blood sample obtained during the clinic visit at 9-12 years old/ blood pressure and anthropometrics measured at the same visit
Sex	Male/Female	Parent questionnaire
Age	Age in years	
Ethnicity	Caucasian/ Other (First-Nation, Métis, Asian, other)	Parent/child questionnaire
History of Food Allergy	Yes/No	Physician visit at age 7-10
Parental History of Atopy or Asthma	History of atopy or asthma present in the mother, father or siblings as Yes/No	Parent questionnaire
Dependent Variable		
Asthma Diagnosis	Yes/No	Diagnosis by a pediatric allergist/ Methacholine challenge test obtained during clinic visits at ages 7-10 and 11-14

Figures

Figure A1-1 Design of the SAGE cohort and case-control study

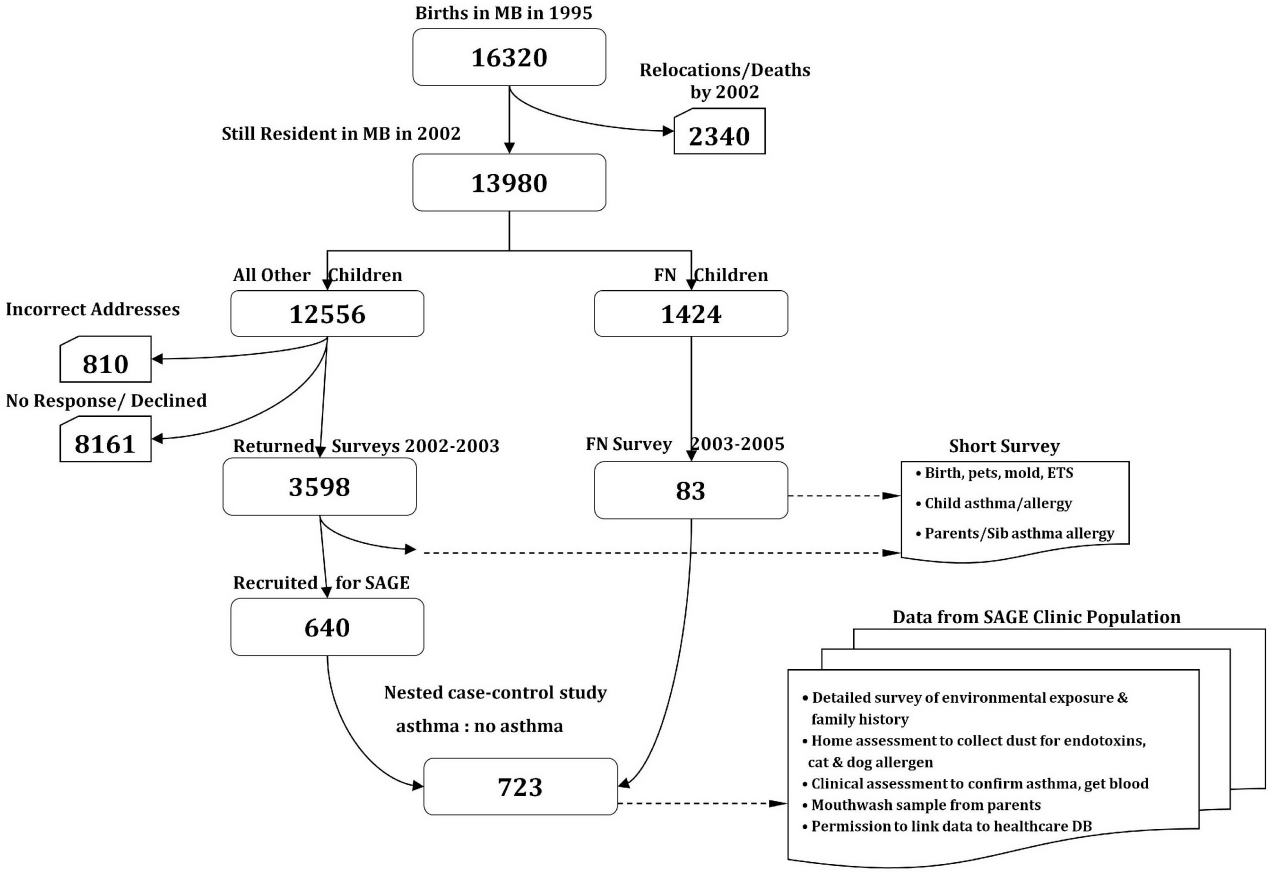
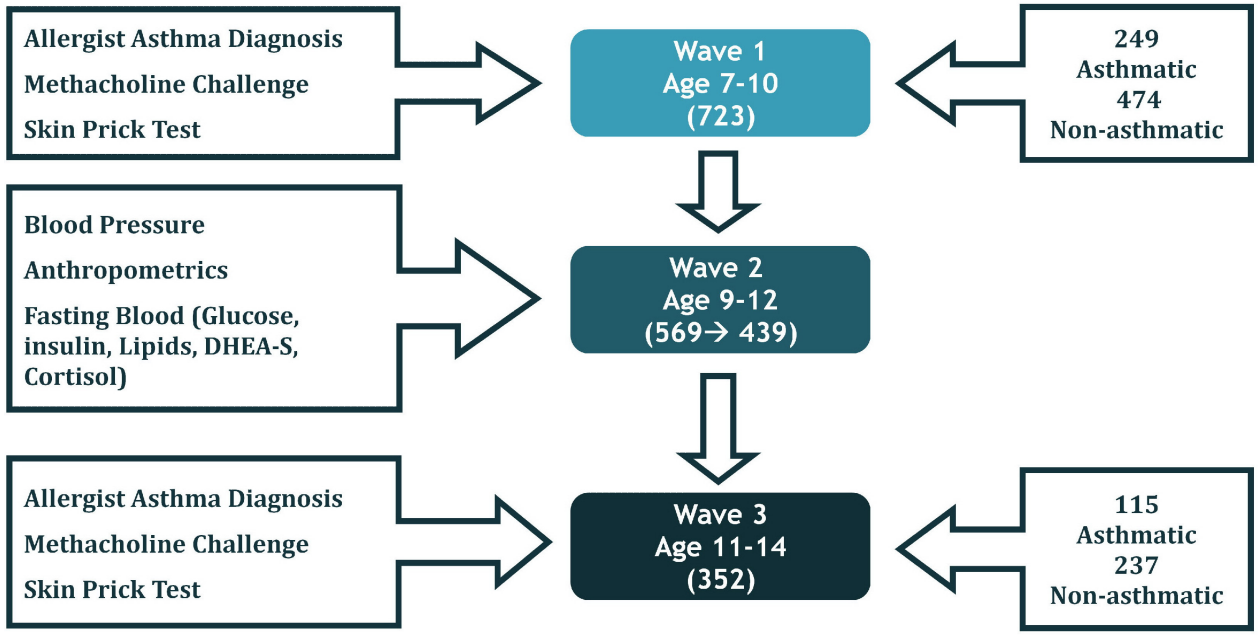


