POLYCYCLIC AROMATIC HYDROCARBON INTERNAL DOSE, OXIDATIVE STRESS, INFLAMMATION, AND ASTHMA EXACERBATION IN ASTHMATIC ADULTS AND CHILDREN IN BALTIMORE CITY

by
Kamau O. Peters

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ABSTRACT

Background: Asthma is a common chronic inflammatory respiratory disease that disproportionately affects children and African Americans residing in urban, low socioeconomic status communities. Polycyclic aromatic hydrocarbons (PAH) are products of incomplete combustion of organic materials and are found in gas phase, and on fine and ultrafine particulate matter (PM). *In vitro*, animal and human studies have shown PAHs to be associated with increased oxidative stress, allergen-mediated inflammatory responses and asthma exacerbation.

Methods: In this study, we examined whether internal dose of PAHs (urinary 1-OHPG) was associated with early effect biomarkers of oxidative stress, inflammation, and asthma-related symptoms in the DISCOVER/Asthma-Diet study (children with asthma in Baltimore) and the Nasal Challenge to Indoor Particulate Matter study (adults with atopic asthma in Baltimore).

Results: We found that children spending time in homes with low air nicotine concentrations showed inverse associations between time spent indoors and urinary 1-OHPG concentrations. In addition, time spent outdoors was independently associated with increased 1-OHPG in boys. Atopy modulated the associations between urinary 1-OHPG and peripheral blood eosinophils and neutrophils, with significantly stronger positive associations among atopic asthmatic children compared to non-atopic asthmatic children. Children with high urinary 1-OHPG concentrations were also at an increased risk for nighttime waking due to asthma symptoms and nighttime rescue medication use. We also found that *GSTM1* genotype modified the associations between 1-OHPG and urinary isoprostane and serum and peripheral blood inflammatory biomarkers in adult

atopic asthmatics, with significantly stronger positive associations among *GSTM1*-null participants compared to *GSTM1*-present participants.

Conclusion: Our results suggest that exposures to second hand smoke, and combustion products in outdoor ambient air are major contributors to internal dose of PAHs in inner city children. In addition, PAH exposures may contribute to allergen-mediated systemic inflammatory responses (with possible adjuvant effects by PAHs in atopic asthmatics), and to asthma exacerbation in inner city children with asthma. Our results also suggest that PAH exposures contribute to increased local and systemic inflammation in adults with atopic asthma in Baltimore, and that individuals with the *GSTM1*-null polymorphism may be more susceptible to inflammatory responses associated with PAH exposures, due to reduced antioxidant capacity.

Thesis readers

Paul T. Strickland, Ph.D., Professor, Department of Environmental Health Sciences

Gregory B. Diette, MD, MHS, Professor, School of Medicine

Ana Navas-Acien, MD, PhD, Associate Professor, Department of Environmental Health Sciences

Roger D. Peng, PhD, Associate Professor, Department of Biostatistics

Elizabeth C. Matsui, MD, Associate Professor, Department of Pediatrics, School of Medicine

Peter Lees, PhD, Professor, Department of Environmental Health Sciences

Eliseo Guallar, MD, DrPH, Professor, Department of Epidemiology

PREFACE

This thesis dissertation is comprised of six chapters. The first chapter is an introduction to the studies performed, with background, specific aims and hypotheses. The second, third and fourth chapters are the extended draft manuscripts for each of the aims. The fifth, and final, chapter discusses the public health relevance and implications of these studies, and the conclusion.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

LIST OF FIGURES

APPENDICES

APPENDIX E: Supplemental Tables for Chapter 4

ABBREVIATIONS

1-OHPG: 1-hydroxypyrene-glucuronide
2-AA: 2-aminoacridone
Ahr: aryl hydrocarbon receptor
AP-1: activator protein-1
CCL20: chemokine (C-C motif) ligand 20
CYP: cytochrome P450
DEP: diesel exhaust particles
GCLC: glutamate-cysteine ligase catalytic subunit
GCLM: glutamate-cysteine ligase regulatory subunit
GRO: growth-regulated oncogene
GRO-α: growth-regulated oncogene-alpha
GSH: glutathione
GST: glutathione-S-transferase
HO-1: heme oxygenase-1
ICAM-1: intercellular adhesion molecule-1
IgE: immunoglobulin E
IL: interleukin
MCP-1: monocyte chemotactic protein-1
MDA: malondialdehyde
MIP-1α: macrophage inflammatory protein-1α
NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells
NADPH oxidoreductase: nicotinamide adenine dinucleotide phosphate oxidoreductase
NAT2: N-acetyl transferase 2
NQO1: NAD(P)H quinone oxidoreductase 1
NO_2: nitrogen dioxide
Nrf2: nuclear factor (erythroid-derived 2)–like 2
O_3: ozone
PAH: polycyclic aromatic hydrocarbons
PBMC: peripheral blood mononuclear cells
PM: particulate matter
PMA: phorbol 12-myristate 13-acetate
PMN: polymorphonuclear leukocytes
RANTES: regulated on activation, normal T-cell expressed and secreted
ROS: reactive oxygen species
SHS: second hand smoke
TBARS: thiobarbituric acid reactive substances
TFF2: trefoil factor 2
Th1: T-helper cell type 1
Th2: T-helper cell type 2
TNF-α: tumor necrosis factor-α
UGT: uridine diphosphoglucuronosyltransferase
VCAM-1: vascular cell adhesion molecule 1
WBCC: white blood cell count

CHAPTER 1: Introduction

Background

Asthma is a common chronic inflammatory respiratory disease with an increasing disease burden in the United States. Asthma prevalence in the US has increased from 7.3% (20 million people) in 2001 to 8.4% (25.7 million people) in 2010, with higher rates among children ages 0-17 (9.5%) compared to adults 7.7% for 2008-2010 (CDC 2011; Akinbami et al. 2012). The medical and social burden of asthma is considerable, with a disproportionate impact on vulnerable groups including young children, African-Americans, and those who are living in inner cities. African American children are especially at risk, as they have double the asthma prevalence (17%) compared to white children (8.5%) (CDC 2011). Sociodemographic disparities in asthma are also evident, as people living in urban environments, and children from families of low socioeconomic status experience higher asthma burdens–11.2% asthma prevalence compared to the national average for children, 9.5% (CDC 2011; Akinbami et al. 2012). Atopy, an allergic phenotype characterized by increased production of IgE in response to specific allergens, is associated with asthma pathogenesis and asthma severity (Pearce et al. 1999; Suh and Koh 2013). Most asthmatics have an atopic constitution and are vulnerable to inflammatory responses from multiple environmental stimuli (Nelson et al. 1999; Eggleston 2000; Lau et al. 2000), as co-exposures to allergens and environmental air pollutants (such as second hand smoke (SHS), particulate matter (PM), ozone (O_3) and nitrogen dioxide (NO_2)) may lead to increased frequency of asthma exacerbations in atopic asthmatics (D'Amato et al. 2002; Diaz-Sanchez et al. 2006).

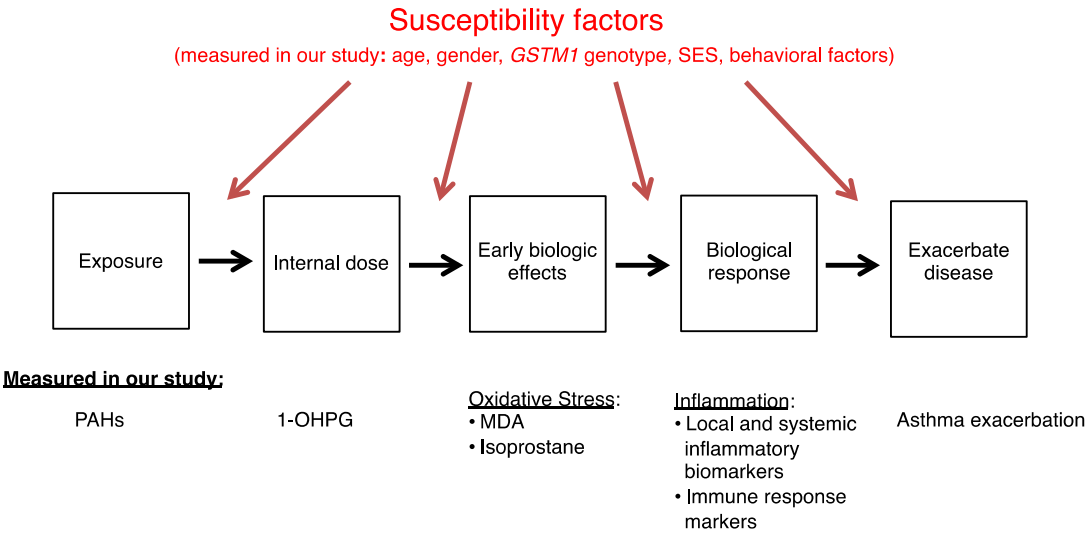
Inner city residents experience increased exposures to agents and conditions (such as PM, allergens, NO_2, O_3, rodent and cockroach infestations and dilapidated housing) that are linked to asthma morbidity (Breysse et al. 2005; McCormack et al. 2009). Exposures to PM from indoor air sources (e.g. cooking, tobacco smoking, and infiltration from outside air) are of particular concern, as people spend over 85% of their day indoors, and the potency (per unit mass) and concentrations of indoor particulates can be significantly greater than outdoor particulates (Klepeis et al. 2000; Wallace et al. 2003; Koenig et al. 2005). Contrasting indoor air exposures in city and suburban settings, Simons et al. (2007) found 2-3 fold higher exposure to indoor PM, and mouse and cockroach allergens in asthmatic children living in inner city Baltimore compared to asthmatic children living in the surrounding suburbs. Sources of ambient air pollution in urban environments include proximity to high vehicular traffic volume on streets and highways, bus and truck depots, waste incinerators and industrial operations (Perera et al. 2002). Urban, inner city populations are therefore more vulnerable due to disproportionately high exposures to PAHs, PM, SHS, and other indoor and ambient air pollutants, and concurrently experiencing disproportionately higher asthma, allergy, cancer and other environmentally related disease burdens (Perera et al. 2002; Breysse et al. 2005; Simons et al. 2007). In addition, the combination of disproportionately higher environmental exposures and generally higher asthma disease burden in urban areas supports the longstanding premise that children living in urban settings are exposed to a more asthmagenic environment (Breysse et al. 2005). These findings highlight both the disparities in asthma morbidity affecting inner city African Americans, and the

importance of elucidating the complex factors that link environmental exposures to asthma exacerbation and increased disease burden.

This study will focus on investigating the links between internal dose of polycyclic aromatic hydrocarbons (PAHs) and biological responses associated with asthma using biomarkers of oxidative stress and allergic and non-allergic inflammatory responses in children living in inner city Baltimore. PAHs are formed from the incomplete combustion or pyrolysis of organic materials (e.g. coal, wood, fuel and oil) and are commonly found on fine (aerodynamic diameter <2.5 μm) and ultrafine particulates (aerodynamic diameter <0.1 μm) (Rosa et al. 2011). Sources of ambient PAHs include motor vehicle emissions (combustion products from diesel and conventional gasoline engines), burning fossil fuels (e.g. coal and oil), and wood burning (ATSDR 1995; Larsen et al. 2003). Tobacco smoking, cooking with gas stoves, heating appliances (e.g. kerosene space heaters), and incense burning are major sources of indoor PAHs (ATSDR 1995; Larsen et al. 2003). Individual PAHs and PAH mixtures have been classified as human carcinogens (e.g. benzo[a]pyrene and coal tar pitch), probable carcinogens, and possible carcinogens by the International Agency for Research on Cancer (IARC), the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR 1995; IARC 2010). Our study, however, will focus on the non-mutagenic, irritant (i.e. oxidative potential) properties of PAHs. In general, particle size is inversely related to the temperature of combustion, surface area to mass ratio, PAH content, and redox activity (Li et al. 2003). Our study is based on the following proposed toxicologic mechanism: exposures to the organic components of PM (i.e. PAHs) increase oxidative stress leading to immunological and physiological responses, and subsequently asthma

related symptoms and exacerbation (Xia et al. 2005) (Figure 1-1). Recent studies suggest that PAHs may be associated with development of asthma (Miller et al. 2004; Jung et al. 2012), neurodevelopmental deficiencies (Perera et al. 2006), and low birth weight (Perera et al. 2003) in inner city urban populations. In addition, experimental evidence suggests that pyrene, a common PAH, enhances allergic immunoglobulin E (IgE) responses in mice (Kanoh et al, 1996; Bommel et al, 2000). Thus, finding significant associations between PAHs, oxidative stress and inflammatory response biomarkers, and asthma exacerbation in our study would support this mechanistic hypothesis.

Figure 1-1. Environmental health paradigm with biomarkers of PAH exposure, effects, and asthma exacerbation



Previous studies have assessed exposure by measuring PAHs isolated from filtered airborne PM samples gathered using personal, active (portable or stationary), and/or outdoor air monitors and estimating internal dose (Choi et al, 2008; Dubowsky et al, 1999; Jedrychowski et al, 2005; Rosa et al, 2011; Tonne et al, 2004). These methods, however, do not assess internal dose or biologically effective dose, as estimates of time spent in the area being sampled, individual activity levels, and temporal fluctuations in

indoor PM concentrations can lead to inaccurate assessments of individual exposures (Breysse et al. 2010). Therefore, we measured the most common urinary metabolite of pyrene, 1-hydroxypyrene-glucuronide (1-OHPG), as a biomarker of internal dose. Urinary 1-OHPG is an accepted biological indicator of PAH exposure, as its parent compound, pyrene, is present in virtually all environmental PAH mixtures and it is a reliable biomarker of low-level exposure (Buchet et al. 1992; Strickland et al. 1994; Strickland et al. 1999; Jongeneelen 2001, Hansen et al, 2008). Previous studies have demonstrated increased urinary 1-OHPG associated with recent exposures through inhalation, ingestion and dermal absorption of PAHs from environmental, occupational, dietary or medicinal sources.

Predictors of urinary 1-OHPG concentrations in the general population include gender: higher in males (Huang et al. 2004, CDC 2005; Lee et al. 2009; Sul et al. 2012), dietary exposures (Fiala et al. 2001; Bostrom et al. 2002; Kang et al. 2005; Lee KH et al. 2009), exposures to SHS (Jongeneelen et al. 1994, van Wijnen et al. 1996; Siwinska et al. 1999; Mucha et al. 2004; Lee KH et al. 2009), exposures to high traffic volume (Taniawiroon et al. 2007; Martinez-Salinas et al. 2010; Fan et al. 2012), and living in an large cities/urban environments (Hansen et al. 2005; Bae et al. 2010). 1-OHPG, or its deconjugated derivative, 1-OHP, have been associated with a variety of exposure/internal dose biomarkers including urinary cotinine (Leem et al. 2010); early effect biomarkers of oxidative stress (8-deoxy-hydroxyguanine and malondialdehyde) (Kang et al. 2005; Hong et al. 2009; Bae et al. 2010); and diseases related to environmental exposures. For example, increased levels of 1-OHPG in children are associated with having asthma (Kim et al. 2005), or experiencing an asthma attack (Leem et al. 2006). Measuring the internal

dose of 1-OHPG therefore allowed us to assess the short-term body burden of PAHs and provide a better understanding of the relationship between these exposures and adverse biochemical, immunological, and physiological responses.

SPECIFIC AIMS

Specific Aim 1

Quantify PAH internal dose in children from the DISCOVER/Asthma-Diet study by measuring urinary 1-OHPG; and examine associations between indoor air pollutants, time spent indoors (at home) or outdoors and urinary 1-OHPG concentrations, and evaluate whether a child's gender and/or adults smoking in the home are effect modifiers of these relationships.

Hypothesis

We hypothesized that urinary 1-OHPG concentrations would be associated with time spent indoors and exposure to indoor air pollutants (such as PM and indoor air nicotine), with more pronounced positive associations between time spent indoors and 1-OHPG among children residing in smoking households.

Specific Aim 2

Examine associations between internal dose of PAHs (by urinary 1-OHPG) and oxidative stress (by analyzing urinary MDA), as well as associations between PAH internal dose and peripheral blood biomarkers of inflammation, asthma-related symptoms, and medication use in children from the DISCOVER/Asthma-Diet study. Evaluate whether atopic constitution is an effect modifier of the associations between internal dose PAHs and inflammatory biomarkers, and asthma symptoms in asthmatic children.

Hypothesis

We hypothesized that urinary 1-OHPG concentrations would be associated with urinary MDA concentrations, increased peripheral blood inflammatory biomarkers, and an

increased risk for asthma-related symptoms. We also hypothesized that atopic asthmatics would have stronger associations between urinary 1-OHPG and peripheral blood biomarkers and asthma-related symptoms, based on synergistic properties of PAHs on allergic inflammatory responses in allergen sensitized individuals.

Specific Aim 3

Evaluate associations between internal dose of PAHs (measured by urinary 1-OHPG) and biomarkers of oxidative stress (urinary isoprostane), gene expression of antioxidants, and peripheral blood, serum and nasal lavage biomarkers of inflammation in the Nasal Challenge to Indoor Particulate Matter study, a study of atopic asthmatic adults in Baltimore City. Evaluate whether *GSTM1* genotype was an effect modifier of the associations between internal dose PAHs and oxidative stress and inflammatory biomarkers.

Hypothesis

We hypothesized that PAH internal dose would be associated with increased internal dose of oxidative stress, increased induction of antioxidant genes (*HO-1, NQO1, GCLC* and *GCLM*), increased serum mediators (ECP, MIP-1α, eotaxin, CCL20, and RANTES), and increased local and systemic inflammation (nasal lavage and peripheral white blood cells, and nasal lavage cytokines and chemokines). We also hypothesized that *GSTM1* genotype would be a significant effect modifier, with *GSTM1*-null participants having stronger positive associations between 1-OHPG and oxidative stress and inflammatory biomarkers compared to *GSTM1*-present participants, due to less antioxidant capacity in individuals who are unable to produce the GSTM1 enzyme.

CHAPTER 2. Predictors of polycyclic aromatic hydrocarbon exposure and internal dose in inner city children with asthma

ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAH) are products of incomplete combustion of organic materials (oil, tobacco, fuel, etc.) and are found in gas phase, and on fine and ultrafine PM. 1-Hydroxypyrene-glucuronide (1-OHPG) is the most common urinary metabolite of pyrene, a semi-volatile PAH that is present in most PAH mixtures. Children spend most of their time indoors and may be exposed to higher PM concentrations than outdoor ambient exposures. These exposures may play an important role in asthma morbidity. We examined time spent indoors and outdoors as predictors of PAH internal dose in inner city Baltimore, and whether a child's gender and/or adults smoking in the home modify the association between urinary 1-OHPG and time spent inside the home or outdoors.

Methods: The DISCOVER/Asthma-Diet study is a longitudinal (panel) study with at total of 118 participants ages 5-13 from Baltimore City. Children were followed for one-week periods in each of four seasons. Urine specimens were collected on day 3 and day 7 of each week, and analyzed for 1-OHPG. Indoor PM and air nicotine were monitored over the week using passive samplers, and questionnaires assessing time spent indoors/outdoors and smoking frequency in the home were administered each day of the week-long study periods.

Results: Most of the children were female (52%), African American (95%), atopic asthmatics (63%), and 53% of the households reported smoking in the home. Time spent in non-smoking homes on days 4-6 was associated with decreased 1-OHPG concentration

in urine collected on day 7 (ß=-0.045, p=0.005). Spending time in homes with low air nicotine concentrations was associated with low 1-OHPG concentrations (ß=-0.075, p=0.003), whereas spending time in homes with high air nicotine concentrations was not associated with changes in 1-OHPG concentration (ß=0.005, p=0.835) (p-value for interaction=0.076). Time spent outdoors on day 4-6 (ß=0.097, p=0.002) was associated with increased 1-OHPG concentrations in urine collected on day 7 in boys only.

Conclusion: Our results suggest that time spent in the home is inversely associated with 1-OHPG concentrations in children's urine and is modified by smoking in the home. Spending time in a non-smoking home confers a more "protective" effect for 1-OHPG concentrations than spending time in a smoking home. In addition, spending time outdoors is positively associated with 1-OHPG, and the effect is more pronounced in boys. Our results suggest that SHS and outdoor ambient air PAH sources contribute to internal dose of PAHs, as measured by urinary 1-OHPG.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are formed from the incomplete combustion or pyrolysis of organic materials (e.g. coal, wood, fuel and oil) and are commonly found on fine and ultrafine particulates, $PM_{2.5}$ (aerodynamic diameter <2.5 μm) and $PM_{0.1}$ (aerodynamic diameter <0.1 μm), respectively (Rosa et al. 2011). Sources of ambient PAHs include motor vehicle emissions (combustion products from diesel and conventional gasoline engines), burning fossil fuels (e.g. coal and oil), and wood burning (ATSDR 1995; Larsen et al. 2003). Tobacco smoking, cooking with gas stoves, heating appliances (e.g. kerosene space heaters), and incense burning are major sources of indoor PAHs (ATSDR 1995; Larsen et al. 2003). Individual PAHs and PAH mixtures have been classified as human carcinogens (e.g. benzo[a]pyrene and coal tar pitch), probable carcinogens (e.g. dibenz[a,h]anthracene and dibenzo[a,l]pyrene) and possible carcinogens (e.g. benz[a]anthracene, benzo[b]fluoranthene and indeno[1,2,3-cd]pyrene) by the International Agency for Research on Cancer (IARC), and the Agency for Toxic Substances and Disease Registry (ATSDR) and the National Toxicology Program (NTP) (ATSDR 1995; IARC 2010; NTP 2011). PAHs have also been associated with development of asthma (Miller et al. 2004; Jung et al. 2012), neurodevelopmental deficiencies (Perera et al. 2006), and low birth weight (Perera et al. 2003) in urban and inner city populations.

Urinary1-hydroxypyrene-glucuronide (1-OHPG) is an accepted biological indicator of PAH exposure, as its parent compound, pyrene, is present in virtually all environmental PAH mixtures and is a reliable biomarker of low-level exposure (Buchet et al. 1992; Jongeneelen 2001). Urinary 1-OHPG concentrations reflect exposures

through inhalation, ingestion, and dermal absorption (from contaminated air and soil) (Jongeneelen 2001). Both 1-hydroxypyrene (1-OHP) and 1-OHPG are commonly used urinary biomarkers of PAH exposure, however 1-OHPG is more sensitive, as the addition of glucuronide confers 3-5 times more fluorescence than 1-OHP alone (Singh et al. 1995; Strickland et al. 1996; Kang et al. 2005). Predictors of 1-OHPG concentrations include gender: higher in males (Huang et al. 2004, CDC 2005; Lee et al. 2009; Sul et al. 2012), dietary exposures (Fiala et al. 2001; Bostrom et al. 2002; Kang et al. 2005; Lee KH et al. 2009), exposures to second hand smoke (Jongeneelen et al. 1994, van Wijnen et al. 1996; Siwinska et al. 1999; Mucha et al. 2004; Lee KH et al. 2009), exposures to high traffic volume (Taniawiroon et al. 2007; Martinez-Salinas et al. 2010; Fan et al. 2012), and living in an large cities/urban environments (Hansen et al. 2005; Bae et al. 2010). 1-OHP(G) is associated with exposure/internal dose biomarkers including urinary cotinine (Leem et al. 2010) and other urinary PAH metabolites (Kang et al. 2005; Fan et al. 2012; Yoon et al. 2012); and biomarkers of oxidative stress and damage (8-deoxy-hydroxyguanine and malondialdehyde) (Kang et al. 2005; Hong et al. 2009; Bae et al. 2010). Elevated levels of 1-OHPG in children are also associated with having asthma (Kim et al. 2005), and experiencing an asthma attack (Leem et al. 2006).

PAHs are commonly found on the surface of fine particulates ($PM_{2.5}$), and are among the most biologically relevant organic constituents of $PM_{2.5}$ because of irritant (i.e. oxidative potential) and carcinogenic properties (Squadrito et al. 2001). Exposure to PM from indoor air sources (such as cooking and smoking) are of particular concern, as people may spend over 85% of their day indoors, and the potency (per unit mass) and concentrations of indoor particulates can be significantly greater than outdoor particulates

(Klepeis et al. 2000; Koenig et al. 2005; Wallace et al. 2003). Sources of ambient air pollution in urban environments include proximity to high vehicular traffic volume on streets and highways, bus and truck depots, waste incinerators and industrial operations (Perera et al. 2002). Urban, inner city populations are therefore more vulnerable due to disproportionately high exposures to PAHs, PM, SHS, and other indoor and outdoor ambient air pollutants, and concurrently experiencing disproportionately higher disease burdens of asthma, allergy, cancer and other environmentally related disease burdens (Perera et al. 2002; Breysse et al. 2005; Simons et al. 2007).

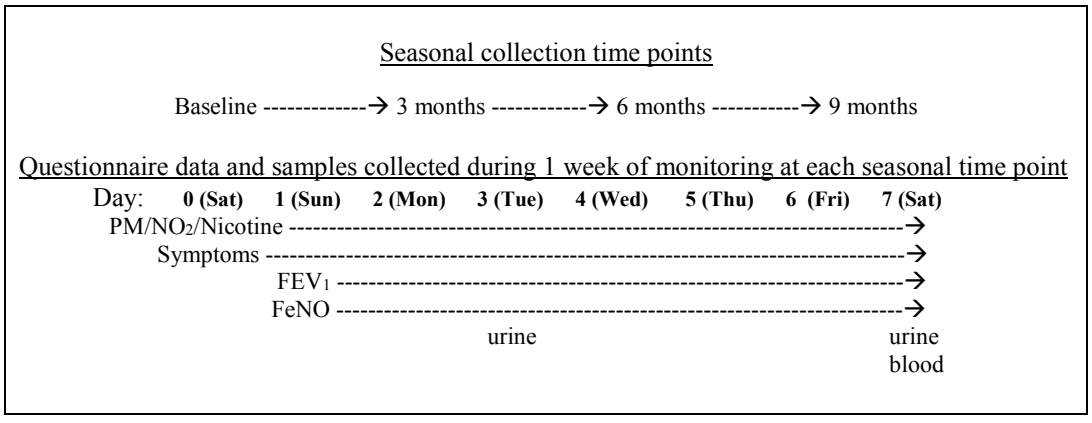
Our overall goal is to quantify PAH internal dose using urinary 1-OHPG, and examine the relationship between predictors of PAH exposure and internal dose of PAHs in inner city Baltimore children participating in the DISCOVER/Asthma Diet study. We also examine associations between indoor air pollutants, time spent indoors (at home) or outdoors and urinary 1-OHPG concentrations, and evaluate whether a child's gender and/or adults smoking in the home are effect modifiers of the these relationships.

MATERIALS AND METHODS

The Role of Particulate Matter and Allergens in Oxidative Stress in Asthma (DISCOVER) and Asthma-Diet (**A S**tudy to understand **Th**e **M**echanisms of **A**sthma-**D**ietary **I**nterventions to protect against **E**nvironmental **T**riggers) studies have the same study design, as the Asthma-Diet study is an extension of the DISCOVER study (with a dietary component). The DISCOVER/Asthma-Diet study is a panel study that enrolled 100 atopic asthmatic and 50 non-atopic asthmatic, and 30 non-asthmatic children ages 5-

12, from nine contiguous zip codes in inner city Baltimore. Indoor PM and allergen exposures, diet, lung function, asthma symptoms, and biomarkers of oxidative stress, and inflammation were assessed for a one week period, in each of four seasons (Figure 2-1) (Dr. Greg Diette, personal communication). Inclusion criteria included: age 5-12 years, physician diagnosis of asthma, symptoms of asthma and/or reliever medication use in past 6 months, and residence in Baltimore city. Children were excluded from the study if they had a current diagnosis of another major pulmonary disease, if they were planning to relocate residence during the study period, were currently taking antioxidant supplements, had a known food allergy, had metabolic or dietary disorders or were pregnant. Each season, the children's homes were monitored for 8 days (labeled as days 0-7); indoor air nicotine, PM, NO_2 concentrations were measured, daily activity questionnaires were administered, and urine was collected on the fourth (day 3) and eight day (day 7). Daily activity diaries asked questions about smoking in the home and where the child spent his/her time (hours spent inside the home, outdoors, and indoors in other buildings and vehicles) (Appendix A: Daily Diary Questionnaire).

Figure 2-1. DISCOVER/Asthma-Diet study framework



Urinary 1-OHPG

Spot urine samples were analyzed for urinary 1-OHPG concentrations using immunoaffinity chromatography (IAC) and synchronous fluorescence spectroscopy (SFS) as previously described (Strickland et al. 1994). Urine samples (2ml) were treated with 0.1N HCl (90°C, 60 min), neutralized, and loaded onto Sep-pak C18 cartridges (Waters). After washing with 30% methanol, the relatively non-polar metabolites were eluted with 4 ml of 80% methanol and the volume of eluate is reduced to 0.5ml by evaporation on a Speedvac. The concentrated samples were diluted to 4ml with 15mM phosphate buffered saline (PBS) and loaded onto immunoaffinity columns prepared with 0.8ml cyanogen bromide activated sepharose 4B (Sigma) coupled with monoclonal antibody 8E11 that recognizes several PAH-DNA adducts and metabolites (Santella et al. 1986). We have previously shown that 1-OHPG binds to these columns (Strickland et al. 1994). After washing the columns three times with 4 ml 15 mM PBS, bound compounds were eluted with 2 ml of 40% methanol in three fractions. Eluate fractions were analyzed by SFS with a Perkin-Elmer LS50 fluorescence spectrometer. The excitation-emission monochromators are driven synchronously with a wavelength difference of 34 nm. Under these conditions 1-OHPG produces a characteristic fluorescence excitation maximum at 347 nm with emission maximum at 381 nm (Strickland et al. 1994). Fluorescence intensity was used to quantify 1-OHPG; in our laboratory the limit of detection was about 0.03 pmol/ml. This level of sensitivity is sufficient to detect urinary 1-OHPG in >90% of subjects with low-level exposure to PAHs (such as in the U.S.), and in 100% of subjects with high exposure. The coefficient of variation of the assay is typically 6-10% (interbatch) in our laboratory.

Urinary Creatinine

Creatinine concentrations were determined using a modified version of the Jaffe reaction using the Creatinine Assay Kit (Cayman Chemical Company; Ann Arbor, MI). Briefly, 150 μl of alkaline picrate solution was added to wells containing 15 μl creatinine standard or urine samples in duplicate, and the solution was mixed for 10 minutes. The absorbance of the solution (due to the reaction between the alkaline picrate and urinary creatinine metabolites) was read at 450-500 nm using a Biotek ELx800 Absorbance Microplate Reader. 5 μl of acid solution was then added to the assay and the absorbance was read again at 450-500 nm, 20 minutes after mixing. The difference in color intensity before and after acidification is proportional to urinary creatinine concentration. Urinary creatinine levels were determined using a creatinine standard curve, which was estimated in each batch from analysis of the standard creatinine.

Particulate matter

Airborne particulate matter monitoring was conducted in the child's bedroom using integrated sampling methods for a 5-7 day period. Air samples for both particulate matter ≤10 microns (PM_{10}) and ≤ 2.5 microns ($PM_{2.5}$) were collected on Teflon® filters (Pall Gelman, Ann Arbor, MI) using SKC Personal Environmental Monitors (SKC, Inc., Eighty-four, PA) and BGI 400S pumps (BGI, Inc., Waltham, MA).

Air nicotine

Two passive sampling badges were placed in the child's bedroom and the TV/family room at 3-5 feet off the floor. The passive air samplers consist of a sodium-bisulfate- treated filter contained in a 37-mm polystyrene cassette covered with a polycarbonate filter diffusion screen. Nicotine content was analyzed using gas

chromatography with a nitrogen-phosphate detector. The limit of detection for the passive air nicotine badges was 0.003 $\mu g/m^3$.

Statistical analysis

Summary statistics were reported for urinary1-OHPG, and indoor $PM_{2.5}$, PM_{10}, $PM_{2.5-10}$ and air nicotine concentrations. 1-OHPG, $PM_{2.5}$, PM_{10}, $PM_{2.5-10}$ and air nicotine concentrations were log-transformed to adjust for positively skewed distributions. Urinary 1-OHPG concentrations on day 3 (Tuesday; n=255) and day 7 (Saturday; n=339) and averages for the monitoring period (n=359) were analyzed separately. Multivariate linear regressions with generalized estimating equations (GEE) were used to assess associations between potential predictors (independent variables) and individual 1-OHPG concentrations (dependent variable), while adjusting for repeated measurements (i.e. monitoring periods) and possible confounders. Explanatory variables were considered possible confounders if the ß coefficient changed by more than 10% after inclusion of the exposure variable (e.g. time spent in the home) in the model or if the variable was significantly associated with the exposure or outcome. Our final models for multivariate linear regression with GEE were adjusted for age, gender, season, atopic status and caregiver educational attainment. Duplicate measures for indoor air nicotine concentration were averaged. $PM_{2.5}$ and PM_{10} measurements were excluded if airflow through the passive monitor was not sufficient, $PM_{2.5}$ concentrations were greater than PM_{10} concentrations or if there were equipment malfunctions. Race was not included in models because 95% of the participants were African American. Urinary 1-OHPG concentrations were adjusted for urinary creatinine by including creatinine concentrations in the model as an independent variable (Barr et al. 2005). Age, 1-OHPG, creatinine, air

nicotine, $PM_{2.5}$, PM_{10}, $PM_{2.5-10}$, and self-reported exposure variables (e.g. average time in the home and average time outdoors) were measured as continuous variables; gender and health insurance were binary variables; BMI percentile and season were categorical variables; and caregiver education and annual household income were measured as ordinal variables. Seasons were defined based on calendar days and heating season was defined as November 1 through March 1 (Jung et al. 2010). Atopic constitution was defined as having allergic responses to at least one of 13 aeroallergens from a skin prick test or by radioallergosorbent test (RAST).

Effect modification was assessed using pairwise interaction terms for independent variables (e.g. the product of average time indoors and smoking in the home). Comparisons of two groups of exposure variables were performed using Wilcoxon sign-rank test and Mann-Whitney U test. Pearson's correlation coefficient and Spearman's rho were used to examine correlations between internal dose biomarkers and indoor air concentrations. Urine collected in the afternoon or early evening of day 3 (Tuesday) was compared to pooled estimates of exposures on days 0-2, representing exposures from 24-96 hours prior to urine collection (24-48 hours for day 2, Monday; 48-72 hours for day 1, Sunday; and 72-96 hours for day 0, Saturday). Urine collected in the morning to early afternoon on day 7 (Saturday) was compared to pooled estimates of exposures on days 4-6, representing exposures from 18-88 hours prior to urine collection. Urinary 1-OHPG concentrations were reported as pmol/ml and μmol/mol creatinine, and concentrations for indoor air nicotine, $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ were reported as $\mu g/m^3$. Missing data varied by the exposure metric (4%-28%) and did not differ substantially by gender (Supplemental Table 2-14). Reasons for missing data included: interviewer was unable to

contact the caregiver and obtain urine samples from the child (especially during the weekday (day 3) and on the first day of the monitoring period (day 0)), incorrect or unusable questionnaire data (e.g. daily time apportionment exceeded 24 hours), and technical problems with the air nicotine and PM monitors. Missing values were not included in the analyses. All data were analyzed using STATA 11.1 (College Station, TX)

RESULTS

Demographics

Children in the DISCOVER/Asthma-Diet study were between 5 and 13 years of age (mean: 10 years of age), and most were female (52%), African American (95%), and from low socioeconomic status households (66% of caregivers had up to a high school diploma, 88% had public health insurance, and 77% had annual household incomes below $25,000) (Table 2-1). Most children were atopic (70%) and asthmatic (86%) and resided in households with adults smoking in the home at some time during the study (53%). Approximately half of the children (46%) were overweight or obese (BMI percentile ≥85%) and about a third (32%) of the children were obese (BMI percentile ≥95%). Most of the obese children (62%) were girls.

Urinary 1-OHPG concentration

1-OHPG concentrations were analyzed in 594 spot urine samples and urinary creatinine (Cr) was measured to adjust for differences in urine dilutions (Table 2-2). Urinary 1-OHPG had an arithmetic mean (standard deviation (SD)) of 1.76 (1.92)

pmol/ml, geometric mean (GM) of 0.88 pmol/ml, median (interquartile range (IQR)) of 1.24 (0.42-2.43) pmol/ml, 95th percentile of 5.35 pmol/ml, and range of 0.05-14.59 pmol/ml. Sixty-seven samples (11%) were below the limit of detection (LOD) of 0.05 pmol/ml. Log-transformed urinary creatinine was significantly associated with age (ß=0.123, p<0.001; 95% CI [0.088-0.158]) and inversely associated with being asthmatic (ß=-0.267, p=0.001; 95% CI [-0.411-(-0.123)]), but was not associated with gender, atopic status, BMI percentile (continuous), season or caregiver's educational attainment (Supplemental Table 2-1). Urinary 1-OHPG concentration (pmol/ml) was divided by urinary creatinine (µmol/ml) to adjust for urine dilution differences, and was reported as µmoles 1-OHPG per mole creatinine (µmol/mol creatinine).

Day 3 vs. Day 7 urine

Urine samples were collected on day 3 (Tuesday) in the afternoon to early evening (3:00-7:00 PM), and day 7 (Saturday) in the morning (8:30 AM-12:00 PM). Diurnal differences in 1-OHPG and creatinine output may therefore be reflected in day 3 and day 7 spot urine samples. GM and median creatinine corrected 1-OHPG concentrations were similar for day 3 (n=255) and day 7 (n=339) samples (Wilcoxon sign-rank test p=0.422) (Supplemental Table 2-2). Individual 1-OHPG concentrations on day 3 and day 7 were correlated using parametric (Pearson's correlation coefficient=0.20, r^2=0.04; p=0.003) and non-parametric (Spearman's rho=0.34; p<0.001) analyses. Log-transformed 1-OHPG concentrations were also significantly associated in multivariate linear regression with GEE to account for repeated measurements in crude (adjusted for urinary creatinine; ß=0.190, p=0.003; 95% CI [0.065-0.316]) and adjusted models (adjusted for urinary creatinine, age, gender, season, atopic and asthma status, caregiver's education; ß=0.204,

p=0.001; 95% CI [0.085-0.323]).

Predictors of 1-OHPG

Overall, girls had higher 1-OHPG concentrations than boys (ß=-0.385; p=0.010; 95% CI [-0.680-(-0.091)]) and the gender difference remained in models without creatinine adjustment (ß=-0.378, p=0.016; 95% CI [0.686-(-0.069)]) (Table 2-3). This translates to 42% higher GM of 1-OHPG concentrations in girls than boys. Significant gender differences within age groups were also reported. Among 13 year olds, gender differences in creatinine adjusted 1-OHPG were more pronounced (ß=-1.821; p<0.001) and remained in analyses of creatinine unadjusted 1-OHPG concentrations (ß=1.511; p<0.001). This translates into 4.5 fold higher GM of creatinine adjusted 1-OHPG concentrations, and 6 fold higher GM of creatinine unadjusted 1-OHPG concentrations in 13 year-old girls than 13 year-old boys. Overall, age, asthma status and atopic constitution, BMI percentile (continuous) and being obese (compared to normal weight) were not significantly associated with 1-OHPG concentrations (Table 2-3). There were also no differences in 1-OHPG levels by season. Comparing individual seasons, 1-OHPG concentrations were highest in the fall, followed by winter and spring, and were lowest in summer (Supplemental Table 2-3). Heating season, defined as November 1 through March 1, was not significantly associated with urinary 1-OHPG concentrations. Type of heating in the home, opening windows in the house, distance from the curb to the street, and type of curb (i.e. parking lot, arterial street or side street) were also not associated with 1-OHPG concentrations (Supplemental Table 2-4). Most homes used a gas stove (85%), and having a gas stove was inversely, though non-significantly associated with urinary 1-OHPG concentrations.

Indoor $PM_{2.5}$

Fine particulate matter ($PM_{2.5}$) was measured over the course of the monitoring week using passive monitors, located predominantly in the child's bedrooms (Table 2-2). Overall, indoor $PM_{2.5}$ concentrations had an arithmetic mean (SD): 28.4 (22.8) $\mu g/m^3$, median (IQR): 21.8 (13.0-34.9) $\mu g/m^3$, 95^{th}%ile: 72.1 $\mu g/m^3$, and range: 3.7-133.2 $\mu g/m^3$. Indoor $PM_{2.5}$ concentrations were associated with adults smoking in the home (ß=0.275, p=0.001; 95% CI [0.119-0.431]) and number of cigarettes smoked in the home each day (ß=0.037 p<0.001; 95% CI [0.023-0.051]) (Supplemental Table 2-5). Daily time with windows open, daily stove use, burning incense or candles, and burning something on the stove were not associated with indoor $PM_{2.5}$ concentrations. Indoor $PM_{2.5}$ concentrations were also not associated with urinary 1-OHPG concentrations from urine collected on day 3, urine collected on day 7 or averaged 1-OHPG concentration (Table 2-4).

Indoor PM_{10}

Particulate matter (PM_{10}) was measured using stationary passive monitors located predominantly in the child's bedroom (Table 2-2). Indoor PM_{10} concentrations had an arithmetic mean (SD): 41.1 (29.0) $\mu g/m^3$, median (IQR): 32.6 (21.6-52.5) $\mu g/m^3$, 95^{th}%ile: 97.5 $\mu g/m^3$, and range: 5.8-268.8 $\mu g/m^3$. Smoking in the home (ß=0.306, p<0.001; 95% CI [0.162-0.450]) and number of cigarettes smoked in the home daily (ß=0.030, p<0.001; 95% CI [0.018-0.041]) were significantly associated with increased indoor PM_{10} (Supplemental Table 2-5). Daily stove use was moderately associated with indoor PM_{10} (ß=0.115, p=0.087), however, daily time with windows open, burning something on the stove and burning incense or candles were not associated with indoor PM_{10} concentrations. Indoor air PM_{10} concentrations did not significantly predict urinary

1-OHPG concentrations, however the associations between 1-OHPG and indoor PM_{10} were stronger than 1-OHPG and indoor $PM_{2.5}$ (Table 2-4).

Indoor $PM_{2.5-10}$

Indoor $PM_{2.5-10}$ (coarse PM) was calculated by subtracting $PM_{2.5}$ from PM_{10} concentrations (Table 2-2). Overall, indoor $PM_{2.5-10}$ concentrations had an arithmetic mean (SD): 12.7 (14.3) $\mu g/m^3$, median (IQR): 9.8 (5.4-15.8) $\mu g/m^3$, 95^{th}%ile: 30.8 $\mu g/m^3$, and range: 0.71-215.3 $\mu g/m^3$. The highest value, 215.3 $\mu g/m^3$ (over 20-fold higher than the median) was considered an outlier and was therefore not included in these analyses. Smoking in the home (ß=0.176, p=0.064; 95% CI[-0.011-0.362]) and stove use (ß=0.158, p=0.072; 95% CI [-0.014-0.330]) were moderately associated with increased $PM_{2.5-10}$ (Supplemental table 2-5). Time with windows open, number of cigarettes smoked in the home, and burning incense or candles, and burning something on the stove were not associated with indoor $PM_{2.5-10}$ concentrations. Indoor $PM_{2.5-10}$ was significantly associated 1-OHPG from urine collected on day 7 (ß=0.177, p=0.021; 95% CI [0.027-0.326]) moderately associated with averaged urinary 1-OHPG (ß=0.117, p=0.074; 95% CI [-0.011-0.246]), but not associated with 1-OHPG from day 3 urine (Table 2-4).

Time spent in the home

Children spent an average of 16 hours in the home each day, with no differences for boys and girls (Table 2-5). More time was spent in the home on days 0-2 (Saturday, Sunday and Monday) (median 17.0 hours), compared to days 4-6 (Wednesday, Thursday and Friday) (median 15.7 hours) (Wilcoxon sign-rank test p=0.005). Spending time in the home on days 4-6 (Wednesday-Friday) showed an inverse association with 1-OHPG from urine collected on day 7 (Saturday) (ß=-0.045, p=0.005 [(95% CI: -0.076-(-0.013)])

(Table 2-6). This translates into a 5% decrease in GM of 1-OHPG concentrations for each hour spent in the home. Spending time in the home on days 0-2 (Saturday-Monday) was moderately associated with decreased 1-OHPG concentrations in urine on day 3 (Tuesday) (ß=-0.026, p=0.076). Associations between time spent in the home and 1-OHPG concentrations were similar, but slightly attenuated, after individually adjusting for indoor $PM_{2.5}$, $PM_{2.5-10}$, and PM_{10} (Supplemental Table 2-7).

Air nicotine

Smoking in the home may contribute to the high levels of 1-OHPG in this cohort, as most households self-reported smoking in the home at some time during the study. Indoor air nicotine concentrations were measured throughout the monitoring week using passive monitors located predominantly in the child's bedroom (Table 2-2). Indoor air nicotine concentrations had an arithmetic mean (SD): 0.76 (1.26) $\mu g/m^3$, median (IQR): 0.13 (0.03-0.96) $\mu g/m^3$, 95^{th}%ile: 3.4 $\mu g/m^3$, and range: 0.005-8.8 $\mu g/m^3$. Using bivariate linear regression analysis with GEE, log transformed air nicotine concentrations were associated with self-reported number of cigarettes smoked in the home per day (ß=0.083, p<0.001; 95% CI [0.055-0.110]) and smoking in the home (ß=1.037, p<0.001; 95% CI [0.703-1.371]) (Supplemental Table 2-6). Air nicotine concentrations were also correlated with indoor $PM_{2.5}$ concentrations (Pearson's correlation coefficient (both log-transformed) r= 0.55, p<0.001; Spearman's rho=0.53, p<0.001), and significantly associated with indoor $PM_{2.5}$ concentrations (ß=0.795, p<0.001; 95% CI [0467-1.124]).

Air nicotine and 1-OHPG

Indoor air nicotine concentrations were correlated with urinary 1-OHPG concentration using parametric (Pearson's r=0.11, p=0.046) and non-parametric

(Spearman's rho=0.12, p=0.031) analyses (Supplemental Table 2-6). Indoor air nicotine was also significantly associated with increased 1-OHPG from urine collected on day 3 (ß=0.129, p=0.007; 95% CI [0.035-0.233]), but not associated with 1-OHPG collected on day 7 and averaged 1-OHPG concentration (Table 2-4). Similar associations between indoor air nicotine and urinary 1-OHPG were found using multivariate linear regression analysis without GEE.

Air nicotine, average time spent in home and 1-OHPG

Overall, average time spent in the home was inversely associated with urinary 1-OHPG in homes with low air nicotine concentration (below the median: 0.13 $\mu g/m^3$) (ß=-0.075, p=0.003; 95% CI [-0.124-(-0.026)]), with a significant trend of decreasing 1-OHPG concentrations with increasing time spent in homes with low air nicotine concentration (Cusick's test for trend p-value=0.029) (Table 2-7, Figure 2-2). Spending time in homes with low air nicotine concentration on days 4-6 was associated with significantly lower urinary 1-OHPG concentrations in urine collected on day 7 (ß=-0.081, p<0.001) (Table 2-8, Figure 2-3). Air nicotine was an effect modifier of the relationship between spending time in the home on days 4-6 and urinary 1-OHPG concentrations from urine collected on day 7, with more pronounced inverse associations between spending time in the home and 1-OHPG in homes with low air nicotine concentration (p-interaction=0.074). Indoor air nicotine concentration also modified the associations between average time spent in the home and 1-OHPG in boys (p-interaction=0.014), but not girls (Table 2-8). Gender differences in effect modification by indoor air nicotine were evident despite lack of gender differences in time spent in the home. Inverse associations between spending time in homes with low air nicotine concentrations and

urinary 1-OHPG were strongest among boys (exposures on days 4-6, 1-OHPG from day 7 urine: ß=-0.088, p=0.005; exposures over the week, averaged 1-OHPG: ß=-0.085, p=0.008), and effect modification by indoor air nicotine concentration was found in boys only (p-interaction=0.014) (Table 2-7).

Indoor air nicotine was significantly associated with indoor $PM_{2.5}$ concentrations and self-reported smoking in the home (Supplemental Table 2-6). These SHS surrogates may therefore have similar interactions with time spent in the home and urinary 1-OHPG concentrations. Models stratified by indoor $PM_{2.5}$ concentrations (dichotomized at the median, 21.57 $\mu g/m^3$) showed significant interactions with time spend in the home, with more pronounced inverse associations between spending time in the home on days 4-6 and 1-OHGP from urine collected on day 7 in homes with low $PM_{2.5}$ concentrations (p-interaction=0.003) (Supplemental Table 2-8). However, indoor $PM_{2.5}$ concentrations did not modify associations between time in the home and 1-OHPG from urine collected on day 3 or averaged urinary 1-OHPG. Self-reported adult smoking in the home also showed effect modification patterns similar to indoor air nicotine and $PM_{2.5}$ (Supplemental Table 2-8).