Figure A1-2 Follow-up visits of the SAGE case-control participants



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APPENDIX 2- TABLES

Additional Tables for Chapter 2

Table A2-1 Distribution allostatic load index among study participants

		All Children		Prevalent Asthma									Incident Asthma		P value
		n	%	All		P value	Atopic Asthma		P value	Non-atopic Asthma		P value	n	Prevalence	
				n	Prevalence		n	Prevalence		n	Prevalence				
All Children	**AL**														
	Low	239	67.9%	78	32.6%	0.98	46	23.6%	0.79	22	12.9%	0.93	36	20.0%	0.50
	High	113	32.1%	37	32.7%		24	25.0%		11	13.3%		13	16.5%	
Girls	**AL**														
	Low	99	64.7%	36	36.4%	0.40	18	24.3%	0.93	14	20.0%	0.17	22	27.5%	0.07
	High	54	35.3%	16	29.6%		12	25.0%		4	10.0%		5	12.8%	
Boys	**AL**														
	Low	140	70.4%	42	30.0%	0.44	28	23.1%	0.78	8	7.9%	0.13	14	14.0%	0.38
	High	59	29.6%	21	35.6%		12	25.0%		7	16.3%		8	20.0%	

Prevalent Asthma

Table 0-2 Risk of asthma subsequent to exposure to risk factors (crude odds ratios)

	Prevalent Asthma	
	Boys	**Girls**
AL		
Low	1.00	1.00
High	1.29 (0.68 to 2.46)	0.74 (0.36 to 1.50)
Existing Asthma		
No	1.00	1.00
Yes	19.64 (10.09 to 38.25)*	17.87 (8.69 to 36.77)*
Atopy		
No	1.00	1.00
Yes	1.38 (1.23 to 1.55)*	1.71 (1.40 to 2.09)*
Age	0.49 (0.30 to 0.82)*	0.39 (0.22 to 0.69)*
Parental History of Asthma		
No	1.00	1.00
Yes	1.94 (1.10 to 3.41)*	1.36 (0.73 to 2.53)
Ethnicity		
Caucasian	1.00	1.00
Other	0.90 (0.48 to 1.70)	1.55 (0.81 to 2.96)
Overweight		
No	1.00	1.00
Yes	0.87 (0.49 to 1.54)	1.13 (0.59 to 2.17)