Time spent outdoors

Children spent approximately 2 hours outdoors each day, and overall boys spent more time outdoors daily than girls (Wilcoxon rank-sum test p=0.053) (Table 2-5). Overall, time spent outdoors on days 0-2 was similar to time spent outdoors on days 4-6 (Wilcoxon sign-rank test p=0.404). Boys spent more time outdoors on days 0-2 and days 4-6 than girls (Wilcoxon sign-rank test p=0.053 and p=0.026, respectively). Average time spent outdoors also varied by season (Kruskal-Wallis rank test p<0.001) (Supplemental

Table 2-9). Children spent the most amount of time outdoors in spring, followed by summer and fall, and were outdoors least during the winter.

The two urine collection days, day 3 (Tuesday) and day 7 (Saturday) may reflect different patterns of exposure because of behavioral and activity differences on the weekday and weekend. The children in the DISCOVER/Asthma-Diet study are of school age (5-13 years old) and will likely spend up to 8 hours in school during the weekday. This is reflected by the difference in average time spent in the home on weekdays (median: 15.4 hours) and weekends (median: 18.0 hours) (Wilcoxon rank sum $p<0.001$); and the corresponding difference in time spent in indoor environments other than the home (including other buildings and vehicles) during weekdays (median: 6.2 hours) compared to weekend (median=1.5 hours) (Wilcoxon rank sum $p<0.001$) (Supplemental Table 2-10). Average time spent outdoors, however, did not differ on weekdays and weekends (Wilcoxon rank sum $p=0.792$).

Average time spent outdoors on days 4-6 (Wednesday, Thursday and Friday) was associated with moderately significant increased urinary 1-OHPG concentrations from urine on day 7 (Saturday) (ß=0.048, $p=0.054$; 95% CI [-0.001-0.098]) (Table 2-9, Figure 2-4). However, time spent outdoors on days 0-2, and average time spent outdoors during the week were not associated with urinary 1-OHPG.

Time spent outdoors and 1-OHPG by gender

Boys spending time outdoors on days 4-6 had statistically significantly increased 1-OHPG concentration from urine collected on day 7 (ß=0.097, $p=0.002$; 95% CI [0.037-0.157]) (Table 2-9). Each hour that boys stayed outdoors on Wednesday through Friday was therefore associated with a 10% increase in geometric mean 1-OHPG concentrations

for urine collected on Saturday. Spending time outdoors was not associated with urinary 1-OHPG in girls. These associations remained after adjusting for indoor air nicotine and $PM_{2.5}$ concentrations (Supplemental Tables 2-11 and 2-12). The gender differences may be explained by differences behaviors, as boys spent more time outdoors than girls during the weekday (median: 2 vs. 1.4 hours; Wilcoxon rank sum $p<0.001$) (Supplemental Table 2-10).

Time spent outdoors and 1-OHPG concentrations by season

The associations between spending time outdoors and urinary 1-OHPG concentrations differed seasonally. Spending time outdoors in the summer (compared to other seasons) was associated with significantly increased urinary 1-OHPG concentrations (ß=0.147, p=0.003; 95% CI [0.048-0.246]) (Supplemental Table 2-13). In addition, summer modulated associations between spending time outdoors and 1-OHPG, as children had significantly higher associations between spending time outdoors and urinary 1-OHPG concentrations in summer than in the other seasons (p-interaction=0.001). During winter, associations were in the opposite direction, with moderately significantly inverse associations between time spent outdoors and urinary 1-OHPG, compared to the other seasons (p-interaction=0.060). Interactions between season and time outdoors for associations with urinary 1-OHPG in spring and fall were not significant. These seasonal interactions remained after individually adjusting for indoor $PM_{2.5}$ and air nicotine concentrations (Supplemental Table 2-13).

DISCUSSION

Urinary 1-OHPG concentrations in this study were higher than a representative sample of the US population (NHANES) and similar international studies, and was influenced by second hand smoke and time spent indoors. 1-Hydroxypyrene-glucuronide (1-OHPG), a urinary metabolite of pyrene, is a short-term biomarker with an estimated half-life of 4-35 hours (Huang et al. 2007). Most 1-OHPG is eliminated in urine in 24-48 hours (Strickland and Kang 1999). The half-life of 1-OHPG may also depend on route of exposure, as the reported half-life from inhalation of pyrene, 6.0-29 hours, is longer than the reported half-life from ingestion, 4.4-12 hours (Jongeneelen et al. 1990; Buchet 1992; Buckley and Lioy 1992; Hu et al. 2006; Li et al. 2012). Our exposure metric, self-reported time apportionment in different environments (e.g. time spent in the home and time spent outdoors) was obtained from daily activity diaries. Time spent in those environments over three days prior to urine collection was averaged to adequately capture the range of attributes (e.g. weekend vs. weekday) and normalize inter-day variability. We hypothesized that having asthma would be associated with 1-OHPG concentrations, as previous studies have shown higher 1-OHP(G) in asthmatics than non-asthmatics (Kim et al. 2005; Cavanaugh et al. 2006). However, in this cohort of mostly asthmatic children, 1-OHPG concentrations did not significantly differ by asthma status. Despite the lack of differences in internal dose PAH biomarkers by asthma status in this study, asthmatics may be more susceptible to adverse health effects from inhaled PAHs (Cho and Moon 2010).

Creatinine correction

Creatinine adjustments of urinary 1-OHPG concentrations to account for

differences in spot urine dilutions were used in some studies (Kang et al. 2002; Epton et al. 2008; Hu et al. 2011; Leyorer et al. 2011; Deziel et al. 2013), and not others (Becker et al. 2003; Leem et al. 2005; Lai et al. 2012; Sul et al. 2012), and some reported both adjusted and unadjusted concentrations (Huang et al. 2004; Huang et al. 2006; Lee et al. 2009; Islami et al. 2012). Urine creatinine output is influenced by age, gender, lean muscle mass, diet (especially meat intake), and kidney function **(**Boeniger et al. 1993**)**. In our study, urinary 1-OHPG concentrations increased, though not significantly, with age. However, when corrected for urinary creatinine (dividing urinary 1-OHPG by urinary creatinine concentrations to adjust for differences in spot urine dilution) these adjusted values showed an inverse relationship with age. Similar trends were seen in children ages 6-19 years old in NHANES III (1999-2000) (Huang et al. 2004), children ages 1-13 years old in Taiwan (Hu et al. 2012), and in a German cohort of children ages 3-17 years old (Heudorf and Angerer 2001). Assuming the children in this study did not have renal dysfunction, the inverse associations between age and creatinine corrected 1-OHPG (1-OHPG/creatinine) were likely influenced by increased PAH burden in younger children (per kilogram body weight) compared to older children, and increased creatinine output in older children (due to increased lean muscle mass, especially during puberty) (Vyskocil et al. 2000). We included urinary creatinine in the model as an independent variable to properly adjust for the relationship between creatinine and the dependent variable (urinary 1-OHPG concentrations), while adjusting for the influence of creatinine on other independent variables (e.g. age and gender) (Barr et al. 2005). 1-OHPG is primarily eliminated from the kidney through the same pathway as creatinine (glomerular filtration and active secretion through the renal tubules), thus including creatinine in the

model may also adjust for variations in 1-OHPG excretion patterns (Viau et al. 2004). Short-term fluctuations in creatinine elimination due to physiological changes in renal functions (e.g. decreased glomerular filtration and increased reabsorption during dehydration events) may also affect 1-OHPG output, and improper or lack of creatinine adjustment may skew analyses of 1-OHPG concentrations. Therefore, we adjusted for urinary creatinine by dividing by urinary creatinine in comparative analyses, and included urinary creatinine as an independent variable in multivariate linear regressions to ensure proper analyses of urinary 1-OHPG concentrations.

Diurnal differences

There were no differences in day 3 (Tuesday) and day 7 (Saturday) urinary 1-OHPG concentrations despite being collected at different times of the day (morning vs. late afternoon, respectively). Diurnal differences in urine 1-OHP(G) concentrations have been previously reported, including higher 1-OHP first void urine concentrations than average daily (24 hour composite) urine (Han et al. 2008), and greater intra-individual variability of 1-OHP concentrations than inter-individual variability (Siwinska et al. 1998; Li et al. 2005). Although 1-OHPG concentrations from Tuesday and Saturday urines were not different, there were differences in urine dilution, as urinary creatinine was significantly higher on day 3. Differences in creatinine excretion throughout the day may reflect dietary and behavioral factors, as creatinine excretion concentration was reported to be higher in late afternoon than early morning, and creatinine elimination rates were significantly higher at night than during the day (Boeniger et al. 1993). Including urinary creatinine as an independent variable in multivariate regression models

therefore normalized urinary 1-OHPG concentrations between the more concentrated Tuesday urine and, the more dilute, Saturday urine.

Comparison to other studies

The geometric mean 1-OHPG concentration for children in this study was higher than that observed in the second (1999-2000), third (2001-2002) and fourth (2003-2004) National Health and Nutrition Examination Surveys (NHANES), nationally representative cross-sectional studies in the United States (Table 2-10, Figure 2-5). Urinary 1-OHPG levels were also higher than most international studies in children. Studies with 1-OHP(G) concentrations that exceeded our study's levels were mostly conducted in regions with major industrial activities (e.g. steel mills, coal-fired power plants and oil refineries) (Kim et al. 2005; Tuntawiroon et al. 2007; Martinez-Salinas et al. 2010; Hu et al. 2011) or high vehicular traffic volume (Kang et al. 2002).

Gender

Urinary 1-OHPG levels were higher in girls than boys in our study. Gender differences remained a significant determinant of 1-OHPG after including creatinine in the model to adjust for urinary dilution. Many studies have higher levels for males (Huang et al. 2004, CDC 2004, CDC 2007, Sul et al. 2012). Similar to our results, a study of South Korean children living close to a steel mill showed higher creatinine corrected 1-OHP concentrations in girls than boys (Lee et al. 2007). The differences, however, were not significant for unadjusted concentrations–highlighting the likely influence of higher urinary creatinine excretion for boys on adjusted 1-OHPG concentrations (Lee et al. 2007). Other studies by Yoon et al. (2012), and Merlo et al. (1998), also showed higher urinary1-OHP(G) in females compared to males. Focusing on 1-OHPG profiles by

age, we found that 13 year-old girls in our study had much higher 1-OHPG levels than boys of the same age. These stark gender differences among 13 year-old children may be due to firsthand cigarette smoking. However, neither the smoking habits of the children or internal dose biomarkers of cigarette smoke exposure (e.g. cotinine) were measured in our study.

Time spent indoors

Time spent indoors showed a "protective" effect for urinary 1-OHPG concentrations. This effect was stronger for urine collected on Saturday (day 7), which reflects weekday (i.e. Wednesday, Thursday and Friday) exposures. Differences in types of exposures, amount of time spent outdoors, and where the children spent time outside of home on the weekday (Saturday urine) and weekend (Tuesday urine) may explain the inverse associations between 1-OHPG and time spent indoors. Children were more likely to be in school for up to 8 hours on the weekdays during the school year. Schools are presumably smoke-free and will likely increase the "protective" effect if the main exposures to PAHs are second hand smoke and outdoor ambient air. In addition, most of the children live in homes with gas or electric heating. Contributions to indoor PAHs from gas furnaces may be less than burning fuels associated with higher PAH concentrations (such as heating oil, coal or kerosene), especially during heating season.

Most of the children (53%) lived in households that reported adults smoking during the study period, which is higher than the US average for children living with a smoker (18%) (CDC 2011), but comparable to the percentage of low-income asthmatic children living with at least on smoker in the home (47-69%) (Butz et al. 2011a). Median indoor air nicotine concentrations in our study were comparable to a similar study of

asthmatic children in Baltimore, MD (median (IQR): 0.13 (0.03-0.96) μg/m^3 vs. 0.12 (0.01-0.61) μg/m^3, respectively) (Matsui et al. 2013). However, mean air nicotine levels in our study were about half as high as a randomized control study in smoking households (at least 5 cigarettes per day) with asthmatic children in Baltimore City (mean (SD): 0.76 μg/m^3 (1.26) vs. 1.43 μg/m^3 (2.16), respectively) (Butz et al. 2011b).

Overall, 1-OHPG levels differed for children exposed to SHS compared to non-exposed children. Similarly, a study by Suwan-ampai et al. (2009) reported that exposures to secondhand smoke were associated with 56-58% higher geometric mean of 1-OHP in children ages 6-19 in the 1999-2000 NHANES. Children spending time in homes with little or no cigarette smoking had significantly lower urinary 1-OHPG concentrations. Residing in non-smoking homes was therefore protective for PAH exposures. Children spending time in smoking homes during the late weekday (Wednesday to Friday) had significantly higher 1-OHPG concentrations (in urine collected on Saturday morning) than children spending time in non-smoking homes. Residing in smoking homes therefore attenuated the "protective" effect.

Indoor air nicotine was significantly associated with elevated 1-OHPG concentrations from day 3 (Tuesday) urine. Indoor air nicotine, however, was not associated with 1-OHPG concentrations from day 7 (Saturday) urine. Trends similar to the associations between tobacco smoke exposures (measured by indoor air nicotine) and 1-OHPG were found using self-reported smoking in the home attained through daily activity questionnaires (i.e. smoking in the home at any time, and average number cigarettes smoked in the home per day) as the SHS exposure metric. These strong associations between SHS exposures (i.e. indoor air nicotine concentrations and self

reported household smoking) and increased 1-OHPG concentrations in our study suggest that SHS contributes to urinary 1-OHPG concentrations. Similar associations between SHS exposure and 1-OHP(G) in children from have been previously reported (van Wijnen et al. 1996, Mucha et al. 2004; Huang et al. 2004; Lee KH et al. 2009; Yoon et al. 2012), while other studies have reported no association (Siwinska et al. 1999; Kang et al. 2002; Lee SM et al. 2009; Hansen et al. 2005). Logistical and behavioral factors may have contributed to the significant influence SHS exposures (as measured by indoor air nicotine) on 1-OHPG in urine collected on an early weekday only. Associations between air nicotine and 1-OHPG in Tuesday urine only may be due to increased exposures to SHS in the home on the weekend and early weekday (Saturday- Monday). Children spent significantly more time in the home during the weekend and early weekday (Saturday-Monday) than during the latter part of the week (Wednesday-Friday). Therefore, increased SHS exposures from increased time spent in smoking homes, may have been reflected in elevated 1-OHPG concentration in Tuesday urine. In addition, the "protective" effect (inverse relationship between spending time in homes with little or no smoking and 1-OHPG concentration) was stronger for Saturday urine (reflecting Wednesday-Friday exposures) than Tuesday urine (reflecting Saturday-Monday exposures), suggesting that children may have been exposed to less cigarette smoke later during the latter half of the week. These intra-week differences in exposure-internal dose relationships may also be due to differences in weekday activities (i.e. children attending school during the day), as well as different behavioral choices by smokers during the weekday/weekend, such as smoking while the child is at school or away from home, smoking outside the home or in rooms other than the monitored room, opening windows

during or after smoking, and smoking by open windows. In addition, children may have been exposed to SHS by spending time in other smoking environments (for example, homes of smoking friends and relatives or in vehicles with smokers), particularly over the weekend, which were not captured by air nicotine monitors in the child's home.

Self-reported smoking in the home and number of cigarettes smoked may give a quantitative assessment of relative frequency and magnitude of smoking. These metrics may, however, introduce reporting bias, which may bias SHS exposure/1-OHPG concentration associations toward the null. In our study, 23 (7%) of the air nicotine samples had levels above the median (0.128 $\mu g/m^3$) despite reporting no cigarette smoking in the home (Figure 2-6a). In addition, 39 samples (13%) had air nicotine levels above 1 $\mu g/m^3$ but reported smoking less than half a pack of cigarettes (10 cigarettes) in the home daily (Figure 2-6b). Similarly, Butz et al. (2011b) reported that 17% of the respondents reported smoking less than 10 cigarettes per day yet had high air nicotine concentrations ($\geq$1 $\mu g/m^3$) in a cohort of asthmatic children from Baltimore, Maryland. In conclusion, self-reported smoking frequency yielded unreliable assessments of second hand smoke exposure. Monitoring of $PM_{2.5}$ and/or air nicotine concentrations (as done in our study) are better metrics for SHS exposure assessment. Internal dose biomarkers of SHS exposure (e.g. urinary cotinine, 3-ethynyl pyridine (3-EP), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)) are best for cigarette smoke exposure assessments because exposures to sources outside of the stationary monitoring sites (e.g. other people's homes, vehicles, and firsthand smoking) are reflected in internal dose measurements (Butz et al. 2011b; Apelberg et al. 2012).

Indoor $PM_{2.5}$

Indoor $PM_{2.5}$ concentrations were not correlated or associated with 1-OHPG concentrations (using multivariate analysis with GEE). Mean (28.5 $\mu g/m^3$) and median (21.7 $\mu g/m^3$) indoor $PM_{2.5}$ concentrations in our study were comparable to other studies in inner city Baltimore, mean: 20.9-45 $\mu g/m^3$ (Breysse et al. 2010; Butz et al. 201lb; McCormack et al. 2009; Sarnet et al. 1999), and median: 20.6-35 $\mu g/m^3$ (Hansel et al. 2008; Hansel et al. 2011). Mean indoor $PM_{2.5}$ levels were below the US Environmental Protection Agency's (EPA) National Ambient Air Quality Standard (NAAQS) for maximum $PM_{2.5}$ concentrations in a 24-hour period, 35 $\mu g/m^3$ (EPA 2013). However, one quarter of the samples, and 36% of the households had $PM_{2.5}$ levels that exceeded the 24-hour standard. Mean indoor $PM_{2.5}$ levels were also about twice the annual standard for ambient $PM_{2.5}$ concentrations, 15 $\mu g/m^3$ (EPA 2013). Nearly 75% of the samples, and 81% of the households had indoor $PM_{2.5}$ levels that exceeded the EPA annual standard during the study. Most children in this study lived in homes that would consistently fail the EPA's standard for maximum annual ambient $PM_{2.5}$ exposure, which may have major implications for health, especially in vulnerable populations such as inner city asthmatic children. Based on the high indoor air PM levels in our study and the correlative associations between PAHs and $PM_{2.5}$, we expected $PM_{2.5}$ concentrations to predict urinary 1-OHPG in multivariate analyses. The absence of an association between indoor $PM_{2.5}$ and 1-OHPG levels may be due to low PAH content on indoor particulates, a possible threshold for airborne PAHs to be reflected in urinary metabolites (that was not reached in this study), or indoor $PM_{2.5}$ concentrations measured in the home did not reflect personal exposures to PAHs on PM, as children may not have been in the

monitored areas for time periods that were sufficient to influence 1-OHPG levels. Our findings of an inverse relationship between children spending time in the home and urinary 1-OHPG in this study therefore suggest that exposures to indoor air PAHs from $PM_{2.5}$ (quantified by indoor air $PM_{2.5}$ concentration) likely had little influence on urinary 1-OHPG concentration.

Time spent outdoors

Overall, time spent outdoors was associated with increased 1-OHPG concentrations, with the strongest associations among day 7 urine samples, reflecting weekday exposures. Specifically, boys who spent time outdoors late in the week had significantly elevated 1-OHPG levels in urine collected on Saturday. The primary source for inhaled PAHs may therefore be outdoors. Similarly, a study of children in Copenhagen, Denmark, showed positive correlations between spending time outdoors on Monday-Thursday and 1-OHP in urine collected the following day (Hansen et al. 2005).

We expected exposures to indoor air pollution (i.e. indoor PM) to be primary predictors of increased urinary 1-OHPG, however we observed the opposite effect, an inverse relationship in non-smoking homes. Exposure to outdoor air may be more potent than indoor air because of higher concentrations of semi-volatile PAHs and particles containing PAHs, and higher PAH content on each particle (i.e. diesel exhaust and particulates from burning of wood and fuel) (Squadrito et al. 2001**)**. Vehicular exhaust, especially diesel exhaust particulates (DEP), are common components of air pollution and are rich in PAH content (Srogi et al. 2007). Larsen et al. (2003) reported that diesel exhaust contributes to 16-26% of the PAH in ambient air in Baltimore City. Exposures to PAHs at school in high traffic areas may also be substantial, as ambient PAH

concentrations from high traffic roads around kindergarten schools were 3-12 times higher than low traffic roads, and indoor PAH concentrations were 6 times higher in kindergarten schools around high traffic roads than low traffic roads in the summertime (Vyskocil et al. 2000; Fiala et al. 2001). Personal monitors measuring PAHs in inner city New York City also reported that hours per day spent outdoors significantly predicated pyrene exposures (Tonne et al. 2004). Inner cities and urban areas, especially poor and mostly African American communities (such as Baltimore City), may therefore experience disproportionately high exposures to ambient air pollution (Miranda et al 2011).

Seasonally, spending time outdoors in summer only, was associated with significantly increased 1-OHPG concentrations in our study. Boys may be driving these associations, as they spend twice as much time outdoors in the summer compared to girls and were likely exposed to more air ambient pollution. A study by Tonne et al. (2004) reported that personal monitors measured higher concentrations of airborne pyrene in the summer compared to the winter in inner city New York City (Tonne et al. 2004). Low molecular weight PAHs (e.g. pyrene and phenanthrene) volatilize with increasing temperature and are found predominantly in gaseous phase (Dimashki et al. 2001). Gaseous phase PAHs and ultrafine particulates are readily deposited in the small airways and alveoli, and have high surface areas per mass, thereby increasing the PAH carrying capacity**,** making them potent toxicants (Squadrito et al. 2001). Exposures to outdoor ambient PAHs may therefore be a significant factor in predicting 1-OHPG levels, as increased exposure to outdoor PAHs (as measured by time spent outdoors) was associated with increased urine 1-OHPG concentrations, especially in boys.

Strengths

To our knowledge, this is the only panel study that examines predictors of 1-OHPG concentrations in inner city Baltimore children. The longitudinal design of the study allowed us to address temporality, seasonal influences, and intra-individual differences. Daily questionnaire administration, measurement of indoor air pollutants throughout the one week monitoring period, and urine specimen collection at multiple time points allowed us to characterize temporally relevant exposures for the outcome, PAH internal dose, measured by urinary 1-OHPG.

Limitations

One drawback to our study is that we did not estimate PAH exposures though ingestion. Dietary intake (e.g. eating roasted, charbroiled or smoked foods) has been shown to explain some of the internal dose of PAH, as ingested PAHs can account for a significant portion of total PAH exposure (Fiala et al. 2000; Vyskocil et al. 2000). In addition, urinary monohydroxylated metabolites of low molecular weight PAHs, including 2-naphthol (naphthalene), 3-hydroxyphenanthrene (phenanthrene) and 9-hydroxyflurorene (fluorine) were not analyzed. These 2-3 ring PAH are predominantly found in gas phase with inhalation as the primary route of exposure, whereas pyrene is introduced to the body through inhalation and ingestion exposures. Incorporation of gas phase PAH metabolites would allow for better assessment of PAH inhalation exposures. Urinary 1-OHPG is a short-term marker of internal dose (half-life of 6-35 h) and may not directly reflect long-term exposure to PAHs.

Outdoor ambient PM and PAHs were also not measured during the one week monitoring period. Although indoor air pollution was the focus of this study, exposure to

outdoor air pollutants may have had a substantial impact on internal dose biomarkers. There also were no assessments of exposures to traffic related pollution, such as geospatial analyses of proximity of the children's homes and schools to major roads, highways, bus and truck depots, and other areas with high traffic volume. Urinary cotinine was also not measured in this study. In addition to quantifying internal dose and SHS exposures, urinary cotinine analysis may identify first hand smokers (which may be a confounder of associations between 1-OHPG and environmental predictors). Finally, gene-environment interactions, such as genetic polymorphisms, were also not assessed in this study. Polymorphisms in phase I (e.g. CYP1A1, CYP1A2) and phase II enzymes (e.g. GSTP1, GSTT1, and GSTM1) may be useful in explaining inter-individual differences in PAH metabolism and urinary 1-OHPG elimination.

Conclusion

Urinary 1-OHPG concentrations in our study was higher than the US national average and many international studies, reflecting the disproportionate burden of exposures to environmental toxicants for inner city predominantly African American communities of low SES. Residing in a non-smoking household confers a "protective" effect for 1-OHPG concentrations compared to residing in a smoking household, as exposures to second hand cigarette smoke contribute to urinary 1-OHPG. Exposures to ambient outdoor PAHs may also influence urinary 1-OHPG concentrations, especially in boys.

TABLES AND FIGURES

Table 2-1. Descriptive characteristics of the DISCOVER/Asthma-Diet study (n=118)

Participant characteristics, total (n=118)	No. (%)
Female	61 (52)
Age, years	10 (5-13)*
Race	
African American	112 (95)
Atopic status	
Non-atopic	33 (30)
Atopic	83 (70)
Asthma status	
Non-Asthmatic	16 (14)
Asthmatic	102 (86)
Caregiver education level (n=116)	
Less than high school	29 (25)
HS diploma	47 (41)
College or higher	40 (34)
Health Insurance (n=113)	
Public	100 (88)
Private or other	16 (12)
Smoking in the house	
No smoking in the house	55 (47)
Smoking in the house	63 (53)
BMI percentile (n=116)	
Normal (BMI percentile 5-85%)	55 (47)
Overweight (BMI percentile 85-95%)	16 (14)
Obese (BMI percentile >95%)	37 (32)
Underweight (BMI percentile <5%)	8 (7)

* Mean (range), at baseline visit

Table 2-2. Descriptive statistics for urinary 1-OHPG, indoor PM and indoor air nicotine concentrations

	1-OHPG (pmol/ml)	1-OHPG (µmol/mol Cr)	Indoor $PM_{2.5}$ ($\mu g/m^3$)	Indoor PM_{10} ($\mu g/m^3$)	Indoor $PM_{2.5-10}$ ($\mu g/m^3$)	Air nicotine ($\mu g/m^3$)
Number of samples	594	594	309	308	280	346
Arithmetic Mean (SD)	1.8 (1.9)	0.17 (0.22)	28.4 (22.8)	41.1 (29.0)	12.7 (14.3)	0.76 (1.26)
Geometric Mean	0.9	0.09	21.8	33.3	9.0	0.17
Median (IQR)	1.2 (0.4-2.4)	0.11 (0.05-0.22)	21.6 (13.0-34.9)	32.6 (21.6-52.5)	9.8 (5.4-15.8)	0.13 (0.03-0.96)
95%ile	5.4	0.54	72.1	97.5	30.8	3.4
Range	0.05-14.59	0.003-2.13	3.7-133.2	5.8-268.8	0.71-215.3	0.005-8.8
% Below LOD	11%	11%	-	-	-	-

Table 2-3. Predictors of urinary 1-OHPG concentrations

	Coef. (β)	P-value	[95% Conf.	Interval]
Urinary creatinine	0.403	**<0.001**	0.259	0.547
Gender (reference: girls)				
Unadjusted for Cr	-0.378	**0.016**	-0.686	-0.069
Adjusted for Cr	-0.385	**0.010**	-0.680	-0.091
Age	-0.023	0.566	-0.100	0.055
Atopic and asthma statuses				
Atopic non-asthmatic (reference)	-	-	-	-
Non-atopic asthmatics	-0.110	0.665	-0.609	0.389
Atopic asthmatic	-0.459	0.057	-0.932	0.014
BMI percentile (continuous)	0.001	0.833	-0.004	0.005
BMI percentile categories				
Normal weight (reference)	-	-	-	-
Overweight	0.007	0.979	-0.495	0.508
Obese	-0.025	0.877	-0.344	0.294
Underweight	-0.162	0.614	-0.790	0.467
Season				
Winter (reference)	-	-	-	-
Spring	0,752	0.572	-0.186	0.336
Summer	-0.072	0.645	-0.377	0.234
Fall	0.079	0.514	-0.158	0.316
Heating season (Nov 1-March 1)	0.081	0.464	-0.135	0.297
Caregiver education				
Some HS (reference)	-	-	-	-
HS diploma	-0.172	0.327	-0.517	0.172
Attended college/college degree	-0.334	0.058	-0.680	0.011
Insurance (ref: public insurance)	-0.071	0.624	-0.356	0.214
Gender; 13 year olds only				
Gender (unadjusted for Cr)*	-1.821	**<0.001**	-2.469	-1.174
Gender (adjusted for Cr) **	-1.511	**<0.001**	-1.928	-1.094

*13 year olds only; Adjusted for atopic/asthma status, season, caregiver's education, BMI percentile
**13 year olds only; Adjusted for urinary creatinine, atopic/asthma status, season, caregiver's education, BMI percentile

Table 2-4. Associations between indoor $PM_{2.5}$, PM_{10}, $PM_{2.5-10}$ and air nicotine concentrations and urinary 1-OHPG concentrations

	Crude*				Adjusted**			
$PM_{2.5}$	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
All samples	0.049	0.605	-0.137	0.235	0.038	0.711	-0.162	0.238
Urine collected on Day 3	0.040	0.757	-0.211	0.290	0.020	0.888	-0.257	0.296
Urine collected on Day 7	0.014	0.902	-0.215	0.244	0.046	0.711	-0.199	0.292
PM_{10}								
All samples	0.140	0.129	-0.041	0.322	0.085	0.385	-0.107	0.278
Urine collected on Day 3	0.143	0.364	-0.165	0.450	0.094	0.580	-0.240	0.429
Urine collected on Day 7	0.142	0.220	-0.085	0.370	0.104	0.390	-0.133	0.342
$PM_{2.5-10}$								
All samples	0.121	0.057	-0.003	0.245	0.117	0.074	-0.011	0.246
Urine collected on Day 3	0.113	0.297	-0.100	0.326	0.083	0.437	-0.127	0.293
Urine collected on Day 7	0.144	0.055	-0.003	0.291	0.177	**0.021**	0.027	0.326
Air nicotine								
All samples	0.050	0.212	-0.028	0.128	0.028	0.464	-0.047	0.103
Urine collected on Day 3	0.157	**0.001**	0.061	0.252	0.129	**0.007**	0.035	0.223
Urine collected on Day 7	0.007	0.923	-0.131	0.144	-0.006	0.928	-0.147	0.134
Air nicotine (MLR, without GEE)								
All samples	0.063	**0.049**	0.000	0.125	0.046	0.171	-0.020	0.111
Urine collected on Day 3	0.117	**0.008**	0.031	0.203	0.097	**0.035**	0.007	0.188
Urine collected on Day 7	0.009	0.815	-0.069	0.088	0.005	0.895	-0.076	0.086

*Adjusted for urinary creatinine
**Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Table 2-5. Average time (hours) spent in the home and time spent outdoors (median (IQR)), and differences by gender

In the Home	All samples	Boys	Girls	P-value[a]	P-value[b]
Avg. time spent in the home on days 0-6 (Sat-Fri)	16.1 (13.6-18.1)	15.9 (12.7-18.0)	16.4 (14.7-18.3)	0.067	0.161
Avg. time spent in the home on days 0-2 (Sat-Mon)	17.0 (13.7-20.3)	16.7 (11.5-19.5)	17.8 (14.3-20.3)	0.059	0.089
Avg. time spent in the home on days 4-6 (Wed-Fri)	15.7 (13.7-17.7)	15.6 (14.0-17.6)	15.7 (13.7-18.0)	0.733	0.704
Difference between days 0-2 and days 4-6	p=**0.005**				
Outdoors					
Avg. time spent outdoors on days 0-6 (Sat-Fri)	1.9 (0.7-3.9)	2.1 (0.8-4.7)	1.6 (0.7-3.3)	0.053	**0.028**
Avg. time spent outdoors on days 0-2 (Sat-Mon)	2.0 (0.5-4.3)	2.0 (0.7-5.3)	1.7 (0.3-3.7)	0.053	**0.033**
Avg. time spent outdoors on days 4-6 (Wed-Fri)	1.5 (0.3-4.0)	1.9 (0.7-4.3)	1.0 (0.3-3.3)	**0.026**	**0.044**
Difference between days 0-2 and days 4-6	p=0.404				

[a] Wilcoxon sign-rank test for difference by gender
[b] Multivariate linear regression with GEE to account for repeated measurements (adjusted for age and season)

Table 2-6. Associations between average time (hours) spent in the home and 1-OHPG concentrations

All Samples	Crude*				Adjusted**			
	β	P>z	[95%	CI]	β	P>z	[95%	CI]
Avg. time in the home all samples	-0.018	0.267	-0.051	0.014	-0.019	0.221	-0.050	0.012
Avg. time in the home days 0-2 (urine day 3)	-0.024	0.105	-0.054	0.005	-0.026	0.076	-0.055	0.003
Avg. time in the home days 4-6 (urine day 7)	-0.038	**0.024**	-0.072	-0.005	-0.045	**0.005**	-0.076	-0.013
By Gender								
Boys								
Avg. time in the home all samples	-0.030	0.176	-0.073	0.013	-0.029	0.209	-0.073	0.016
Avg. time in the home days 0-2 (urine day 3)	-0.043	**0.022**	-0.079	-0.006	-0.045	**0.010**	-0.079	-0.011
Avg. time in the home days 4-6 (urine day 7)	-0.041	0.105	-0.090	0.008	-0.051	**0.038**	-0.099	-0.003
Girls								
Avg. time in the home all samples	-0.014	0.543	-0.059	0.031	-0.023	0.275	-0.065	0.019
Avg. time in the home days 0-2 (urine day 3)	0.014	0.555	-0.033	0.061	0.011	0.668	-0.038	0.059
Avg. time in the home days 4-6 (urine day 7)	-0.047	**0.033**	-0.091	-0.004	-0.046	**0.013**	-0.082	-0.010

*Adjusted for urinary creatinine
**Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Table 2-7. Associations between time spent in the home (continuous and tertiles) and urinary 1-OHPG, stratified by indoor air nicotine

	Low air nicotine				High air nicotine			
Average time spent in the home	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Average time spent in the home (continuous)	-0.075	**0.003**	-0.124	-0.026	0.005	0.835	-0.039	0.048
Average time spent in the home								
5-12 hours (reference)	-	-	-	-	-	-	-	-
13-18 hours	-0.231	0.417	-0.789	0.327	0.117	0.263	-0.258	0.612
19-24 hours	-0.451	0.108	-1.001	0.099	0.372	0.134	-0.083	0.827
Trend*		**0.029**				0.084		

*Cusick's trend test p-value

Figure 2-2. Comparison of associations between urinary 1-OHPG concentration and tertiles of time spent in the home, by indoor air nicotine

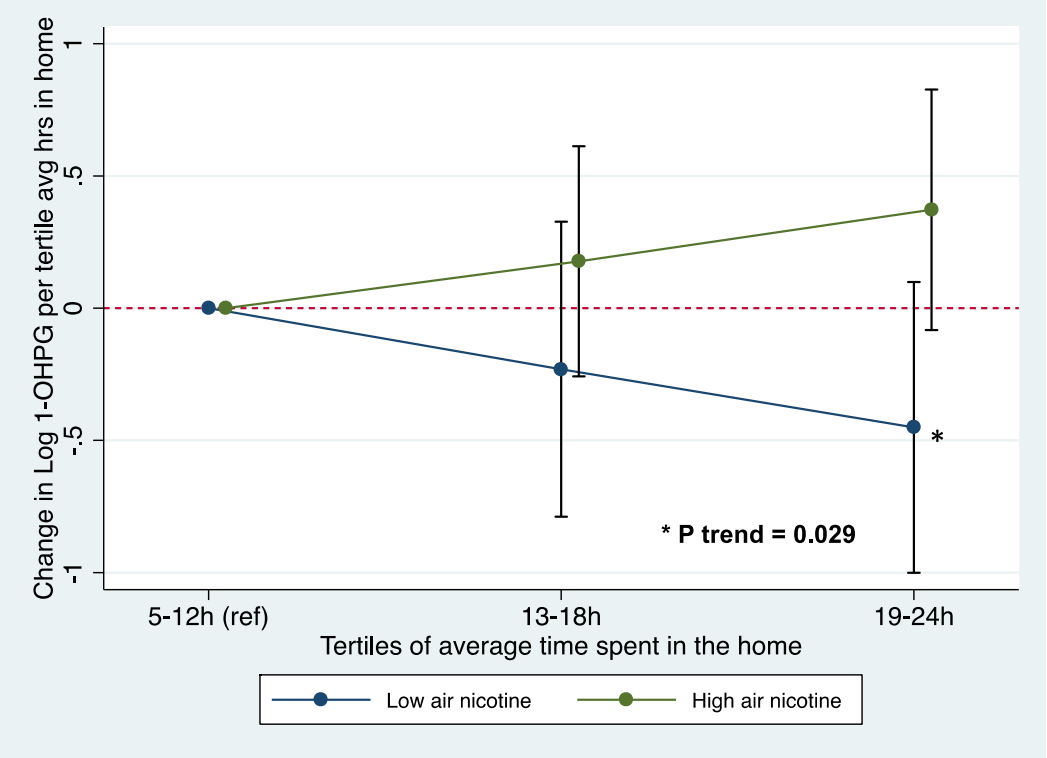


Table 2-8. Associations between time spent in the home and 1-OHPG, stratified by indoor air nicotine

	Low air nicotine				High air nicotine				
	β	P>z	[95%	CI]	β	P>z	[95%	CI]	$P_{interaction}$
Avg. time in home days 0-2 (urine day 3)	-0.034	0.167	-0.083	0.014	-0.008	0.658	-0.041	0.026	0.357
Avg. time in home days 4-6 (urine day 7)	-0.081	**<0.001**	-0.121	-0.041	-0.024	0.285	-0.069	0.020	**0.074**
By Gender									
Boys									
Avg. time in the home all samples	-0.085	**0.008**	-0.148	-0.022	0.018	0.583	-0.047	0.083	**0.014**
Avg. time in home days 0-2 (urine day 3)	-0.055	0.078	-0.116	0.006	-0.023	0.221	-0.061	0.014	0.264
Avg. time in home days 4-6 (urine day 7)	-0.088	**0.005**	-0.149	-0.027	-0.053	0.164	-0.127	0.021	0.623
Girls									
Avg. time in the home all samples	-0.008	0.837	-0.080	0.065	-0.038	0.166	-0.092	0.016	0.693
Avg. time in home days 0-2 (urine day 3)	0.011	0.743	-0.056	0.079	0.032	0.442	-0.049	0.113	0.478
Avg. time in home days 4-6 (urine day 7)	-0.067	**0.011**	-0.119	-0.016	-0.024	0.363	-0.075	0.027	0.381

Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Figure 2-3. Comparison of 1-OHPG concentrations by time spent in the home (days 0-2 and 4-6), stratified by smoking in the home

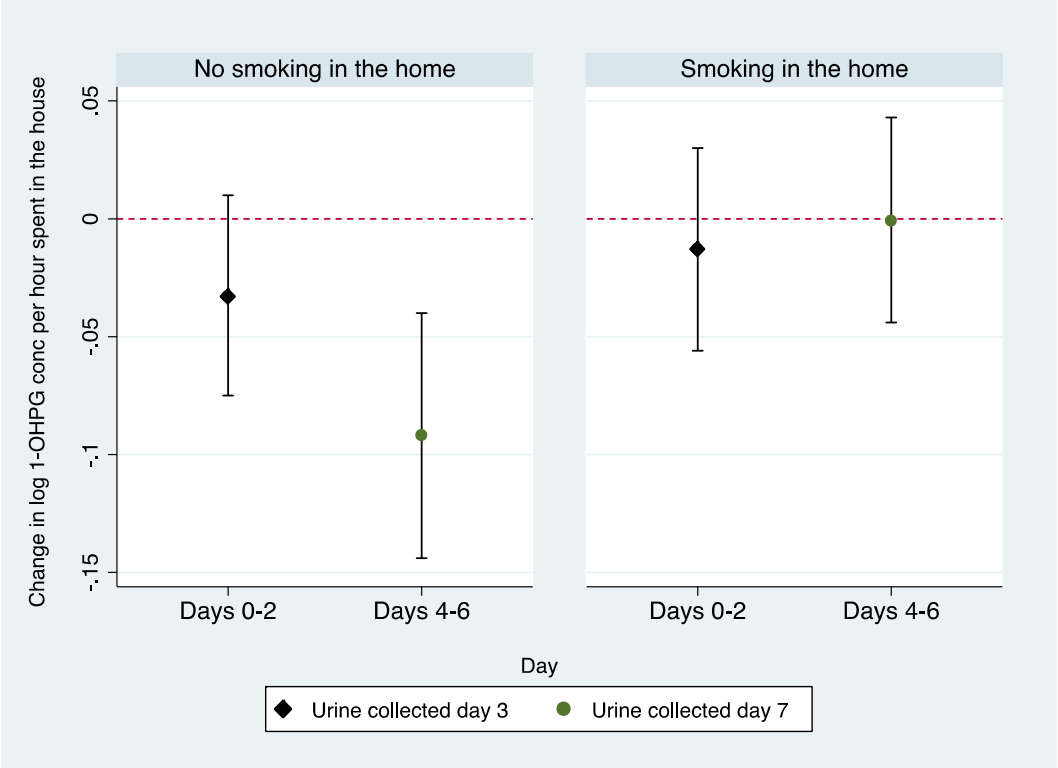


Table 2-9. Associations between average time spent outdoors and urinary 1-OHPG

	Crude*				Adjusted**			
All Samples	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Avg. time in the home all samples	-0.001	0.971	-0.048	0.047	0.014	0.618	-0.040	0.067
Avg. time in home days 0-2 (urine day 3)	0.002	0.939	-0.061	0.066	0.019	0.635	-0.058	0.096
Avg. time in home days 4-6 (urine day 7)	0.030	0.178	-0.014	0.075	0.048	0.054	-0.001	0.098
By Gender								
Boys								
Avg. time in the home all samples	-0.006	0.854	-0.074	0.061	0.004	0.919	-0.073	0.081
Avg. time in home days 0-2 (urine day 3)	-0.009	0.798	-0.082	0.063	-0.008	0.861	-0.095	0.080
Avg. time in home days 4-6 (urine day 7)	0.085	**0.005**	0.026	0.144	0.097	**0.002**	0.037	0.157
Girls								
Avg. time in the home all samples	0.020	0.545	-0.046	0.087	0.031	0.426	-0.045	0.108
Avg. time in home days 0-2 (urine day 3)	0.034	0.585	-0.088	0.156	0.060	0.417	-0.085	0.206
Avg. time in home days 4-6 (urine day 7)	-0.019	0.588	-0.087	0.049	-0.019	0.552	-0.080	0.043

* Adjusted for urinary creatinine
** Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Figure 2-4. Comparison of 1-OHPG concentrations by time spent outdoors (days 0-2 and 4-6), stratified by gender

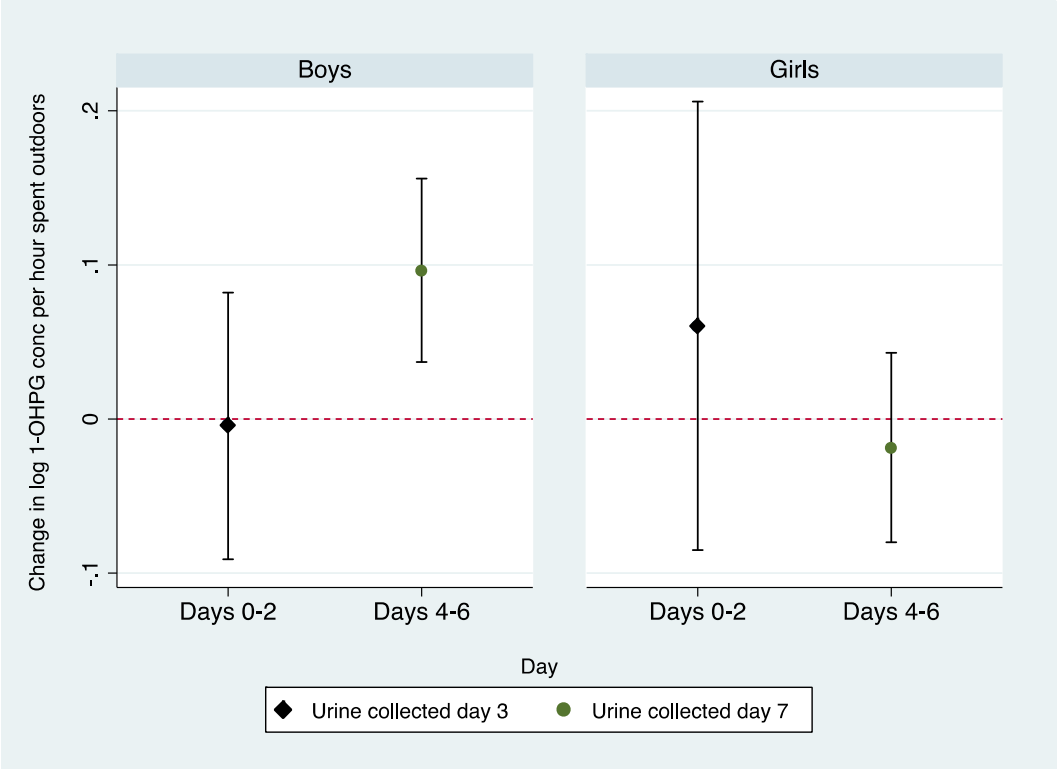
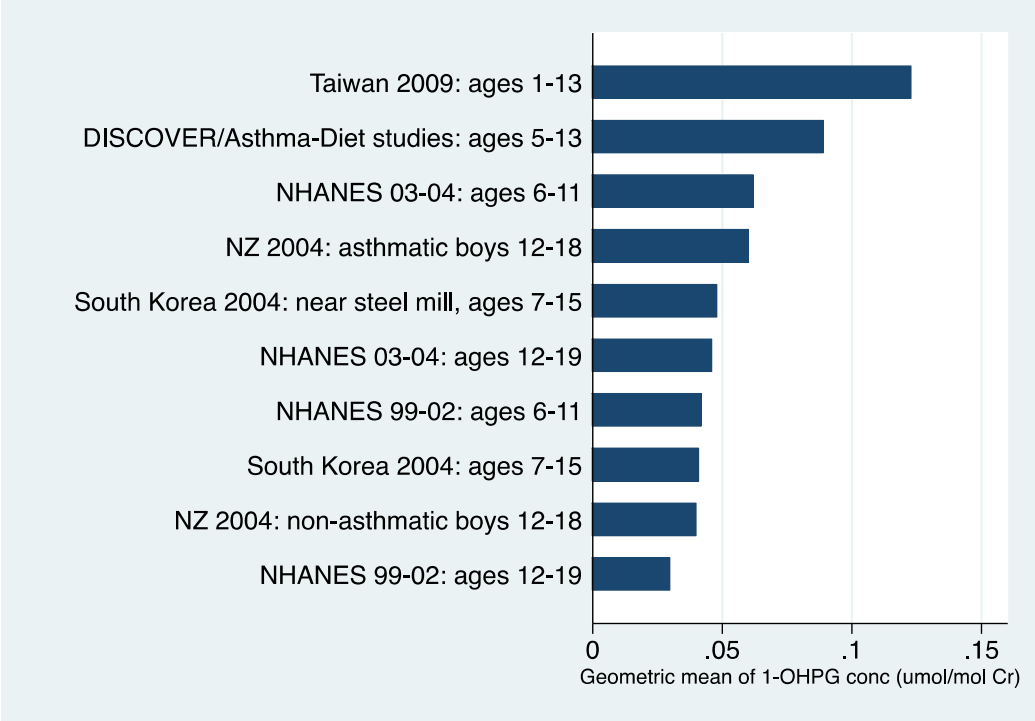


Table 2-10. Urinary 1-OHPG concentrations in children (µmol/mol creatinine)

Study	Year	Age/Gender	Mean (SD) (µmol/mol Cr)	Median (IQR) (µmol/mol Cr)
DISCOVER/Asthma-Diet (n=118)	2008-2012	all: ages 5-13	GM: 0.089 AM: 0.172 (0.207)	0.113 (0.048-0.225)
		boys	GM: 0.072 AM: 1.43 (1.51)	0.097 (0.035-0.203)
		girls	GM: 0.109 AM: 2.07 (2.20)	0.142 (0.064-0.238)
NHANES (US)*	1999-2000	ages 6-11	GM: 0.049	0.047
		ages 12-19	GM: 0.037	0.037
	2001-2002	ages 6-11	GM: 0.035	0.031
		ages 12-19	GM: 0.023	0.023
	Average 1999-2002	ages 6-11	GM: 0.042	0.039
		ages 12-19	GM: 0.030	0.03
	2003-2004	ages 6-11	GM: 0.062	0.058
		ages 12-19	GM: 0.046	0.042
NHANES (Huang et al. 2004)	1999-2000	Non-smokers 12-19	GM: 0.031	
		Smokers 12-19	GM: 0.079	
Christchurch, NZ (Upton et al. 2008) (n=98)	2004 (Autumn) control	boys ages 12-18	GM: 0.019	0.020 (0.01–0.04)
	2004 (Winter) control	boys ages 12-18	GM: 0.023	0.025 (0.01–0.04)
	2004 (Winter I) high pollution	boys ages 12-18	GM: 0.040	0.043
	2004 (Winter II) high pollution	boys ages 12-18	GM: 0.039	0.042
	2004 (Autumn) control	asthmatic boys 12-18	AM: 0.027 (0.021)	0.023
	2004 (Winter) control	asthmatic boys 12-18	AM: 0.032 (0.018)	0.028
	2004 (Winter I) high pollution	asthmatic boys 12-18	AM: 0.060 (0.033)	0.058
	2004 (Winter II) high pollution	asthmatic boys 12-18	AM: 0.060 (0.065)	0.042
South Korea (Kang et al. 2002) (n=137)	1997	ages 11-13	GM: 0.10	0.09
Mexico (Sanchez-Guerra et al. 2012) (n=82)	2008	ages 6-10	GM: 0.38	0.37

Taiwan (Hu et al. 2011)	2009 all samples (n=387)	ages 1-13	GM: 0.123 (2.19)	
	High exposure community 1		GM: 0.153 (2.03)	
	High exposure community 2		GM: 0.141 (2.12)	
	Low exposure community 1		GM: 0.085 (2.28)	
	Low exposure community 2		GM: 0.096 (2.01)	
San Louis Potosi, Mexico (Martinez-Salinas et al. 2010)	Tercera, 75 brick kilns (n=30)	ages 3-13	AM: 0.2 (0.2)	0.2 (0.09-0.4)
	Domingo, heavy traffic (n=17)	ages 3-13	AM: 0.2 (0.2)	0.1 (<LOD-0.2)
	Centro, urban moderate traffic (n=39)	ages 3-13	AM: 0.08 (0.2)	0.08 (0.04-0.1)
Thailand (Tuntawiroon et al. 2007)	Urban school children day 1	boys ages 9-13		0.16
	Rural school children day 1	boys ages 9-13		0.11
Guangzhou, China (Fan et al. 2012)	Control (n=35)	ages 6-7	AM: 0.14 (0.11)	0.1
	Polluted area (n=39)	ages 4-6	AM: 0.65 (0.07)	0.55
South Korea (Lee SM et al. 2009)	2004 all samples (n=72)	ages 7-15	GM: 0.050 (2.42)	
Samples taken over 3 consecutive days	Close to steel mill (n=38)		GM: 0.062 (2.12)	
	Remote site (n=34)		GM: 0.040 (2.65)	
South Korea (Lee SM et al. 2009)	2004 all samples (n=1012)	ages 7-15	GM: 0.041 (2.23)	
	Close to steel mill (n=406)		GM: 0.048 (1.88)	
	Remote site (n=606)		GM: 0.041 (2.43)	
Germany (Heudorf and Angerer 2001)	Children (n=261)	ages 6-12		0.06
Denmark (Hansen et al. 2005)	Urban children (n=204)	ages 3-13		0.1
Yonsei, South Korea (Kim et al. 2005)	Asthmatic children (n=30)	mean age: 4.9	GM: 0.430 (0.343)	
	Non-asthmatic children (n=30)	mean age: 5.5	GM: 0.239 (0.175)	

Figure 2-5. Comparison of geometric means of 1-OHPG concentrations



Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Figure 2-6a. Comparison of indoor air nicotine concentration (µg/m^3) with average number of cigarettes per day. Subjects identified in the square have high air nicotine concentrations (> 0.128 µg/m^3) and no reported cigarette smoking in the home.