* Statistically significant at $p<0.05$

Table 0-3 Risk of asthma phenotypes subsequent to exposure to risk factors (crude odds ratios)

	Prevalent Atopic Asthma		Prevalent Non-atopic Asthma	
	Boys	**Girls**	**Boys**	**Girls**
AL				
Low	1.00	1.00	1.00	1.00
High	1.11 (0.51 to 2.41)	1.04 (0.45 to 2.41)	2.26 (0.76 to 6.69)	0.44 (0.14 to 1.46)
Existing Asthma				
Healthy	1.00	1.00	1.00	1.00
Non-atopic Asthma	2.79 (0.54 to 14.52)	1.70 (0.19 to 15.40)	17.85 (5.10 to 62.49)*	17.82 (6.13 to 51.78)*
Atopic Asthma	35.43 (14.73 to 85.23)*	61.60 (20.84 to 182.12)*	5.27 (1.31 to 21.15)*	1.40 (0.16 to 12.35)
Age	0.53 (0.29 to 0.97)*	0.41 (0.20 to 0.81)*	0.20 (0.10 to 0.83)*	0.43 (0.19 to 0.95)*
Parental History of Asthma				
No	1.00	1.00	1.00	1.00
Yes	1.90 (0.98 to 3.69)	1.46 (0.68 to 3013)	0.96 (0.33 to 2.77)	0.95 (0.36 to 2.48)
Food Allergy				
No	1.00	1.00	1.00	1.00
Yes	8.34 (3.65 to 19.07)*	6.83 (2.44 to 19.13)*	-	-
Ethnicity				
Caucasian	1.00	1.00	1.00	1.00
Other	0.76 (0.34 to 1.70)	1.58 (0.73 to 3.44)	2.08 (0.72 to 5.99)	1.44 (0.54 to 3.82)
Overweight				
No	1.00	1.00	1.00	1.00
Yes	0.79 (0.40 to 1.58)	0.95 (0.41 to 2.18)	1.22 (0.44 to 3.38)	1.64 (0.64 to 4.19)

* Statistically significant at $p<0.05$

Table 0-4 Risk of asthma subsequent to high AL in boys (adjusted odds ratios)

	Adjusted OR (Model 1)	**Adjusted OR (Model 2)**
AL		
Low	1.00	1.00
High	2.70 (1.01 to 7.23)*	4.01 (1.37 to 11.80)*
Existing Asthma		
No	1.00	1.00
Yes	15.83 (6.35 to 39.44)*	16.57 (6.50 to 42.22)*
Atopy		
No	1.00	1.00
Yes	1.18 (1.00 to 1.39)*	1.20 (1.01 to 1.41)*
Age	0.69 (0.29 to 1.60)	0.65 (0.28 to 1.53)
Parental History of Asthma		
No	1.00	1.00
Yes	2.70 (1.12 to 6.47)*	2.85 (1.17 to 6.98)*
Ethnicity		
Caucasian	1.00	1.00
Other	0.69 (0.20 to 2.35)	0.77 (0.22 to 2.67)
Overweight		
No	-	1.00
Yes	-	0.36 (0.13 to 1.02)

* Statistically significant at $p<0.05$

Table 0-5 Risk of asthma subsequent to high AL in girls (adjusted odds ratios)

	Adjusted OR (Model 1)	**Adjusted OR (Model 2)**
AL		
Low	1.00	1.00
High	0.33 (0.11 to 1.04)	0.32 (0.10 to 1.02)
Existing Asthma		
No	1.00	1.00
Yes	14.92 (5.14 to 43.30)*	14.94 (5.15 to 43.34)*
Atopy		
No	1.00	1.00
Yes	1.53 (1.18 to 1.97)*	1.53 (1.18 to 1.98)*
Age	0.76 (0.30 to 1.95)	0.76 (0.30 to 1.93)
Parental History of Asthma		
No	1.00	1.00
Yes	0.73 (0.27 to 1.99)	0.73 (0.27 to 1.99)
Ethnicity		
Caucasian	1.00	1.00
Other	2.60 (0.80 to 8.42)	2.55 (0.78 to 8.27)
Overweight		
No	-	1.00
Yes	-	1.21 (0.39 to 3.80)