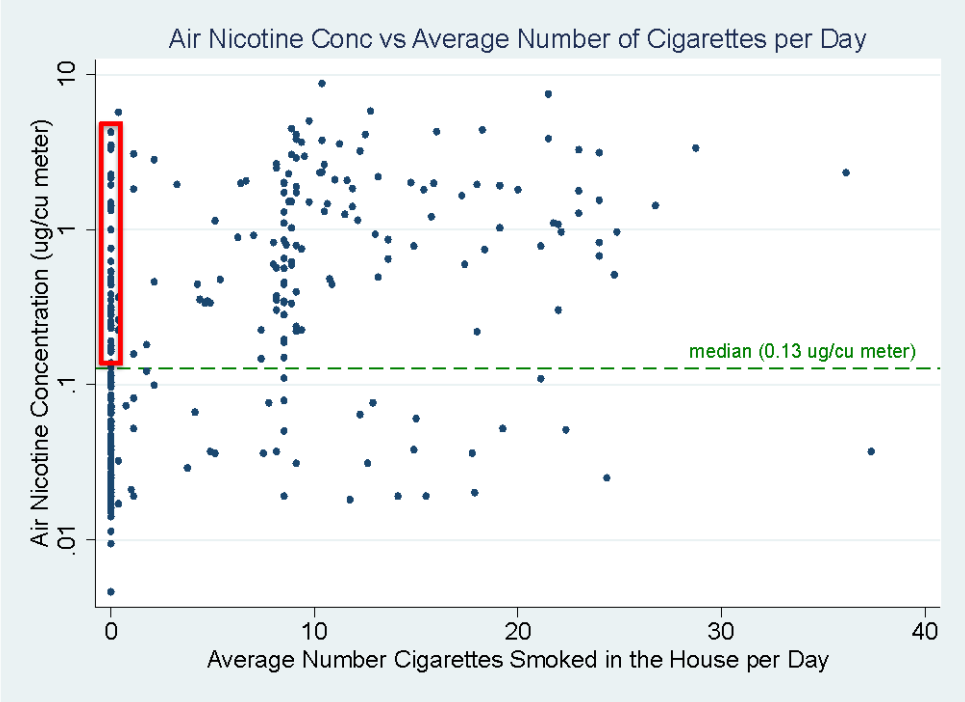
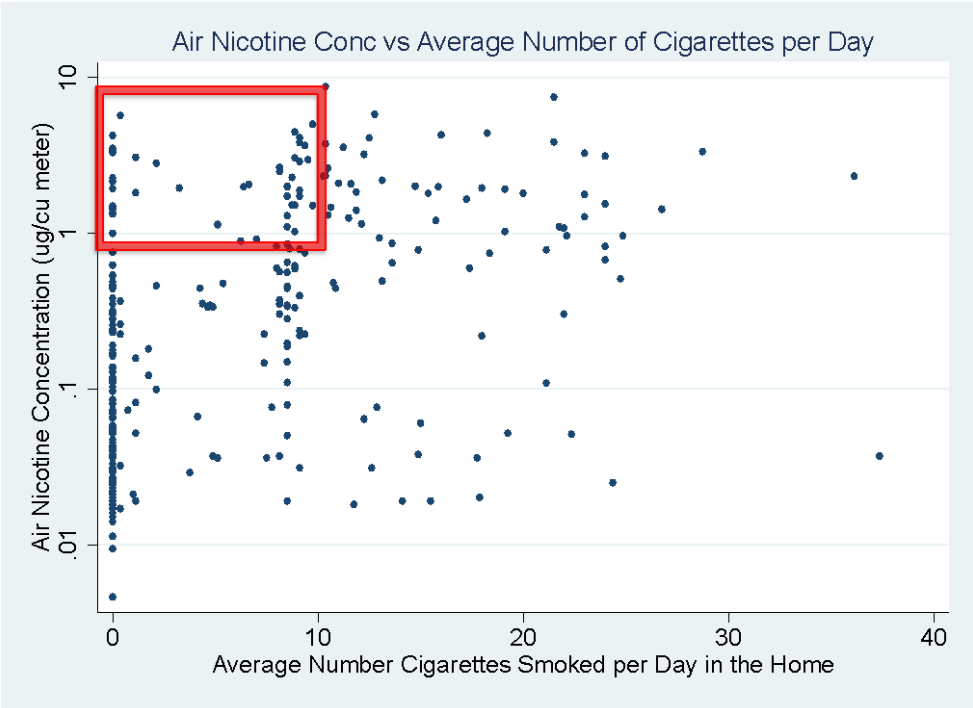


Figure 2-6b. Comparison of indoor air nicotine concentration (µg/m^3) with average number of cigarettes per day. Subjects identified in the square have air nicotine concentrations >1 µg/m^{3}and average ≤10 cigarettes smoked per day.



CHAPTER 3: Polycyclic aromatic hydrocarbon internal dose, oxidative stress, inflammatory biomarkers, and symptoms in asthmatic children

ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAH) are products of incomplete combustion of organic materials. PAH internal dose is commonly measured using urinary 1-hydroxypyrene-glucuronide (1-OHPG) or its deconjugated derivative, 1-hydroxypyrene (1-OHP), the most common urinary metabolite of pyrene. PAHs have been reported to have adjuvant activity in allergic inflammatory responses, and PAHs have been associated with increased oxidative stress, inflammation and asthma-related symptoms. We examined whether PAH internal dose was associated with systemic oxidative stress and systemic inflammation in inner city asthmatic children in Baltimore, and whether being atopic modified these associations.

Methods: The DISCOVER/Asthma-Diet study is a longitudinal (panel) study with a total of 118 participants ages 5-14 from Baltimore City. Children were followed for one-week periods in each of four seasons. Urine specimens were collected on day 3 and day 7 of each week, and analyzed for 1-OHPG and malondialdehyde (MDA); blood was collected on day 7 and analyzed for peripheral blood biomarkers. Day and nighttime asthma-related symptoms and medication use were assessed for using daily diary questionnaires.

Results: Urinary 1-OHPG was not significantly associated with the oxidative stress biomarker, urinary MDA, but was associated with biomarkers of systemic inflammation: peripheral white blood cell count (ß=0.019, p=0.030), and eosinophil count (ß=0.061, p=0.012) and percent (ß=0.415, p=0.002). Atopy modified the associations between urinary 1-OHPG and eosinophil and neutrophil counts, with significantly higher

associations among atopic asthmatic children compared to non-atopic asthmatic children (p-interaction=0.052, and p-interaction=0.057, respectively). Children with high urinary 1-OHPG concentration were at a three to four-fold higher risk of waking during the night due to asthma symptoms in the 2-4 days prior to urine collection, and had a three to five-fold higher risk of nighttime waking due to asthma symptoms during the 2-4 days after urine collection (on Tuesday) than children with low 1-OHPG concentration. Having high 1-OHPG concentration also was also associated with a two-fold higher risk of nighttime rescue medication (ß-agonist) use both during the 2-4 days prior to urine collection and over the 4 days following urine collection.

Conclusions: Exposures to PAHs were associated with biomarkers systemic inflammation, and were modulated by atopy, with more pronounced inflammatory responses among atopic asthmatic children compared to non-atopic asthmatic children. In addition, PAH exposures were associated with increased risk of nighttime waking due to asthma symptoms and rescue medication use. Our results suggest that exposures to combustion products in the form of PAHs may contribute to systemic inflammation and asthma exacerbation.

INTRODUCTION

Asthma

Asthma is a chronic inflammatory disease of the airways characterized by reversible airway obstruction, sensitivity of the nerve endings and an influx of inflammatory cells and cellular elements (i.e. eosinophils, neutrophils, macrophages, mast cells and T-lymphocytes) (Delfino et al. 2002, Dozor et al. 2010). Asthma is the most prevalent chronic disease in children, afflicting 7 million children (9.5%) in the United States in 2010 (Akinbami et al. 2012). African-American children have a disproportionate asthma burden, with higher prevalent asthma (11.1%) than the national average (Akinbami et al. 2012**)**. Atopy is an allergic phenotype characterized by increased production of IgE in response to specific allergens, and diagnosed with skin prick test (Pearce et al. 1999; Suh and Koh 2013). Most asthmatic children have an atopic constitution and are therefore vulnerable to inflammatory responses from multiple stimuli (Nelson et al. 1999; Eggleston 2000; Lau et al. 2000; Breysse et al. 2005). Co-exposures to allergens and asthma triggers (such as second hand smoke (SHS), particulate matter (PM), ozone and nitrogen dioxide (NO_2)) in atopic asthmatics may lead to increased frequency of asthma exacerbations (D'Amato et al. 2002; Diaz-Sanchez et al. 2006). Inner city children are a particularly vulnerable population, as they experience both higher asthma burden and increased exposures to substances associated with asthma exacerbation and severity, including SHS, indoor and outdoor PM, indoor allergens (e.g. mouse, cockroach, and dust mite allergens), endotoxin, mold and fungus (Wallace et al. 2003; Breysse et al. 2005; McCormack et al. 2007; Simons et al. 2007; Matsui et al. 2008).

Oxidative stress and MDA

Oxidative stress, caused by an abundance of reactive oxygen species (ROS) (such as superoxide, peroxide and hydroxyl radicals), is associated with asthma pathogenesis and exacerbation (Saleh et al. 1998; Leem et al. 2005; Ercan et al. 2006; Nadeem et al. 2008; Cho et al. 2010; Dozor et al. 2010). Malondialdehyde (MDA), a by-product of lipid peroxidation of polyunsaturated fatty acids, is commonly analyzed in urine and serum to assess oxidative stress **(**Kang et al. 2005). Systemic oxidative stress, measured by serum MDA, is associated with asthma and increased asthma severity, with asthmatics having higher concentrations than non-asthmatics (Jacobson et al. 2007; Ercan et al. 2006; Nadeem et al. 2003). Urinary MDA is also correlated with internal dose polycyclic aromatic hydrocarbons (PAHs), measured by urinary 1-hydroxypyrene-glucuronide (1-OHPG) (Kang et al. 2005; Bae et al. 2010; Yoon et al. 2012). Mechanisms for asthma exacerbations due to PAH exposures (from PM and diesel exhaust particles (DEP)), that are mediated by oxidative stress, have also been reported (Pandya et al. 2002; Li et al. 2003). The proposed mechanism for biomolecular and physiological responses to oxidative stress is multi-tiered, depending on levels oxidative stress generated and the capacity to counteract oxidative stress through antioxidant pathways. Low levels of oxidative stress may lead antioxidant responses through the induction of the Nrf2 pathway and phase II enzymes. At moderate levels, oxidative stress may overwhelm antioxidant responses and trigger pro-inflammatory activator protein-1 (AP-1) and nuclear factor kappa-B (NF-kB) pathways, which are associated with increased cytokines, chemokines and immune mediators (e.g. interleukin (IL)-8, IL-13, macrophage inflammatory protein-1α (MIP-1α), monocyte chemotactic protein-3 (MCP-3) and

regulated on activation, normal T-cell expressed and secreted (RANTES)) (Li et al. 2003). High levels of oxidative stress may lead to superoxide generation, mitochondrial damage, necrosis and apoptosis (Nel et al. 2001; Gilmour et al. 2006; Li and Nel 2006; Reidl et al. 2008; Cho and Moon 2010). Moderate to high ROS burdens from PAH exposures may therefore elicit inflammatory cascades and cytotoxicity, which may contribute to airway inflammation and asthma exacerbations.

PAHs

Polycyclic aromatic hydrocarbons are formed from the incomplete combustion or pyrolysis of organic materials (e.g. coal, wood, fuel and oil) and are commonly found on fine and ultrafine particulates, $PM_{2.5}$ (aerodynamic diameter <2.5 μm) and $PM_{0.1}$ (aerodynamic diameter <100 ng), respectively (Rosa et al. 2011). Sources of airborne PAH exposures include motor vehicle emissions (combustion products from diesel and conventional gasoline engines), burning fossil fuels (e.g. coal and oil), wood burning, tobacco smoking, cooking with gas stoves, heating appliances (e.g. kerosene space heaters), and incense burning (ATSDR 1995; Larsen et al. 2003). Diesel exhaust particulates (DEP), which are present in the fine ($PM_{2.5}$) and ultrafine ($PM_{0.1}$) particulate fractions have high concentrations of volatile and semi-volatile PAHs (i.e. naphthalene, fluorine, phenanthrene, pyrene) on the particulate surface. DEP are potent inducers of allergic and non-allergic inflammatory responses due to high oxidation potential of activated PAHs, formed through the conversion of PAHs to oxidative reactive intermediates (such as quinones, semiquinones and epoxides) by cytochrome P450 (CYP) (Nel et al. 2001; Reidl et al. 2008). PAHs are primarily known for their carcinogenicity, as PAHs and PAH mixtures have been categorized as human carcinogens (i.e.

benzo[a]pyrene and coal tar pitch), probable carcinogens (e.g. dibenzo[a,l]pyrene and dibenz[a,h]anthracene) and possible carcinogens (e.g. benz[a]anthracene, benzo[b]fluoranthene and indeno[1,2,3-cd]pyrene) by the International Agency for Research on Cancer (IARC), and the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR 1995; IARC 2010). PAHs, have also been associated with the development of asthma (Miller et al. 2004; Jung et al. 2012), neurodevelopmental deficiencies (Perera et al. 2006), and low birth weight (Perera et al. 2003) in inner city children, most likely through non-mutagenic inflammatory mechanisms.

1-OHPG

Urinary 1-hydroxypyrene-glucuronide (1-OHPG) is an accepted biological indicator of PAH exposure, as its parent compound, pyrene, is present in virtually all environmental PAH mixtures and is a reliable biomarker of low-level exposure (Buchet et al. 1992; Jongeneelen 2001). Urinary 1-OHPG concentrations reflect exposures through inhalation, ingestion, and dermal absorption (from contaminated air and soil) (Jongeneelen 2001). 1-hydroxypyrene (1-OHP) and 1-OHPG are commonly used urinary biomarkers, however 1-OHPG is more sensitive, as the glucuronide molecule confers 3-5 times more fluorescence than 1-OHP (Strickland et al. 1996; Kang et al. 2005). 1-OHPG levels have been associated with increased levels of oxidative stress biomarkers (i.e. urinary MDA and 8-OHdG), decreased lung function, and having asthma, and higher 1-OHP concentrations have been reported in asthmatic children than non-asthmatic children (Kim et al. 2005; Leem et al. 2005; Bae et al. 2010; Hong et al. 2009). Molecular pathways for (oxidative stress and allergic inflammation mediated) asthma-related responses to PAH exposures by diesel exhaust particulates have been explored in *in vitro*,

in vivo and human studies. The proposed mechanism for PAH-asthma exacerbation is thought to involve high concentrations of quinones, semiquinones and epoxides derived from biotransformed PAHs overriding protective pathways (i.e. antioxidants and phase II enzymes), leading to increased oxidative damage in cells and the surrounding tissues, and subsequent biomolecular, immunological and physiological responses (e.g. airway inflammation and airway hyperreactivity) associated with asthma exacerbations (Nel et al. 2001; Ercan et al. 2006; Park et al. 2006; Cho and Moon 2010). PAHs can also target eosinophils, basophils, neutrophils, and antigen presenting cells, and act as adjuvants for allergic sensitization (Nel et al. 1998; Delfino et al. 2002). In epidemiologic studies, exposures to PAHs early in life have been shown to act as adjuvants to the development of allergies to cockroach proteins (Bla g 2) in an inner city birth cohort (Perzanowski et al. 2009); and adjuvant activities by PAHs have been shown to increase allergic inflammatory responses among atopic individuals challenged with PAH-rich DEP and allergens intranasally in randomized crossover studies (Diaz-Sanchez et al. 1997; Diaz-Sanchez et al. 1999).

Our overall goal was to evaluate associations between internal dose of PAHs (i.e. 1-OHPG) and oxidative stress (by analyzing urinary MDA), as well as associations between PAH internal dose and peripheral blood biomarkers of inflammation, and asthma-related symptoms and medication use. We also evaluated whether atopic constitution is an effect modifier of the associations between internal dose PAHs and inflammatory biomarkers, and asthma symptoms in asthmatic children. We hypothesize that urinary 1-OHPG concentrations will be associated with urinary MDA concentrations, increased peripheral blood inflammatory biomarkers, and an increased risk for asthma-

related symptoms. We also hypothesize that atopic asthmatics will have stronger associations between urinary 1-OHPG and peripheral blood biomarkers and asthma-related symptoms, based on synergistic properties of PAHs on allergic inflammatory responses in allergen sensitized individuals.

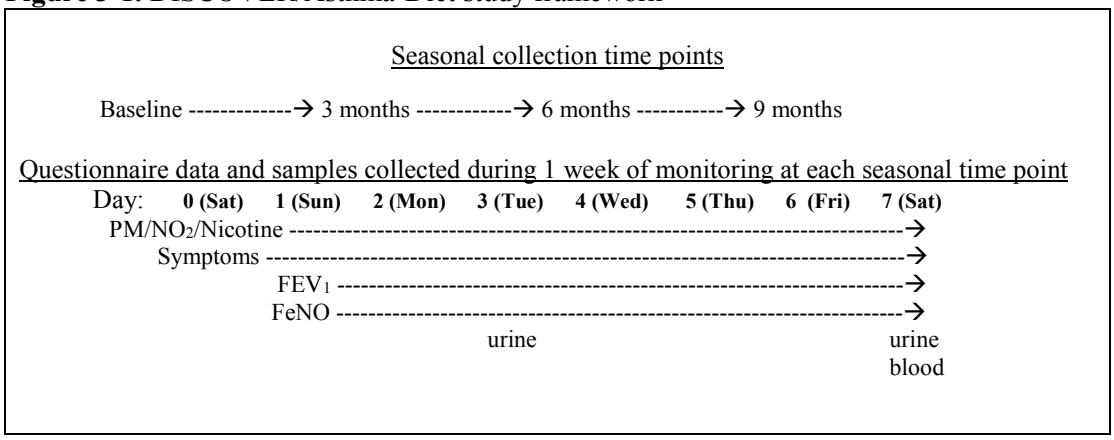
METHODS

Study

The Role of Particulate Matter and Allergens in Oxidative Stress in Asthma (DISCOVER) and Asthma-Diet (**A S**tudy to understand **Th**e **M**echanisms of **A**sthma-**D**ietary **I**nterventions to protect against **E**nvironmental **T**riggers) studies have the same study design, as the Asthma-Diet study is an extension of the DISCOVER study (with a dietary component). The DISCOVER/Asthma-Diet study is a panel study that enrolled 100 atopic asthmatic and 50 non-atopic asthmatic, and 30 non-asthmatic children ages 5-12, from nine contiguous zip codes in inner city East Baltimore. Indoor PM and allergen exposures, diet, lung function, asthma symptoms, and biomarkers of oxidative stress and inflammation were assessed for a one week period, in each of 4 seasons (Figure 3-1) (Dr. Greg Diette, personal communication). Inclusion criteria included: age 5-12 years, physician diagnosis of asthma, symptoms of asthma and/or reliever medication use in past 6 months, and residence in Baltimore city. Children were excluded from the study if they had a current diagnosis of another major pulmonary disease, if they were planning to relocate residence during the study period, were currently taking antioxidant supplements, had a known food allergy, had metabolic or dietary disorders or were

pregnant. Each season, the children's homes were monitored for 8 days (day 0-7), air nicotine, $PM_{2.5}$, $PM_{2.5-10}$, PM_{10}, and NO_2 concentrations were measured. Asthma-related symptom questionnaires were administered (in the morning and evening); urine was collected on the fourth (day 3, Tuesday) and eighth day (day 7, Saturday), and blood was collected on the eighth day (day 7). The morning (AM) and night (PM) symptom diaries asked questions about sleep interruption due to asthma, inhaler use, trouble breathing, doctor of emergency room (ER) visits for asthma, prednisone treatment, and physical activity limitation due to asthma (see Appendix B).

Figure 3-1. DISCOVER/Asthma-Diet study framework



Malondialdehyde

Malondialdehyde (MDA) concentrations were analyzed using high-performance liquid chromatography (HPLC) with fluorescence detection measured as modified from Giera et al. (2011).

MDA standard preparation

10mM malondialdehyde standard was prepared using a modified method previously described by Kang et al. (2006). 0.084 ml 1,1,3,3-tetramethoxypropane (TMP) was

mixed with 1ml 1.0N HCl in a 40°C bath for 10 minutes, with frequent shaking. 1mL 1.0N NaOH was added to increase the pH. 48 ml of 0.1M phosphate buffer was then added to bring the total volume to 50 ml.

2-Aminoacridone preparation

5nM stock solution 2-aminoacridone (2-AA) was prepared weekly. Briefly, 9 mg of 2-AA was mixed with 860 acetonitrile (ACN).

Assay

MDA concentrations were analyzed with HPLC and laser-induced fluorescence (LIF). 0.5 ml of the urine sample was mixed with 40µL 2-AA and 460 µL 0.1M citrate buffer (pH 4.0) and incubated in a water bath for 90 minutes at 40°C. Five microliters of the sample were injected into an HPLC system consisting of a Rheodyne injector with a 5 mm loop, Agilent 1100 series pumps, Series 56 helium-cadmium Omnichrome laser, and a Picometrics Zetalif LIF detector (P/N1701- 201) set to 325 nm. Separation was achieved with a Zorbax SB C18 5 mm, 150 0.5 mm column. The sample was eluted using a 20-minute linear gradient with acetylnitrile (ACN) and water (25%–90% ACN) followed by a cleaning regimen (90% ACN over 5 minutes). This system was operated at a flow rate of 5 mL/min. Area under the peak at the retention time of 12 minutes was determined by manually integrating the peaks using ChemStation Software Rev. A.08.04 (Agilent Technologies). MDA concentrations, reported as pmol/ml, were calculated by dividing fluorescence peak area by the slope of 1.5 from MDA standard curves (water spiked with 0, 50, 100 and 200 pmol/ml). The limit of detection was 33 pmol/ml. The MDA range in our study was <33-726 pmol/ml (mean: 96.2 pmol/ml). Giera et al. (2011) reported a range 50-500 pmol/ml with mean of approximately 150 pmol/ml (personal

communication with Giera).

Urinary 1-OHPG

Spot urine samples were analyzed for urinary 1-OHPG concentrations using immunoaffinity chromatography (IAC) and synchronous fluorescence spectroscopy (SFS), as previously described (Strickland et al. 1994). Urine samples (2ml) were treated with 0.1N HCl (90°C, 60 min), neutralized, and loaded onto Sep-pak C18 cartridges (Waters). After washing with 30% methanol, the relatively non-polar metabolites were eluted with 4 ml of 80% methanol and the volume of eluate is reduced to 0.5ml by evaporation on a Speedvac. The concentrated samples were diluted to 4ml with 15mM phosphate buffered saline (PBS) and loaded onto immunoaffinity columns prepared with 0.8ml cyanogen bromide activated sepharose 4B (Sigma) coupled with monoclonal antibody 8E11 that recognizes several PAH-DNA adducts and metabolites (Santella et al. 1986). We have previously shown that 1-OHPG binds to these columns (Strickland et al. 1994). After washing the columns three times with 4 ml 15 mM PBS, bound compounds were eluted with 2 ml of 40% methanol in three fractions. Eluate fractions were analyzed by SFS with a Perkin-Elmer LS50 fluorescence spectrometer. The excitation-emission monochromators are driven synchronously with a wavelength difference of 34 nm. Under these conditions 1-OHPG produces a characteristic fluorescence excitation maximum at 347 nm with emission maximum at 381 nm (Strickland et al. 1994). Fluorescence intensity was used to quantify 1-OHPG; in our laboratory the limit of detection was about 0.03 pmol/ml. This level of sensitivity is sufficient to detect urinary 1-OHPG in >90% of subjects with low-level exposure to PAHs (such as in the U.S.), and in 100% of subjects

with high exposure. The coefficient of variation of the assay is typically 6-10% (interbatch) in our laboratory.

Complete blood counts

Peripheral blood total cell counts, differential counts and platelet counts and were done using an automated counter.

Urinary creatinine

Creatinine concentrations were determined using a modified version of the Jaffe reaction using the Creatinine Assay Kit (Cayman Chemical Company; Ann Arbor, MI). Briefly, 150 µl of alkaline picrate solution was added to wells containing 15 µl creatinine standard or urine samples in duplicate, and the solution was mixed for 10 minutes. The absorbance of the solution (due to the reaction between the alkaline picrate and urinary creatinine metabolites) was read at 450-500 nm using a Biotek ELx800 Absorbance Microplate Reader. 5 µl of acid solution was then added to the assay and the absorbance was read again at 450-500 nm, 20 minutes after mixing. The difference in color intensity before and after acidification is proportional to urinary creatinine concentration. Urinary creatinine levels were determined using a creatinine standard curve, which was estimated in each batch from analysis of the standard creatinine.

Air nicotine

Two passive sampling badges were placed in the child's bedroom or TV/family room at 3–5 feet off the floor. The passive air samplers consist of a sodium-bisulfate treated filter contained in a 37-mm polystyrene cassette covered with a polycarbonate filter diffusion screen. Nicotine content was analyzed using gas chromatography with a

nitrogen-phosphate detector. The limit of detection for the passive air nicotine badges was 0.003 $\mu g/m^3$.

Statistical analysis

Summary statistics were reported for urinary1-OHPG and MDA concentrations. 1-OHPG and MDA concentrations were log-transformed to adjust for positively skewed distributions. Urinary 1-OHPG and MDA concentrations on day 3 (Tuesday; n=255) and day 7 (Saturday; n=339) and averages for the monitoring period (n=359) were analyzed separately. Individual day (day 3 or day 7) 1-OHPG and MDA values were used instead of the average when urine samples for both days were not collected. Multivariate linear regressions with generalized estimating equations (GEE) were used to assess associations between the primary predictor (e.g. urinary 1-OHPG) and outcome (dependent) variables (i.e. urinary MDA, peripheral blood biomarkers, asthma-related symptoms) while adjusting for repeated measurements (i.e. monitoring periods) and possible confounders. Analyses of relationships between 1-OHPG and peripheral blood biomarkers and asthma symptoms were performed for asthmatics only. Explanatory variables were considered possible confounders if the ß-coefficient changed by more than 10% after inclusion of the exposure variable (e.g. time spent in the home) in the model or if the variable was significantly associated with the exposure or outcome. Our final models for multivariate linear regression with GEE were adjusted for age, gender, season, atopic status and caregiver educational attainment. Asthma symptom analyses were also adjusted for indoor air nicotine concentration. Urinary 1-OHPG and MDA concentrations were adjusted for urinary creatinine by including creatinine concentrations in the model as an independent variable (Barr et al. 2005). Age, 1-OHPG, MDA, creatinine, air nicotine,

peripheral blood biomarkers were measured as continuous variables; asthma symptoms, gender and atopic status were binary variables; BMI percentile and season were categorical variables; and caregiver education was measured as ordinal variables. Atopic constitution was defined as having allergic responses to at least one of 13 common aeroallergens (dust mite mix, rat and mouse epithelia, dog hair/dander, cat dander, American and German cockroach, three pollens (Eastern Oak mix, grass mix, ragweed mix) and four molds (*Helminthosporium, Alternariam, Penicillium* and *Aspergillus*)) from skin prick tests or having anti-mouse IgE using radioallergosorbent test (RAST).

Effect modification was assessed using pairwise interaction terms for independent variables (e.g. the product of 1-OHPG concentrations and atopic status) and interaction terms were considered statistically significant at p-value<0.1. Comparisons of two groups were performed using Wilcoxon sign-rank test and Mann-Whitney U test was used for comparisons of exposure variables. Pearson's correlation coefficient and Spearman's correlation coefficient examined correlations between internal dose biomarkers and indoor air concentrations. Two-tailed p-value<0.05 was used to determine statistical significance. Urine was collected in the afternoon or early evening of day 3 (Tuesday), and in the morning to early afternoon on day 7 (Saturday). 1-OHPG and MDA concentrations were reported as both pmol/ml and μmol/mol creatinine, and concentrations for air nicotine was reported as $\mu g/m^3$, and blood biomarkers were reported as percent or count density ($cells/mm^3$). Reasons for missing data included inability to contact the caregiver or obtain urine samples (especially during the weekday (day 3)) and blood samples from the child and incorrect or unusable questionnaire data.

Missing values were not included in analyses. All data were analyzed using STATA 11.1 (College Station, TX)

RESULTS

Overall MDA

Urinary malondialdehyde concentrations were analyzed for 594 spot urine samples (Table 3-1). Urinary MDA had an arithmetic mean (standard deviation (SD)) of 10.4 (9.6) µmol/mol creatinine (Cr) and a median (IQR) of 7.6 (4.4-12.9) µmol/mol Cr. Fifty-nine samples (10%) were below the limit of detection of 33 pmol/ml. Log-transformed creatinine corrected MDA concentrations from urine collected on day 3 (Tuesday) and day 7 (Saturday) were significantly correlated (Pearson's r: 0.25, $p<0.001$) and associated (ß=0.204, p=0.001; 95% CI [0.086-0.322]) (Tables 3-2 and 3-3). Using multivariate linear regression analyses with GEE to account for repeated measurements, log transformed urinary MDA concentrations were associated with urinary creatinine (ß=0.246, $p<0.001$; 95% CI [0.153-0.339]), and obesity (ß=0.151, p=0.054; 95% CI [-0.003-0.305]), particularly in asthmatics, but not with gender, age, atopic status, season, or caregiver's educational status (Table 3-2).

Overall MDA and 1-OHPG

Urinary 1-OHPG concentrations from 594 spot urine samples had an arithmetic mean (SD) of 0.17 (0.22) µmol/mol Cr and a median (IQR) of 0.11 (0.05-0.22) µmol/mol Cr (Table 3-1). Creatinine-corrected urinary MDA was correlated with urinary 1-OHPG concentrations (Pearson's r: 0.11, p=0.042) (Table 3-3). However, urinary MDA was not

associated with 1-OHPG concentrations in urine collected on day 3, day 7 or in the averaged urinary measurements, using multivariate linear regression analysis (accounting for repeated measurements and adjusting for possible confounders) (Table 3-5).

MDA in asthmatics

Urinary MDA concentrations were analyzed in 532 spot urine samples from asthmatic children (Table 3-1). Urinary MDA had an arithmetic mean (SD) of 10.6 (9.8) µmol/mol Cr and a median (IQR) of 7.8 (4.6-13.0) µmol/mol Cr. Forty-nine samples (9%) were below the limit of detection of 33 pmol/ml. Log-transformed creatinine corrected MDA concentrations from urine collected on day 3 were significantly correlated (Pearson's r: 0.25, $p<0.001$) and associated (ß=0.187, p=0.004; 95% CI [0.061-0.314]) with MDA concentrations from day 7 (Tables 3-2 and 3-4). Log transformed MDA concentrations were also associated with urinary creatinine (ß=0.248, $p<0.001$; 95% CI [0.155-0.340]) and being obese (compared to normal weight) (ß=0.181, p=0.024; 95% CI [0.024-0.338]), but not with gender, age, atopic status, asthma status, or caregiver's educational status (Table 3-2).

MDA and 1-OHPG in asthmatics

Urinary 1-OHPG concentrations in 532 spot urine samples from asthmatic children had an arithmetic mean (SD) of 0.17 (0.21) µmol/mol Cr and a median (IQR) of 0.11 (0.05-0.23) µmol/mol Cr. (Table 3-1). Creatinine adjusted urinary MDA concentrations were correlated with urinary 1-OHPG concentrations (Pearson's correlation coefficient: 0.12, p=0.033) (Table 3-4). MDA concentrations were not associated with 1-OHPG concentrations in urine collected on day 3, day 7 or in the averaged urinary measurements, using multivariate linear regression analysis (accounting

for repeated measurements and adjusting for possible confounders) (Table 3-5). However, creatinine unadjusted urinary MDA was significantly associated with creatinine unadjusted 1-OHPG concentration in urine collected on day 3 in asthmatic children (ß=0.089, p=0.024; 95% CI [0.012-0.165]) (Table 3-5).

Peripheral blood biomarkers summary

Peripheral blood eosinophil count (Wilcoxon rank-sum test: $p<0.001$), and eosinophil, basophil and leukocyte percent were higher in atopic asthmatic children than non-atopic asthmatic children (Wilcoxon rank-sum test: $p<0.001$, $p<0.001$ and $p=0.045$, respectively) (Table 3-6). Peripheral blood neutrophil count and percent (Wilcoxon rank-sum test: $p=0.024$ and $p=0.001$, respectively) and monocyte count (Wilcoxon rank-sum test: $p=0.037$), were higher in non-atopic asthmatics than atopic asthmatics.

1-OHPG and peripheral blood biomarkers

Peripheral blood biomarkers from blood draws on day 7 (Saturday) were compared to urinary 1-OHPG concentrations from urine collected on day 3 (Tuesday) and day 7 (Saturday), and an average of the two samples (averaged 1-OHPG) (Table 3-7). Urinary 1-OHPG concentrations on day 3 were significantly associated with increased peripheral blood eosinophil counts (ß=0.061, p=0.012; 95% CI [0.014-0.108]), eosinophil percent (ß=0.415, p=0.002; 95% CI [0.154-0.676]), and white blood cell count (ß=0.019, p=0.030; 95% CI [0.002-0.035]) from blood collected on day 7 (Table 3-7). A dose-dependent relationship between quartiles of 1-OHPG from day 3 urine and peripheral blood eosinophil counts was also observed (Table 3-8, Figure 3-2). 1-OHPG concentration in urine collected on day 7, and 1-OHPG concentration averaged over the

one-week monitoring period, however, were not associated with peripheral blood biomarkers (Table 3-7).

Effect modification by atopic status

Atopy was examined as a possible effect modifier of associations between urinary 1-OHPG concentrations and peripheral blood inflammatory biomarkers in asthmatic children only (Tables 3-9 to 3-11; Figures 3-3 to 3-6).

Associations with 1-OHPG from urine collected on day 3

1-OHPG concentrations in urine from day 3 were significantly associated with increased peripheral blood eosinophil counts (ß=0.079, p=0.001; 95% CI [0.033-0.125]) and eosinophil percent (ß=0.524, p<0.001; 95% CI [0.258-0.790]) in atopic asthmatic children (Table 3-9; Figure 3-3 and 3-4). Urinary 1-OHPG concentrations were also significantly associated with decreased monocyte percent (ß=-0.229, p=0.030; 95% CI [-0.436-(-0.022)]) and marginally associated with decreased basophil percent (ß=-0.017, p=0.096; 95% CI[-0.037-(-0.003)]) in non-atopic asthmatic children.

Atopic status modified associations between urinary 1-OHPG and peripheral blood eosinophil percent, with atopic asthmatics experiencing more pronounced increases in eosinophil percent associated with urinary 1-OHPG (ß=0.524, p<0.001) than non-atopic children (ß=0.016, p=0.860) (p-interaction=0.052) **(**Table 3-9, Figure 3-4**)**. Similar patterns were seen with 1-OHPG concentrations and peripheral blood neutrophil counts (atopic asthmatic children: ß=0.019, p=0.343; non-atopic asthmatic children: ß=0.004, p=0.864) (p-interaction=0.062). Atopy also modified the associations between urinary 1-OHPG concentration on day 3 and peripheral blood monocyte count and percent, with associations by atopic status in opposite directions (Table 3-9, Figure 3-3). 1-OHPG was

positively (though non-significantly) associated with peripheral blood monocyte counts in atopic asthmatics (ß=0.018, p=0.294; 95% CI [-0.016-0.053]), whereas non-atopic asthmatics had inverse (though non-significant) associations between 1-OHPG and peripheral blood monocyte counts (ß=-0.021, p=0.152; 95% CI [-0.049-0.008]) (p-interaction=0.083). Similarly, atopy modified the associations between 1-OHPG and peripheral blood monocyte percent, with non-atopic asthmatic children showing significantly stronger inverse associations than atopic asthmatic children (non-atopic asthmatics: ß=-0.229, p=0.030; atopic asthmatics: ß=-0.013, p=0.864) (p-interaction=0.001) (Table 3-9).

Associations with 1-OHPG from urine collected on day 7

Atopy modified associations between urinary 1-OHPG concentration and peripheral blood monocyte percent from urine and blood samples collected on the same day (day 7 (Saturday)), with associations in opposite directions (Table 3-10, Figure 3-6). Atopic asthmatics showed marginally significant positive associations between 1-OHPG and monocyte percent (ß=0.131, p=0.085; 95% CI [0.018-0.279]), whereas 1-OHPG and monocyte percent were non-significantly inversely associated in non-atopic asthmatics (ß=0.072, p=0.618; 95% CI [-0.354-0.210]) (p-interaction=0.016). Atopic status also modified inverse associations between urinary 1-OHPG concentration and peripheral blood neutrophil counts (Figure 3-5). Atopic asthmatics had stronger non-significant inverse associations between 1-OHPG and neutrophil counts (ß=-0.023, p=0.322; 95% CI [0.068-0.022]) compared to non-atopic asthmatics (ß=-0.001, p=0.968; 95% CI [-0.052-0.050]) (p-interaction=0.096).

1-OHPG and symptoms

Asthma-related symptoms and medicine use were assessed each day of the weeklong monitoring period, days 0-7 and compared to urinary 1-OHPG concentrations. Associations were analyzed for creatinine adjusted 1-OHPG (dichotomized at the median) and symptoms two, three and four days prior to spot urine collection on day 3 (Tuesday) and day 7 (Saturday); two and four days after urine collection on Tuesday; as well as over the entire week (Tables 3-12 and 3-13; Supplemental Tables 3-1 to 3-5). High 1-OHPG concentrations from urine collected on Tuesday were associated with approximately 3-4 times higher risk of waking during the night due to asthma symptoms (such as wheeze) in the 2-4 days prior to urine collection, and children with high 1-OHPG were 3-5 times more likely to wake during the night due to asthma symptoms during the 2-4 days after urine collection (Table 3-12, Figure 3-7). Children with high urinary 1-OHPG concentration from urine collected on Saturday were approximately 3 times more likely to be woken at night due to asthma during the previous 4 nights (Thursday-Saturday) than children with low 1-OHPG (OR=2.98, p=0.015; 95% CI [1.239-7.147]). Children with high 1-OHPG from the weekly averaged urine concentration were 3 times as likely to be woken due to asthma symptoms from Wednesday to Saturday (days 4-7) than children with low urinary 1-OHPG (OR=3.36, p=0.019; 95% CI [1.223-9.214]) (Table 3-12, Figure 3-8).

1-OHPG concentrations from urine collected on Tuesday were also associated with 2 times higher risk of nighttime rescue medication (ß-agonist) use during the previous 2-4 days compared to children with low 1-OHPG (OR=2.05, p=0.048; 95% CI [1.005-4.181]) (Table 3-13, Figure 3-9). Children with high 1-OHPG from Tuesday urine

were also approximately two times more likely to use rescue medication during the night or early morning over the next 4 days (Tuesday-Saturday) (OR=2.48, p=0.014; 95% CI [1.201-5.104]) (Table 3-13, Figure 3-9).

There was little evidence for associations between 1-OHPG and other asthma-related symptoms (Supplemental Tables 3-1 to 3-11).

DISCUSSION

Urinary 1-OHPG and MDA

The proposed mechanism for exacerbation of asthma-related symptoms involves exposures to PAH (measured as internal dose, urinary 1-OHPG) inducing oxidative stress (measured by urinary MDA) leading local and systemic inflammation and subsequently asthma exacerbation. Although urinary 1-OHPG and MDA were correlated in our study, they were not associated using both cross-sectional multivariate linear regression (MLR) and longitudinal analyses (MLR with GEE, with urinary creatinine included in the model as an independent variable, and adjusting for possible confounders). Leem et al. (2003) reported similar lack of a relationship between internal dose PAHs (urinary 1-OHPG) and urinary MDA in asthmatic children. Other studies have reported associations between 1-OHP(G) and MDA (Kang et al. 2005; Bae et al. 2010; Yoon et al. 2012), however, these reports may be misleading because the thiobarbituric acid reactive substances (TBARS) (used to measure urinary MDA) is a non-specific assay and may underestimate concentrations and suggest relationships that are not representative of oxidative stress. In our study, MDA was analyzed using a recently developed assay that measured the

fluorescence of 2-aminoacridone (2-AA) covalently bound to MDA (2-AA-MDA) (Giera et al. 2011). TBARS assays were commonly used to analyze urinary MDA concentrations because the assay is time and cost efficient (i.e. TBARS can be measured using fluorescence from multi-welled plates) (Janero et al. 1990; Esterbauer et al. 1991; Kang et al. 2005). The 2-AA-MDA assay is more specific for MDA than TBARS because 2-thiobarbituric acid (TBA) may cross-react with other aldehydes, sugars, amino acids, oxidized lipids, and by-products resembling MDA–formed through the breakdown of various compounds from the harsh conditions of TBA reactions (incubation in acidic solution at high temperatures (90°C) for up to an hour) (Esterbauer et al. 1991; Draper et al. 2001; Giera et al. 2011). MDA can be measured in two forms, free (unbound) MDA–as measured in this study, and total MDA (free MDA+bound MDA)–measured by TBARS (de Vecchi et al. 2009). Analysis of urinary free MDA concentration quantifies recent lipid peroxidation activity and current oxidative stress, whereas TBARS concentrations, although up to 10 fold higher, are less specific and reflect old oxidative stress events (de Vecchi et al. 2009). Analysis of short-term biomarkers of internal dose PAHs (urinary 1-OHPG) and oxidative stress biomarkers (free MDA concentrations) may therefore give a snapshot of recent PAH exposures and early biologic effects. Analysis of other oxidative stress biomarkers, such as urinary isoprostane (8-iso-$PGF_{2\alpha}$) and urinary 8-hydroxy-deoxyguanosine (8-OHdG), in conjunction with urinary free MDA analysis (using urinary 2-AA-MDA) may improve assessments of systemic oxidative stress and oxidative damage, and better reflect early biological effects in individuals exposed to environmental pollutants.

1-OHPG and peripheral blood biomarkers

In this study, 1-OHPG concentrations from urine collected on Tuesday showed more significant associations with peripheral blood biomarkers collected on Saturday than urinary 1-OHPG and peripheral blood markers both collected on Saturday. Peripheral blood biomarkers assess systemic inflammation, and the short half-lives of circulating neutrophils (6-8 hours), eosinophils (8-18 hours), and basophils (a few days) are similar to urinary 1-OHPG (6-35 hours, mean 15.4 hours) (Stock et al. 2000; Hu et al. 2006; Stone et al. 2009). Associations between urinary 1-OHPG and peripheral blood biomarkers may therefore reflect short-term inflammatory responses to exogenous exposures**.**

Urinary 1-OHPG concentration was independently associated with increased peripheral white blood cell count, highlighting the relationships between internal dose PAHs and systemic inflammation. Similar positive associations were found in cross-sectional, nationally representative studies in the United States from 1999-2004 (the National Health and Nutrition Surveys (NHANES II, III and IV)) (Alshaaraway et al. 2013). In our study, PAH internal dose (from urine collected on Tuesday) was also independently associated with increased peripheral blood eosinophil counts and percent in atopic asthmatics children, and atopy modified these associations, with significantly stronger PAH-eosinophil count associations in atopic asthmatics than non-atopic asthmatics. This relationship between PAH internal dose and peripheral eosinophils supports the concept of PAH induced inflammation mechanisms and has implications for asthma exacerbation, as eosinophilic inflammation (eosinophilia) is an integral component of allergen-mediated inflammatory responses that are associated with asthma-

related symptoms (Stone et al. 2009). Peripheral blood eosinophils are associated with total serum IgE and positive skin prick test responses, which is consistent with an allergic phenotype in children (Oryszczyn et al. 2007). Eosinophilia is also an integral component of asthma attacks and asthma severity in children. A meta-analysis showed half of childhood asthma cases were attributed to eosinophilic airway inflammation (Douwes et al. 2002). Bossley et al. (2012) reported that severe asthmatic children overwhelmingly present eosinophilic asthma phenotype. In this study, significant independent associations between internal dose PAHs and increased peripheral blood eosinophils suggest that PAHs influence eosinophilia in atopic asthmatic children, and effect modification by atopy also suggests significant interaction between PAHs and allergen-mediated inflammatory responses in atopic asthmatic children.

We did not measure exposures to aeroallergens in this study, however, previous studies quantifying allergen exposures in the homes of inner city asthmatic children in New York City and Baltimore City have reported high background levels of mouse urinary protein (Mus m 1), dust mite allergen (Der f 1), and cockroach allergen (Bla g 2) (Perzanowski et al. 2009; Matsui et al. 2006; Matsui et al. 2010). It is therefore likely that the participants in our study (inner city asthmatic children from Baltimore City) experienced similarly high exposures to aeroallergens, and co-exposures with indoor and ambient outdoor PAHs. The significantly increased levels of peripheral blood eosinophils in atopic asthmatic children with high PAH exposures, and comparatively higher peripheral neutrophils and monocytes in atopic asthmatics than non-atopic asthmatics suggests a synergistic relationship between PAHs and allergens, with PAHs acting as adjuvants for allergic inflammatory responses. PAH adjuvant properties have been

reported in human nasal provocation studies with (PAH rich) diesel exhaust particulates and allergens (e.g. ragweed and keyhole limpet hemocyanin (KLH)) in atopic individuals. In those studies, allergic inflammatory responses were enhanced in individuals exposed to both DEP and allergens, leading to marked increases in allergen specific IgE, increases in Th2-type cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13), and induction of chemokines related to eosinophil recruitment (RANTES, MIP-1α, and MCP-3) (Diaz-Sanchez et al. 1994; Diaz-Sanchez et al. 1997a; Diaz-Sanchez et al. 1997b; Nel et al. 1998; Bastain et al. 2003). PAHs can also target macrophages, leading to increased IL-8, RANTES, and MIP-1α, induction of adhesion molecules, initiation of chemokine and cytokine cascades, and recruitment of pro-inflammatory cells (e.g. eosinophils and neutrophils) (Nel et al. 1998; Nel et al. 2001; Pandya et al. 2002). The comparatively higher peripheral blood monocyte levels in atopic asthmatic children in our study may therefore reflect interactions between PAHs and monocytes. Peripheral blood monocytes differentiate into antigen presenting cells (macrophages and dendritic cells) in the tissues and are integral in enhancing allergen-mediated inflammatory responses.