* Statistically significant at $p<0.05$

Table 0-6 Risk of non-atopic asthma subsequent to high AL in boys (adjusted odds ratios)

	Adjusted OR (Model 1)	**Adjusted OR (Model 2)**
AL Low High	 1.00 3.72 (1.03 to 13.48)*	 1.00 3.82 (0.91 to 16.08)
Existing Asthma Healthy Non-atopic asthma Atopic Asthma	 1.00 18.55 (4.20 to 81.98)* 3.28 (0.72 to 14.88)	 1.00 18.23 (4.07 to 81.67)* 3.23 (0.71 to 14.73)
Age	0.33 (0.10 to 1.09)	0.34 (0.10 to 1.10)
Overweight No Yes	 - -	 1.00 0.98 (0.23 to 4.13)

* Statistically significant at p<0.05

Table 0-7 Risk of non-atopic asthma subsequent to high AL in girls (adjusted odds ratios)

	Adjusted OR (Model 1)	**Adjusted OR (Model 2)**
AL Low High	 1.00 0.45 (0.11 to 1.78)	 1.00 0.33 (0.07 to 1.52)
Existing Asthma Healthy Non-atopic asthma Atopic Asthma	 1.00 15.38 (4.19 to 56.42)* 1.39 (0.14 to 13.89)	 1.00 15.86 (4.24 to 59.30)* 1.54 (0.15 to 15.76)
Age	0.91 (0.33 to 2.51)	0.88 (0.32 to 2.40)
Overweight No Yes	 - -	 1.00 2.15 (0.53 to 8.68)

* Statistically significant at p<0.05

Table 0-8 Risk of atopic asthma subsequent to high AL in boys (adjusted odds ratios)

	Adjusted OR (Model 1)	**Adjusted OR (Model 2)**
AL		
Low	1.00	1.00
High	2.30 (0.62 to 8.56)	4.58 (1.04 to 20.16)*
Existing Asthma		
Healthy	1.00	1.00
Non-atopic asthma	3.86 (0.54 to 27.69)	6.59 (0.85 to 50.97)
Atopic Asthma	50.57 (13.08 to 195.59)*	73.61 (15.89 to 340.94)*
Age	0.60 (0.21 to 1.73)	0.53 (0.18 to 1.57)
Parental History of Asthma		
No	1.00	1.00
Yes	4.21 (1.19 to 23.66)*	5.25 (1.38 to 20.01)*
Food Allergy		
No	1.00	1.00
Yes	5.67 (1.36 to 23.66)*	6.68 (1.51 to 29.5)*
Overweight		
No	-	1.00
Yes	-	0.18 (0.04 to 0.80)*

* Statistically significant at $p<0.05$

Table 0-9 Risk of atopic asthma subsequent to high AL in girls (adjusted odds ratios)

	Adjusted OR (Model 1)	**Adjusted OR (Model 2)**
AL		
Low	1.00	1.00
High	0.63 (0.17 to 2.36)	0.55 (0.14 to 2.13)
Existing Asthma		
Healthy	1.00	1.00
Non-atopic asthma	3.22 (0.30 to 34.79)	3.78 (0.34 to 42.64)
Atopic Asthma	45.27 (11.52 to 178.00)*	50.94 (12.18 to 213.04)*
Age	1.01 (0.29 to 3.52)	0.86 (0.24 to 3.08)
Parental History of Asthma		
No	1.00	1.00
Yes	0.88 (0.23 to 3.33)	0.76 (0.19 to 2.99)
Food Allergy		
No	1.00	1.00
Yes	7.32 (0.87 to 61.32)	7.77 (0.89 to 68.04)
Overweight		
No	-	1.00
Yes	-	2.14 (0.46 to 10.05)

* Statistically significant at $p<0.05$

Incident Asthma

Table 0-10 Prevalence of high allostatic load and incident asthma among covariates

	All Children		Children with High AL Score		P value	Children with Incident Asthma		P value
	n	%	n	Prevalence		n	Prevalence	
Atopy								
No	175	67.8%	60	34.0%	0.06	33	18.9%	0.88
Yes	83	32.2%	19	22.9%		15	18.1%	
Food Allergy								
No	242	93.4%	97	31.8%	0.08	42	17.4%	0.01*
Yes	17	6.6%	2	11.8%		7	42.2%	
Sex								
Female	119	45.9%	40	28.6%	0.46	22	15.7%	0.15
Male	140	54.1%	39	32.8%		27	22.7%	
Parental History								
No	129	56.1%	46	35.7%	0.15	19	14.7%	0.04*
Yes	101	43.9%	27	26.7%		26	25.7%	
Ethnicity								
Caucasian	191	76.4%	53	26.9%	0.04*	34	17.3%	0.20
Other	61	23.6%	25	41.0%		15	24.6%	
Overweight								
No	178	69.0%	35	19.7%	<0.001*	36	20.2%	0.45
Yes	80	31.0%	43	53.8%		13	16.3%	
	Mean		**Mean**		0.05*	**Mean**		0.001*
Age (Years)	9.00		9.10			8.77		