Among non-atopic asthmatic children in our study, 1-OHPG was independently associated with decreased peripheral blood monocytes, and atopic status modified the associations, with non-atopic asthmatics showing stronger inverse associations than atopic asthmatics. Interactions between PAHs and suppression of blood monocytes in non-atopic asthmatics likely has a different mechanism than the adjuvant activities of PAH among atopic individuals, however, further research is needed to explore the mechanism. Fahy et al. (1999) reported that peripheral blood mononuclear cells (PBMC) exposed to DEP-PAH showed dose-dependent decreases in MCP-1 concentrations, and

increases in IL-8 and RANTES (which are associated with neutrophil and eosinophil recruitment, respectively), suggesting a preference for neutrophils and eosinophils induction, and a suppression of monocytes and macrophages in response to DEP-PAH exposures. In non-atopic asthmatics, asthma pathogenesis and exacerbation have been associated with a neutrophilic inflammatory phenotype, which differs from the mostly eosinophilic inflammatory phenotype in atopic asthmatics (Douwes et al. 2002). Neutrophilic inflammation and airway obstruction is characterized by non-allergenic, innate immune inflammatory responses, and has been shown to be induced by PM, NO_2, ozone, endotoxin and viruses. Exposures to DEP have also been shown to increase neutrophil mobilization and neutrophilic inflammation through the NF-kB inflammatory pathway (Nightinggale et al. 2000; Mukae et al. 2001; Douwes et al. 2002).

Associations between internal dose of PAHs and peripheral blood monocytes were modified by atopic status in our study. On both urine collection days, monocyte percent was significantly modified by atopy, with more pronounced positive associations between 1-OHPG and peripheral blood monocytes in atopic asthmatics than non-atopic asthmatics. *In vitro* studies have shown diminished differentiation and maturation of monocyte derived dendritic cells (Laupeze et al. 2002) and macrophages (van Grevenynghe et al. 2003) after PAH exposures, suggesting altered trafficking of monocytes. However, the mechanism for altered peripheral blood monocyte levels by PAH exposures, and the influence of atopy on this pathway, is not known. Similarly, atopy marginally modified the positive associations between peripheral blood eosinophil counts and urinary 1-OHPG, with more pronounced associations in atopic asthmatics compared to non-atopic asthmatics. Atopy was an effect modifier for 1-OHPG-peripheral

blood biomarker associations involving urine collected four days prior to blood collection, but not for urine and blood collected on the same day. These differences in effect modification by sample collection day (for statistically similar 1-OHPG concentrations in Tuesday and Saturday urines) may be due to differences in the types of PAH exposures (e.g. outdoor, indoor or SHS exposures), differences in the relative potencies of the PAH mixtures (such as, inflammatory, adjuvant and immunotoxicity potentials), and differences in routes of PAH exposures (inhalation or ingestion) on those days. PAHs reflected in urine collected on Tuesday may be largely from airborne sources with high oxidative potential (i.e. from DEP and SHS), which may confer increased local and systemic inflammatory responses, and increased adverse pulmonary outcomes in asthmatic children. These differences in PAH internal dose-peripheral blood biomarker associations by urine collection day may also reflect differences in temporality of the inflammatory responses to PAH exposures. Acute inflammatory responses may be captured in urine and blood collected on the same day, while longer-term inflammatory responses may be captured in blood collected four days after urine collection. Inflammation may last for hours to days after PAH exposures, and may increase over time, especially if the PAH exposures persist and if there are co-exposures to aeroallergens–which may potentiate the effects of PAHs in atopic asthmatics.

1-OHPG and asthma-related symptoms

In this study, significant associations were found between urinary 1-OHPG and nighttime and early morning asthma symptoms. Children with high exposures to PAHs were at an increased risk to wake up during the night due to wheezing and other asthma symptoms, and use ß-agonist (Albuterol) rescue inhalers during the night and early

morning. Associations between PAH internal dose and symptoms were found on days prior to, and after urine collection on Tuesday, suggesting both prolonged exposures to PAHs in the beginning of the week (i.e. Saturday through Tuesday), and lasting effects of those exposures on asthma related outcomes for the rest of the week (Wednesday through Saturday). In a study of the effects of daily PAH exposures on asthma symptoms, Gale et al. (2012) reported that increased exposures to ambient PAHs were associated with higher odds of wheeze in asthmatic children ages 6-11 years old in Fresno, CA (Gale et al. 2012). Exposures to phenanthrene, a semi-volatile PAH, were associated with significant increases in odds of wheeze with lags for 1-4 days, and for 2 day and 5 day moving averages (Gale et al. 2012). Nighttime asthma symptoms and medication use may be particularly important to the health and well being of asthmatic children, as nighttime awakening is a predictor of poor asthma control and may lead to decreased sleep, missed school and poor performance in school (Diette et al. 2000; Hansel et al. 2011). ß-agonist use among asthmatic children was also shown to be an indicator of poorly controlled asthma and asthma severity (Diette et al. 2000).

Asthma-related responses to PAH exposures differed by atopic phenotype, with non-atopic asthmatic children at increased risk for asthma symptoms. Internal dose of PAHs were independently associated with overall risk of nighttime waking due to asthma and ß-agonist use in non-atopic asthmatics only. 1-OHPG from urine collected on Saturday and nighttime waking due to asthma and ß-agonist use during the previous 2-3 days (Thursday and Friday) showed slightly stronger positive associations in non-atopic asthmatic children than atopic asthmatic children (with moderate interactions by atopic status; p-interaction<0.2). Jung et al. (2012) reported significantly higher odds of wheeze

in non-atopic asthmatic children ages 5-6 years old who were repeatedly exposed to PAHs (prenatally and during a two week monitoring period at ages 5-6 years old) in an inner city birth cohort. Asthma symptoms during the night may involve non-allergen mediated mechanisms, which may result in increased symptoms in non-atopic asthmatics compared to asthmatic children. In a review of studies characterizing lung eosinophilia in asthmatics, Douwes et al. (2002) found that about half of childhood asthma cases were attributed to non-eosinophilic inflammation. The mechanism for non-atopic asthma exacerbations involved innate immune responses linked to environmental exposures (such as particulates, ozone and NO_2, eotaxin and viruses) and NF-kB mediated neutrophilic inflammation (Douwes et al. 2002). Non-atopic asthmatic children also showed greater bronchial hyperresponsiveness after methacholine challenges than atopic asthmatics, and may therefore be more susceptible to pulmonary irritants (Mochizuki et al. 1999). In addition, Li et al. (2006) showed that cough frequency was associated with sputum neutrophil cell counts in atopic children with mild asthma, highlighting the importance of possible independent (non-allergen mediated) inflammatory pathways for symptoms in asthmatic children (Li et al. 2006). Neutrophilic inflammation may therefore be important in both susceptibility to exposures to airborne pollutants such as PAHs, and subsequent increased asthma-related outcomes in non-atopic asthmatic children.

Our study showed associations between PAH internal dose and early effect peripheral blood biomarkers of inflammation (i.e. eosinophil count and percent, and white blood cell count), and an increased risk of nighttime asthma symptoms and rescue medication use. Systemic eosinophilic inflammation–a key contributor to the

pathophysiology of asthma–was associated with PAH internal dose in atopic asthmatics, suggesting a synergistic relationship between PAH exposures and allergen mediated inflammatory responses (e.g. skewing toward Th2-type pathways, increased IgE production, and increased cytokine and chemokine production). These findings support the proposed mechanisms for PAH-asthma exacerbation involving adjuvant PAH activities and allergic inflammation in atopic asthmatics. However, there were no significant associations between PAH internal dose and asthma-related symptoms in atopic asthmatics. PAH adjuvant activity may therefore predispose atopic asthmatics to increased inflammation, but progression to asthmatic symptoms may also include mechanisms (such as neutrophilic inflammation) that are not directly related to allergen-mediated responses. More research is needed to quantify PAH exposures (e.g. airborne PAH monitoring and multiple internal dose biomarkers) and inflammation (e.g. inflammatory biomarkers from various biospecimens); evaluate allergic and asthma related responses in epidemiologic studies of affected populations; and to further investigate mechanisms linking PAH exposures, local and systemic inflammatory responses, and asthma exacerbation.

Strengths

This is the first panel study to examine associations between internal dose of PAHs, measured by urinary 1-OHPG concentrations, peripheral blood biomarkers, and asthma-related symptoms in inner city Baltimore children. The longitudinal design of the study allowed us to assess associations over a one-year period, account for seasonal differences, and intra-individual differences over time. Daily diaries of symptoms and urine specimen collection at multiple time points also allowed us to assess

exposure/outcome relationships for temporally relevant time periods. This study also adds to the growing literature on effects of PAHs on inflammation and asthma-related outcomes. This study also focuses on a vulnerable population (predominantly African American children from low SES neighborhoods in inner city Baltimore) with concomitantly high air pollutant exposures and asthma burdens.

Limitations

One drawback to our study is that we could not apportion PAH exposures through inhalation and ingestion. Dietary intake of PAHs from eating roasted, charbroiled or smoked foods can account for a significant portion of total PAH exposure (Fiala et al. 2000; Vyskocil et al. 2000). In addition, measurement of urinary monohydroxylated metabolites of low molecular weight PAHs (predominantly found in gas phase), including 2-naphthol (naphthalene), 3-hydroxyphenanthrene (phenanthrene) and 9-hydroxyflurorene (fluorine) may give a better assessment of internal dose from inhaled PAHs. We also did not measure other air pollutants and biological inhalants (such as ozone, NO_2, endotoxin and aeroallergen) or assess viral infections. These possible co-exposures are associated with inflammation and asthma exacerbation, and may confound the relationships found in our analyses. We did not measure aeroallergen content in settled dust or quantitate serum anti-allergen immunoglobulin (i.e. total serum IgE, anti-mouse IgE, anti-cockroach IgE, anti-dust mite IgE, etc.). More comprehensive exposure assessments and early effect biomarker analyses would allow us to better assess the influence of allergens and allergic immune responses to inflammation and asthma exacerbations. We did not measure biomarkers of inflammation in other fluids (e.g. sputum, nasal lavage fluid and bronchiolalveolar lavage fluid) and tissues. Quantification

of inflammatory biomarkers in these biospecimens would give insight into local and tissue specific inflammatory responses. We also did not account for medications (other than ß-agonist use), which may influence frequency and severity of asthma attacks, as well as peripheral eosinophil and other blood biomarker levels. Gene-environment interactions, such as genetic polymorphisms, were also not assessed in this study. Polymorphisms in phase I (e.g. CYP1A1, CYP1A2) and phase II enzymes (e.g. GSTP1, GSTT1, and GSTM1) may be important in explaining inter-individual differences in PAH metabolism and urinary 1-OHPG elimination. The study cohort was comprised of mostly African American children residing exclusively in neighborhoods of low socioeconomic status in inner city Baltimore. This study may therefore not be generalizable to the pediatric asthmatic populations or children not living in inner city neighborhoods of low socioeconomic status.

Conclusion

PAH internal dose was not associated with urinary MDA, a marker of oxidative stress, but was associated with increased peripheral blood eosinophils, neutrophils, and monocytes. Stronger associations were found in PAH internal dose biomarkers collected 4 days prior to peripheral blood biomarkers than same day comparisons, suggesting differences in PAH exposures, differences in inflammatory responses over time or sustained effects from prolonged exposures. PAHs may also exhibit adjuvant effects with allergens, leading to more pronounced allergic inflammation (e.g. eosinophilia) in atopic asthmatic children. Internal dose PAHs were also associated with increased risks of nighttime symptoms and rescue medication use in days prior to, and after urine collection, suggesting prolonged effects of PAH exposures on asthma-related symptoms.

TABLES AND FIGURES

Table 3-1. Descriptive statistics for urinary MDA and 1-OHPG

	All Samples		Asthmatics Only	
	MDA (µmol/mol Cr)	**1-OHPG (µmol/mol Cr)**	**MDA (µmol/mol Cr)**	**1-OHPG (µmol/mol Cr)**
Number of samples	594	594	532	532
Arithmetic Mean (SD)	10.4 (9.6)	0.17 (0.22)	10.6 (9.8)	0.17 (0.21)
Geometric Mean	7.6	0.09	7.8	0.09
Median (IQR)	7.6 (4.4-12.9)	0.11 (0.05-0.22)	7.9 (4.6-13.0)	0.11 (0.05-0.23)
95%ile	28.5	0.54	28.8	0.54
Range	0.7-75.5	0.003-2.13	0.7-75.5	0.003-2.13
% Below LOD	10%	11%	9%	11%

Table 3-2. Predictors of urinary MDA concentrations

	All Samples				Asthmatics only			
	β	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Urinary creatinine	0.246	**<0.001**	0.153	0.339	0.248	**<0.001**	0.155	0.340
Log-1-OHPG	-0.009	0.736	-0.061	0.043	0.011	0.676	-0.041	0.064
Age	0.010	0.880	-0.122	0.142	0.030	0.680	-0.111	0.170
Gender	-0.023	0.237	-0.060	0.015	-0.027	0.184	-0.067	0.013
Atopic status	0.055	0.426	-0.080	0.190	0.065	0.349	-0.071	0.200
Season								
Winter (reference)	-	-	-	-	-	-	-	-
Spring	0.058	0.510	-0.115	0.232	0.059	0.543	-0.131	0.248
Summer	-0.049	0.556	-0.211	0.114	-0.076	0.379	-0.247	0.094
Fall	-0.145	0.094	-0.314	0.025	-0.133	0.130	-0.304	0.039
BMI percentile category								
Normal weight (reference)	-	-	-	-	-	-	-	-
Overweight	0.139	0.197	-0.072	0.351	0.177	0.173	-0.077	0.431
Obese	0.151	0.054	-0.003	0.305	0.181	**0.024**	0.024	0.338
Underweight	0.142	0.195	-0.073	0.357	0.094	0.407	-0.128	0.316
Caregiver's education attainment								
Less than HS (reference)	-	-	-	-	-	-	-	-
HS graduate	-0.029	0.695	-0.172	0.114	-0.045	0.544	-0.188	0.099
College or higher	-0.143	0.091	-0.308	0.023	-0.118	0.178	-0.290	0.054
Asthma status	0.040	0.703	-0.167	0.247	-	-	-	-
Constant	4.238	<0.001	3.793	4.683	4.254	**<0.001**	3.790	4.717
MDA day 3 vs. day 7 samples								
Cr. adjusted MDA day 3 vs. day 7	0.204	**0.001**	0.086	0.322	0.187	**0.004**	0.061	0.314
Cr. unadjusted MDA day 3 vs. day 7	0.227	**<0.001**	0.110	0.344	0.220	**<0.001**	0.097	0.344

Adjusted for creatinine, age, gender, season, atopic status and caregiver's education

Table 3-3. Correlations between urinary log MDA and log 1-OHPG concentrations in all samples (n=594)[a]

	Log MDA/Cr (average)	Log MDA/Cr day 3	Log MDA/Cr day 7	Log 1-OHPG/Cr (average)	Log 1-OHPG/Cr day 3	Log 1-OHPG/Cr day 7
Log MDA/Cr (average)	-					
Log MDA/Cr day 3	**0.76****	-				
Log MDA/Cr day 7	**0.84****	**0.25****	-			
Log 1-OHPG/Cr (average)	**0.11***	**0.17***	0.07	-		
Log 1-OHPG/Cr day 3	0.06	0.109	-0.02	**0.78****	-	
Log 1-OHPG/Cr day 7	0.101	0.105	0.1	**0.83****	**0.27****	-

[a]Pearson's correlation
*p<0.05
**p<0.001

Table 3-4. Correlations between urinary log MDA and log 1-OHPG concentrations, asthmatic children (n=532)[a]

	Log MDA/Cr (average)	Log MDA/Cr day 3	Log MDA/Cr day 7	Log 1-OHPG/Cr (average)	Log 1-OHPG/Cr day 3	Log 1-OHPG/Cr day 7
Log MDA/Cr (average)	-					
Log MDA/Cr day 3	**0.76****	-				
Log MDA/Cr day 7	**0.84****	**0.25****	-			
Log 1-OHPG/Cr (average)	**0.12***	**0.17***	0.09	-		
Log 1-OHPG/Cr day 3	0.10	**0.15***	0.03	**0.77****	-	
Log 1-OHPG/Cr day 7	**0.12***	**0.14***	**0.13***	**0.84****	**0.27****	-

[a]Pearson's correlation
*p<0.05
**p<0.001

Table 3-5. Associations between urinary log MDA and log 1-OHPG concentrations (urine samples from day 3, day 7 and averaged values)

All Samples (n=594)	**Crude**				**Adjusted***			
Cr adjusted log MDA and 1-OHPG**	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Log 1-OHPG day 3	0.034	0.379	-0.041	0.109	0.028	0.445	-0.045	0.101
Log 1-OHPG day 7	0.013	0.635	-0.040	0.066	0.000	0.997	-0.054	0.054
Log 1-OHPG (average)	0.002	0.937	-0.057	0.062	-0.003	0.922	-0.058	0.052
Cr unadjusted log MDA and 1-OHPG								
Log 1-OHPG day 3	0.053	0.167	-0.022	0.127	0.049	0.195	-0.025	0.122
Log 1-OHPG day 7	0.034	0.188	-0.017	0.085	0.026	0.335	-0.027	0.079
Log 1-OHPG (average)	0.046	0.123	-0.012	0.105	0.040	0.156	-0.015	0.096

*Adjusted for, air nicotine, age, gender, atopic status, season, and caregiver's education (and creatinine)
**creatinine included in model as an independent variable

Asthmatics only (n=532)	**Crude**				**Adjusted***			
Cr adjusted log MDA and 1-OHPG**	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Log 1-OHPG day 3	0.062	0.129	-0.018	0.143	0.064	0.107	-0.014	0.142
Log 1-OHPG day 7	0.016	0.585	-0.042	0.074	0.008	0.790	-0.051	0.067
Log 1-OHPG (average)	0.014	0.661	-0.048	0.075	0.012	0.663	-0.043	0.068
Cr unadjusted log MDA and 1-OHPG								
Log 1-OHPG day 3	0.087	**0.027**	0.010	0.164	0.089	**0.024**	0.012	0.165
Log 1-OHPG day 7	0.042	0.131	-0.013	0.096	0.033	0.272	-0.026	0.091
Log 1-OHPG (average)	0.030	0.317	-0.029	0.089	0.055	0.057	-0.002	0.112

* Adjusted for air nicotine, age, gender, atopic status, season, and caregiver's education
**Creatinine included in model as independent variable

Table 3-6. Peripheral blood biomarkers in asthmatic children, stratified by atopic status

Asthmatics Only	All samples		Non-Atopic		Atopic		
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	p-value*
White blood cell count (K cells/mm^3)	6.03 (2.16)	5.87 (4.80-6.85)	6.04 (1.89)	6.01 (4.80-6.84)	6.02 (2.26)	5.72 (4.80-6.85)	0.62
Platelet count (cells/mm^3)	304 (610	296 (262-341)	304 (48)	301 (271-329)	303 (66)	294 (258-345)	0.627
Monocyte percent	7.9 (2.2)	7.8 (6.5-9.1)	8.4 (2.6)	7.7 (6.4-10)	7.8 (2.0)	7.8 (6.5-8.9)	0.43
Lymphocyte percent	42.8 (11.7)	43.1 (34.7-50.2)	41.1 (12.2)	39.2 (33.2-48.3)	43.5 (11.4)	45.1 (36.5-50.9)	**0.045**
Neutrophil percent	43.7 (22.3)	41.5 (33.7-51.5)	46.3 (13.6)	47.2 (38.7-54.9)	42.8 (24.6)	39.6 (33.6-49.3)	**0.001**
Eosinophil percent	6.0 (3.9)	5.3 (2.9-7.9)	3.3 (2.3)	2.7 (1.8-4.2)	7.1 (3.8)	6.5 (4-9.4)	**<0.001**
Basophil percent	0.58 (0.31)	0.5 (0.4-0.8)	0.46 (0.24)	0.4 (0.3-0.6)	0.63 (0.32)	0.6 (0.4-0.8)	**<0.001**
Lymphocyte count (K cells/mm^3)	2.48 (737)	2.39 (1.91-2.94)	2.41 (0.648)	2.26 (1.96-2.87)	2.52 (0.757)	2.50(1.91-2.99)	0.287
Monocyte count (cells/mm^3)	472 (187)	445 (360-540)	493 (142)	465 (415-575)	463 (202)	430 (340-535)	**0.037**
Neutrophil count (K cells/mm^3)	2.68 (1.61)	2.32 (1.72-3.32)	2.98 (1.58)	3.04 (1.87-3.61)	2.56 (1.61)	2.18 (1.64-3.13)	**0.024**
Eosinophil count (cells/mm^3)	358 (281)	280 (170-470)	190 (128)	160 (110-225)	427 (297)	360 (240-560)	**<0.001**

*Wilcoxon rank-sum test

Table 3-7. Associations between urinary 1-OHPG concentrations (day 3, day 7, and average) and peripheral blood biomarkers (from day 7 blood draw) in asthmatic children

	Day 3				Day 7				Average			
	β	P>z	[95%	CI]	β	P>z	[95%	CI]	β	P>z	[95%	CI]
White blood cell count	0.019	**0.03**	0.002	0.035	-0.005	0.611	-0.026	0.015	0.011	0.273	-0.009	0.031
Platelet count	0.007	0.356	-0.008	0.021	0.007	0.371	-0.008	0.022	0.012	0.066	-0.001	0.024
Monocyte percent	-0.078	0.210	-0.200	0.044	0.053	0.414	-0.074	0.181	0.010	0.904	-0.154	0.174
Lymphocyte percent	-0.299	0.292	-0.854	0.257	0.217	0.473	-0.376	0.810	-0.089	0.828	-0.898	0.720
Neutrophil percent	0.011	0.974	-0.633	0.654	-0.155	0.637	-0.798	0.488	-0.037	0.936	-0.937	0.863
Eosinophil percent	0.415	**0.002**	0.154	0.676	-0.145	0.106	-0.320	0.031	0.105	0.467	-0.178	0.388
Basophil percent	-0.006	0.601	-0.027	0.016	-0.003	0.701	-0.02	0.014	-0.005	0.634	-0.024	0.015
Lymphocyte count	0.013	0.128	-0.004	0.031	-0.005	0.603	-0.023	0.013	0.006	0.551	-0.013	0.024
Monocyte count	0.011	0.395	-0.014	0.036	-0.01	0.364	-0.031	0.011	0.007	0.556	-0.016	0.029
Neutrophil count	0.02	0.226	-0.012	0.051	-0.008	0.659	-0.046	0.029	0.012	0.572	-0.030	0.054
Eosinophil count	0.061	**0.012**	0.014	0.108	-0.037	0.191	-0.093	0.019	0.007	0.821	-0.056	0.071

Adjusted for creatinine, air nicotine (continuous), age, gender, atopic status, season, and caregiver's education

Table 3-8. Associations between quartiles of urinary 1-OHPG and peripheral blood eosinophil counts in asthmatic children

Log Eosinophil counts	Crude*				Adjusted**			
	β	P>z	[95%	CI]	β	P>z	[95%	CI]
1-OHPG quartile 1 (ref.)	-	-	-	-	-	-	-	-
1-OHPG quartile 2	0.080	0.462	-0.132	0.291	0.094	0.315	-0.089	0.277
1-OHPG quartile 3	0.230	0.076	-0.024	0.485	0.342	**0.013**	0.074	0.610
1-OHPG quartile 4	0.325	**0.026**	0.039	0.611	0.398	**0.002**	0.146	0.650

* Adjusted for urinary creatinine
** Adjusted for urinary creatinine, air nicotine, age, gender, atopic status, season, and caregiver's education

Figure 3-2. Peripheral blood eosinophil counts by quartiles of urinary 1-OHPG concentrations in asthmatic children