* Statistically significant at p<0.05

Table 0-11 Risk of incident asthma subsequent to exposure to risk factors (crude odds ratios)

	Incident Asthma	
	Boys	**Girls**
AL Low High	 1.00 1.54 (0.59 to 4.01)	 1.00 0.39 (0.13 to 1.12)
Atopy No Yes	 1.00 0.69 (0.30 to 1.60)	 1.00 1.86 (0.79 to 4.40)
Age	0.35 (0.16 to 0.79)*	0.34 (0.17 to 0.70)*
Parental History of Asthma No Yes	 1.00 1.81 (0.78 to 4.20)	 1.00 1.68 (0.75 to 3.80)
Food Allergy No Yes	 1.00 1.96 (0.50 to 7.72)	 1.00 3.50 (0.89 to 13.86)
Ethnicity Caucasian Other	 1.00 1.03 (0.43 to 2.59)	 1.00 1.86 (0.82 to 4.21)
Overweight No Yes	 1.00 0. 67 (0.28 to 1.61)	 1.00 1.19 (0.52 to 2.73)

* Statistically significant at $p<0.05$

Table 0-12 Risk of incident asthma subsequent to high AL in boys (adjusted odds ratios)

	Adjusted OR (Model1)	**Adjusted OR (Model2)**
AL		
Low	1.00	1.00
High	2.06 (0.73 to 5.83)	4.35 (1.19 to 15.93)*
Atopy		
No	1.00	1.00
Yes	0.30 (0.08 to 1.14)	0.24 (0.06 to 1.03)
Food Allergy		
No	1.00	1.00
Yes	4.84 (0.80 to 29.19)	8.04 (1.16 to 55.80)*
Age	0.33 (0.12 to 0.92)*	0.30 (0.10 to 0.85)*
Overweight		
No	-	1.00
Yes	-	0.24 (0.06 to 0.98)*

* Statistically significant at p<0.05

Table 0-13 Risk of incident asthma subsequent to high AL in girls (adjusted odds ratios)

	Adjusted OR (Model1)	**Adjusted OR (Model2)**
AL		
Low	1.00	1.00
High	0.48 (0.16 to 1.48)	0.44 (0.14 to 1.39)
Atopy		
No	1.00	1.00
Yes	1.23 (0.40 to 3.78)	1.32 (0.43 to 4.07)
Food Allergy		
No	1.00	1.00
Yes	7.44 (1.00 to 56.18)*	7.62 (1.00 to 58.43)*
Age	0.44 (0.19 to 0.99)*	0.42 (0.19 to 0.97)*
Overweight		
No	-	1.00
Yes	-	1.63 (0.57 to 4.69)

* Statistically significant at p<0.05

APPENDIX 3- ETHICS APPROVAL

Re-Approval Form

Date:	January 31, 2011	
Amendment/Renewal ID:	Pro00012073_REN1	
Study ID:	MS1_Pro00012073	
Study Title:	Does Chronic Stress Predict Asthma Development in Pre-adolescents?	
Principal Investigator:	Anita Kozyrskyj	
Sponsor/Funding Agency:	Women and Children's Health Research Institute	WCHRI
Approval Expiry Date:	January 31, 2012	

The Health Research Ethics Board - Health Panel has reviewed the renewal request and file for this project and found it to be acceptable within the limitations of human research.

The re-approval for the study as presented is valid for one year. It may be extended following completion of the annual renewal request before the approval expires. Beginning at 45 days prior to expiration, you will receive notices that the study is about to expire. Once the study has expired, you will have to resubmit. Any proposed changes to the study must be submitted to the Health REB for approval prior to implementation.

For studies where investigators must obtain informed consent, signed copies of the consent forms must be retained, as should all study related documents, so as to be available to the Health REB upon request. They should be kept for the duration of the project and for at least five (5) years following study completion.

Sincerely,

Dr. Jana Rieger
Chair, Health Research Ethics Board - Health Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).