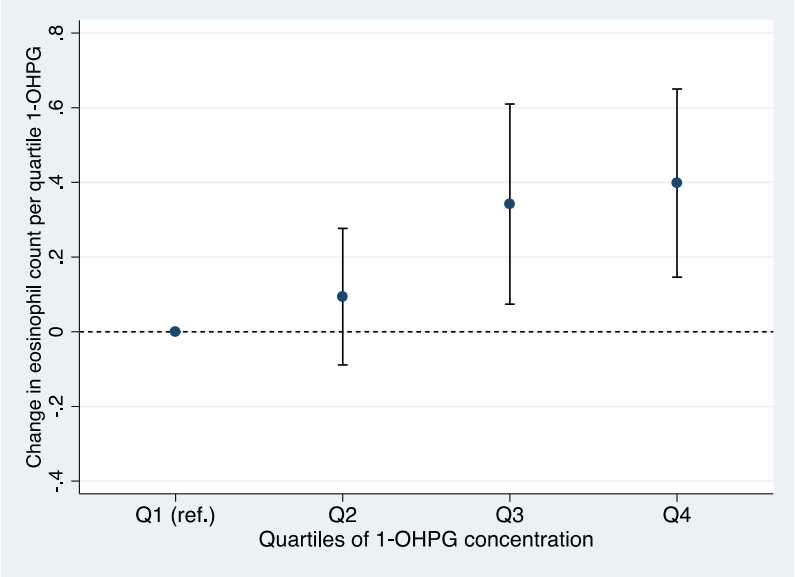
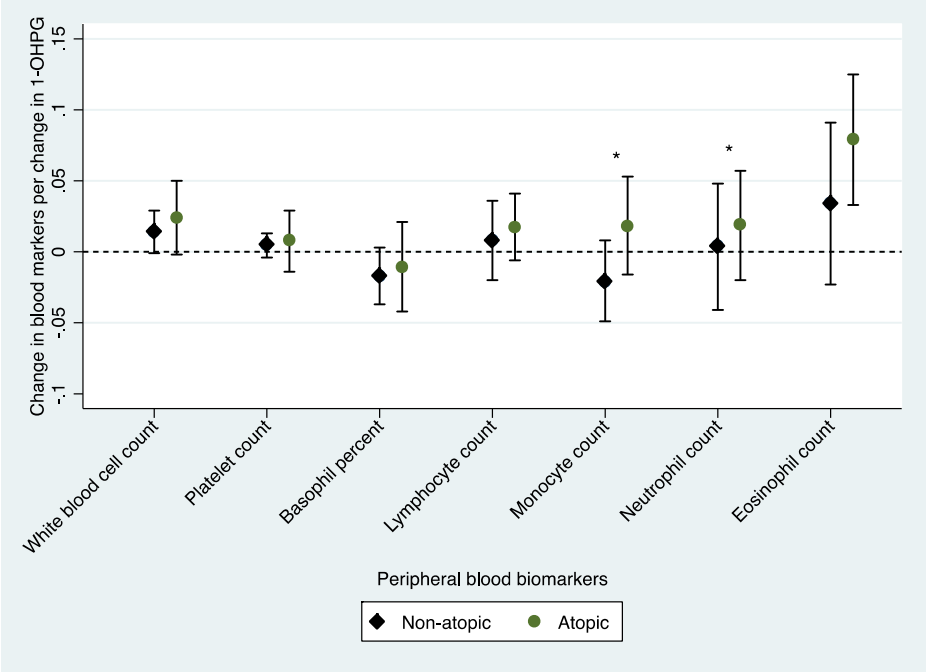


Table 3-9. Atopic status as an effect modifier of associations between 1-OHPG concentrations from urine collected on day 3 (Tuesday) and peripheral blood biomarkers from blood collected on day 7 (Saturday) in asthmatic children

	Non-atopic				Atopic				
	β	P>z	[95%	CI]	β	P>z	[95%	CI]	P-interaction
White blood cell count	0.014	0.069	-0.001	0.029	0.024	0.066	-0.002	0.050	0.638
Platelet count	0.005	0.281	-0.004	0.013	0.008	0.469	-0.014	0.029	0.916
Monocyte percent	-0.229	**0.030**	-0.436	-0.022	-0.013	0.870	-0.168	0.142	**0.001**
Lymphocyte percent	0.315	0.632	-0.975	1.604	-0.492	0.141	-1.146	0.163	0.995
Neutrophil percent	-0.444	0.502	-1.739	0.851	-0.019	0.958	-0.732	0.693	0.143
Eosinophil percent	0.016	0.860	-0.164	0.196	0.524	**<0.001**	0.258	0.790	0.052
Basophil percent	-0.017	0.096	-0.037	0.003	-0.011	0.504	-0.042	0.021	0.836
Lymphocyte count	0.008	0.567	-0.020	0.036	0.017	0.140	-0.006	0.041	0.753
Monocyte count	-0.021	0.152	-0.049	0.008	0.018	0.294	-0.016	0.053	0.083
Neutrophil count	0.004	0.864	-0.041	0.048	0.019	0.343	-0.020	0.057	0.062
Eosinophil count	0.034	0.243	-0.023	0.091	0.079	**0.001**	0.033	0.125	0.789

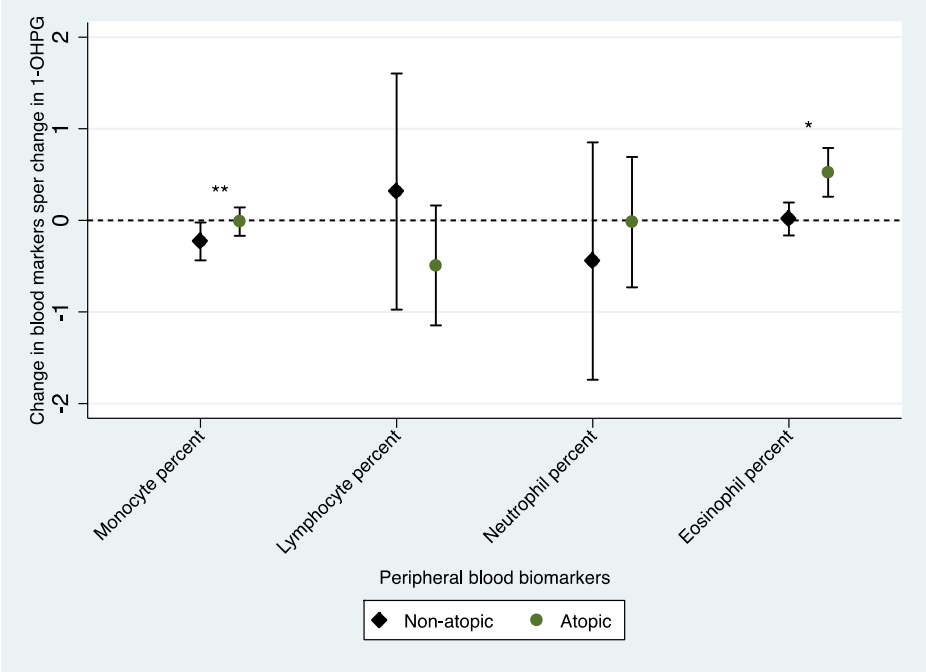
Adjusted for urinary creatinine, air nicotine (continuous), age, gender, atopic status, season, and caregiver's education

Figure 3-3. Associations between 1-OHPG concentration from Tuesday (day 3) urine and peripheral blood biomarkers in asthmatic children, stratified by atopic status



*p-interaction<0.1

Figure 3-4. Associations between 1-OHPG concentration from Tuesday (day 3) urine and peripheral blood biomarkers in asthmatic children, stratified by atopic status (continued)



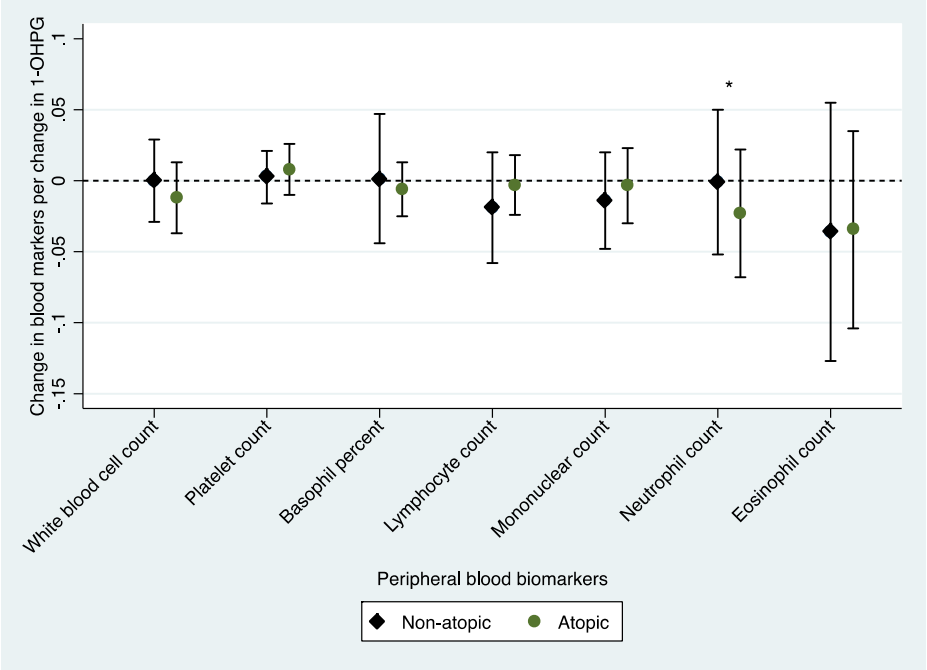
*p-interaction<0.1
**p-interaction<0.05

Table 3-10. Atopic status as an effect modifier of associations between 1-OHPG concentrations from urine collected on day 7 (Saturday) and peripheral blood biomarkers from blood collected on day 7 (Saturday) in asthmatic children

	Non-Atopic				Atopic				
	β	P>z	[95%	CI]	β	P>z	[95%	CI]	P-interaction
White blood cell count	0.000	0.991	-0.029	0.029	-0.012	0.347	-0.037	0.013	0.284
Platelet count	0.003	0.783	-0.016	0.021	0.008	0.376	-0.010	0.026	0.214
Monocyte percent	-0.072	0.618	-0.354	0.210	0.131	0.085	-0.018	0.279	**0.016**
Lymphocyte percent	0.281	0.720	-1.255	1.817	0.317	0.355	-0.355	0.990	0.171
Neutrophil percent	-0.067	0.931	-1.585	1.451	-0.339	0.370	-1.080	0.402	0.143
Eosinophil percent	-0.177	0.172	-0.430	0.077	-0.126	0.254	-0.342	0.090	0.539
Basophil percent	0.001	0.960	-0.044	0.047	-0.006	0.527	-0.025	0.013	0.620
Lymphocyte count	-0.019	0.346	-0.058	0.020	-0.003	0.779	-0.024	0.018	0.518
Monocyte count	-0.014	0.410	-0.048	0.020	-0.003	0.807	-0.030	0.023	0.212
Neutrophil count	-0.001	0.968	-0.052	0.050	-0.023	0.322	-0.068	0.022	0.096
Eosinophil count	-0.029	0.554	-0.126	0.068	-0.034	0.334	-0.104	0.035	0.773

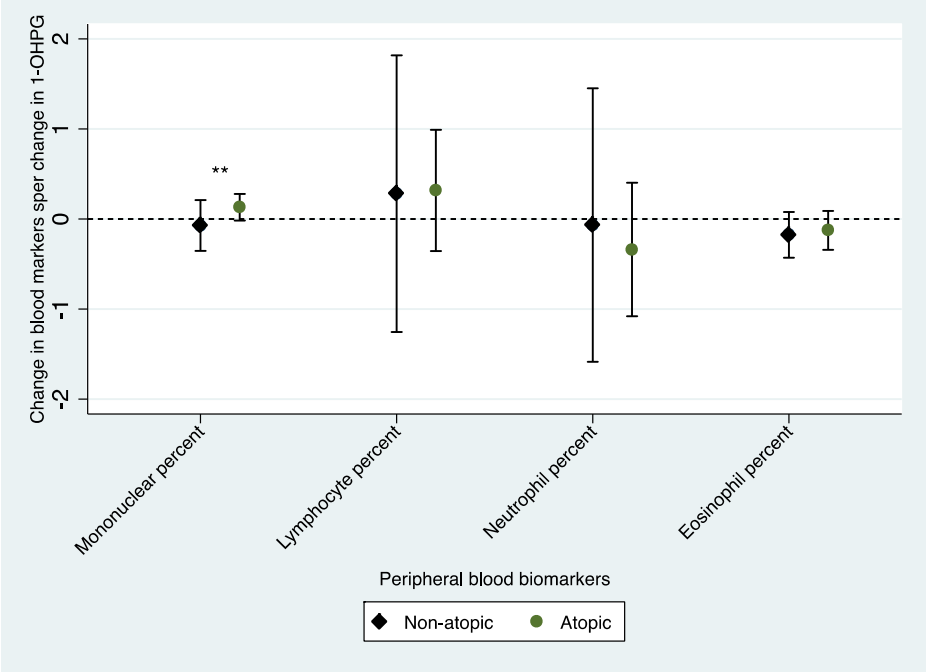
Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Figure 3-5. Associations between 1-OHPG concentration from Saturday (day 7) urine and peripheral blood biomarkers in asthmatic children, stratified by atopic status



* p-interaction<0.1

Figure 3-6. Associations between 1-OHPG concentration from Saturday (day 7) urine and peripheral blood biomarkers in asthmatic children, stratified by atopic status (continued)



* p-interaction<0.1
**p-interaction<0.05

Table 3-11. Atopic status as an effect modifier of associations between averaged urinary 1-OHPG concentrations and peripheral blood biomarkers collected on day 7 (Saturday) in asthmatic children

	Non-atopic				Atopic				
	β	P>z	[95%	CI]	β	P>z	[95%	CI]	P-interaction
White blood cell count	0.013	0.102	-0.003	0.029	0.006	0.738	-0.028	0.039	0.318
Platelet count	0.007	0.105	-0.001	0.015	0.016	0.126	-0.005	0.037	0.286
Monocyte percent	-0.209	0.078	-0.441	0.024	0.177	0.056	-0.004	0.358	**<0.001**
Lymphocyte percent	-0.018	0.980	-1.399	1.363	0.105	0.845	-0.950	1.161	0.186
Neutrophil percent	0.109	0.871	-1.208	1.426	-0.494	0.330	-1.487	0.499	**0.018**
Eosinophil percent	-0.048	0.632	-0.243	0.148	0.209	0.285	-0.174	0.592	0.121
Basophil percent	-0.005	0.768	-0.041	0.030	-0.007	0.612	-0.033	0.019	0.994
Lymphocyte count	-0.012	0.424	-0.042	0.018	0.011	0.387	-0.014	0.035	0.337
Monocyte count	-0.011	0.535	-0.045	0.024	0.020	0.199	-0.011	0.051	**0.093**
Neutrophil count	0.013	0.594	-0.034	0.060	-0.006	0.842	-0.060	0.049	**0.038**
Eosinophil count	-0.003	0.920	-0.070	0.063	0.023	0.584	-0.060	0.106	0.414

Adjusted for urinary creatinine, air nicotine (continuous), age, gender, atopic status, season, and caregiver's education

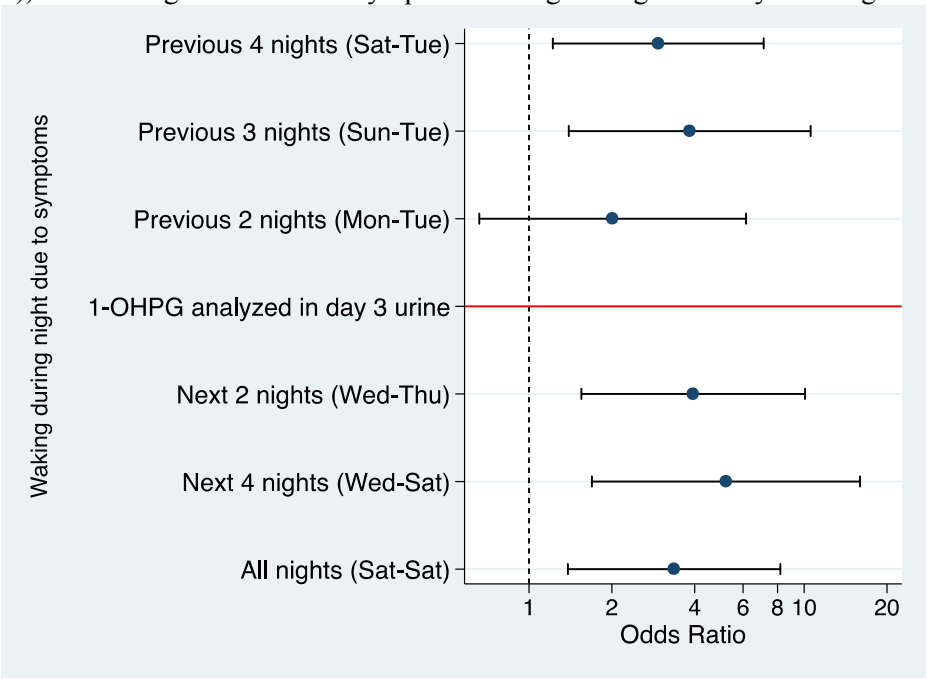
Symptoms

Table 3-12. Associations between urinary 1-OHPG concentrations and waking due to asthma symptoms during the night or early morning

	Crude*				Adjusted**			
1-OHPG conc. day 3	**OR**	**P>z**	**[95%**	**CI]**	**OR**	**P>z**	**[95%**	**CI]**
Woken by asthma during previous 4 nights (0-3)	2.631	**0.013**	1.230	5.626	2.950	**0.016**	1.220	7.132
Woken by asthma during previous 3 nights (1-3)	3.667	**0.003**	1.560	8.621	3.838	**0.009**	1.395	10.559
Woken by asthma during previous 2 nights (2-3)	2.106	0.138	0.787	5.634	2.014	0.219	0.660	6.149
Woken by asthma during next 2 nights (4-5)	3.391	**0.006**	1.419	8.100	3.950	**0.004**	1.550	10.068
Woken by asthma during next 4 nights (4-7)	5.110	0.001	1.922	13.589	5.198	**0.004**	1.693	15.961
Woken by asthma any night (0-7)	3.311	0.003	1.484	7.384	3.368	**0.007**	1.385	8.192
1-OHPG conc. day 7								
Woken by asthma during previous 2 nights (6-7)	2.638	0.045	1.020	6.824	1.885	0.299	0.569	6.239
Woken by asthma during previous 3 nights (5-7)	1.992	0.088	0.902	4.396	1.643	0.313	0.626	4.313
Woken by asthma during previous 4 nights (4-7)	2.872	**0.005**	1.370	6.021	2.976	**0.015**	1.239	7.147
Woken by asthma any night (0-7))	1.396	0.317	0.726	2.683	1.425	0.365	0.663	3.062
1-OHPG conc. average of samples								
Woken by asthma on nights 0-3	1.842	0.056	0.985	3.447	1.863	0.078	0.932	3.724
Woken by asthma on nights 4-7	3.290	**0.002**	1.544	7.012	3.357	**0.019**	1.223	9.214
Woken by asthma on nights 0-7	1.919	0.055	0.985	3.736	1.886	0.103	0.879	4.043

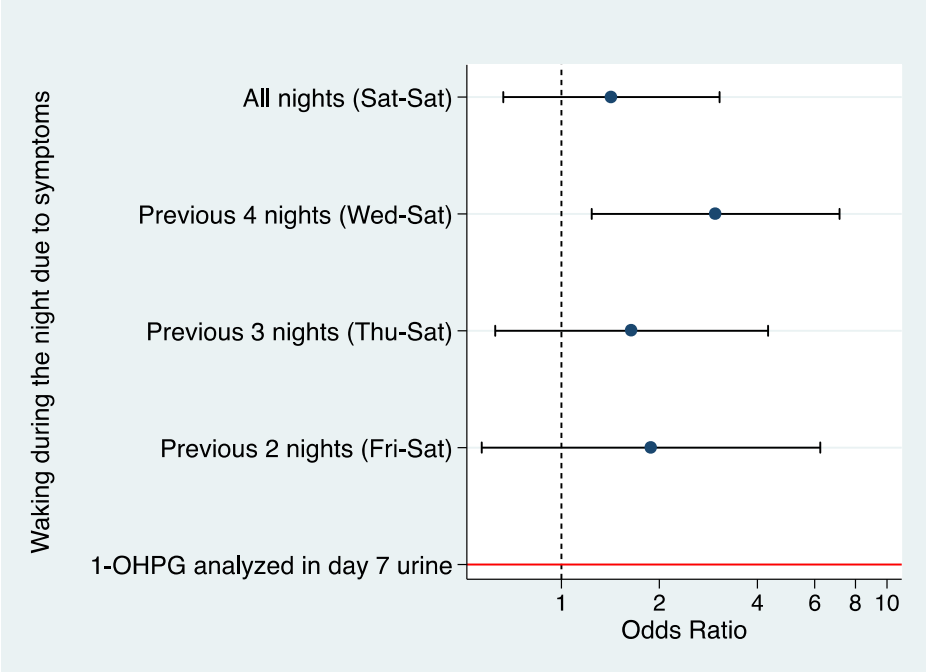
*Unadjusted
**Adjusted for air nicotine (continuous), age, gender, atopic status, season, and caregiver's education

Figure 3-7. Associations between 1-OHPG concentration (from urine collected on Tuesday (day 3)) and waking due to asthma symptoms during the night or early morning.



Urinary 1-OHPG dichotomized at the median (0.11 µmol/mol Cr)

Figure 3-8. Associations between 1-OHPG concentration (from urine collected on Saturday (day 7)) and waking due to asthma symptoms during the night or early morning.



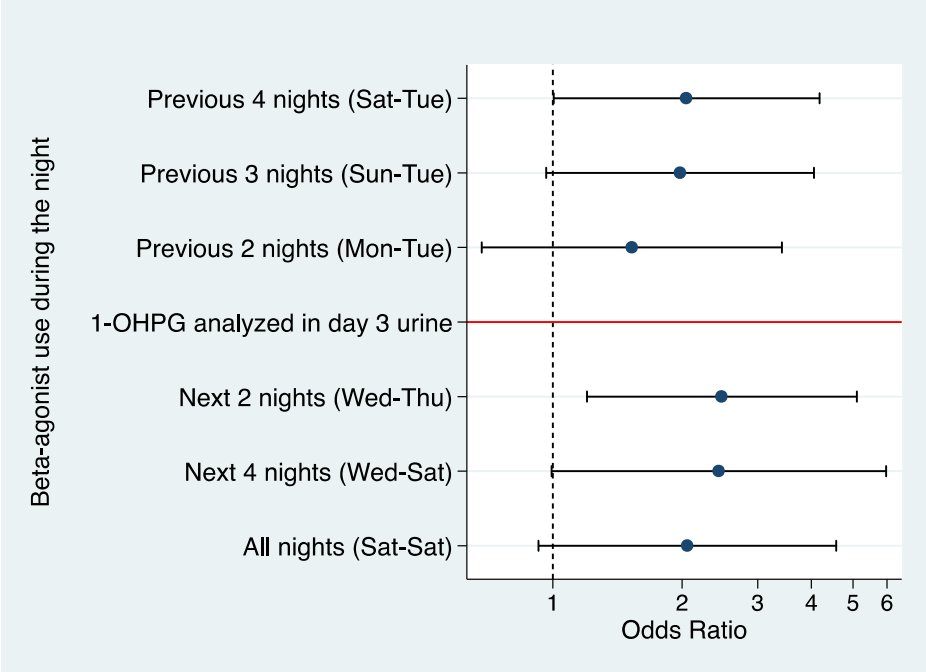
Urinary 1-OHPG dichotomized at the median (0.11 µmol/mol Cr)

Table 3-13. Associations between urinary 1-OHPG concentrations and ß-agonist use during the night or early morning

	Crude*				Adjusted**			
1-OHPG conc. day 3	**OR**	**P>z**	**[95%**	**CI]**	**OR**	**P>z**	**[95%**	**CI]**
ß-agonist use during previous 4 nights (0-3)	1.612	0.108	0.900	2.887	2.050	**0.048**	1.005	4.181
ß-agonist use during previous 3 nights (1-3)	1.460	0.195	0.824	2.586	1.978	0.063	0.965	4.055
ß-agonist use during previous 2 nights (2-3)	1.068	0.849	0.543	2.098	1.528	0.302	0.683	3.416
ß-agonist use during next 2 nights (4-5)	1.902	**0.049**	1.004	3.604	2.476	**0.014**	1.201	5.104
ß-agonist use during next 4 nights (4-7)	2.197	**0.033**	1.066	4.528	2.437	0.051	0.994	5.971
ß-agonist use any night (0-7)	1.892	0.056	0.983	3.639	2.057	0.077	0.926	4.569
1-OHPG conc. day 7								
ß-agonist use during previous 2 nights (6-7)	1.219	0.580	0.604	2.463	1.107	0.803	0.497	2.466
ß-agonist use during previous 3 nights (5-7)	0.911	0.789	0.459	1.808	0.918	0.825	0.430	1.961
ß-agonist use during previous 4 nights (4-7)	0.960	0.905	0.496	1.860	0.966	0.926	0.462	2.018
ß-agonist use any night (0-7)	0.967	0.908	0.549	1.705	1.030	0.932	0.521	2.037
1-OHPG conc. average of samples								
ß-agonist use on nights 0-3	1.269	0.315	0.798	2.018	1.430	0.187	0.840	2.435
ß-agonist use on nights 4-7	1.345	0.351	0.721	2.510	1.390	0.366	0.681	2.837
ß-agonist use on nights 0-7	1.324	0.277	0.798	2.197	1.472	0.230	0.783	2.768

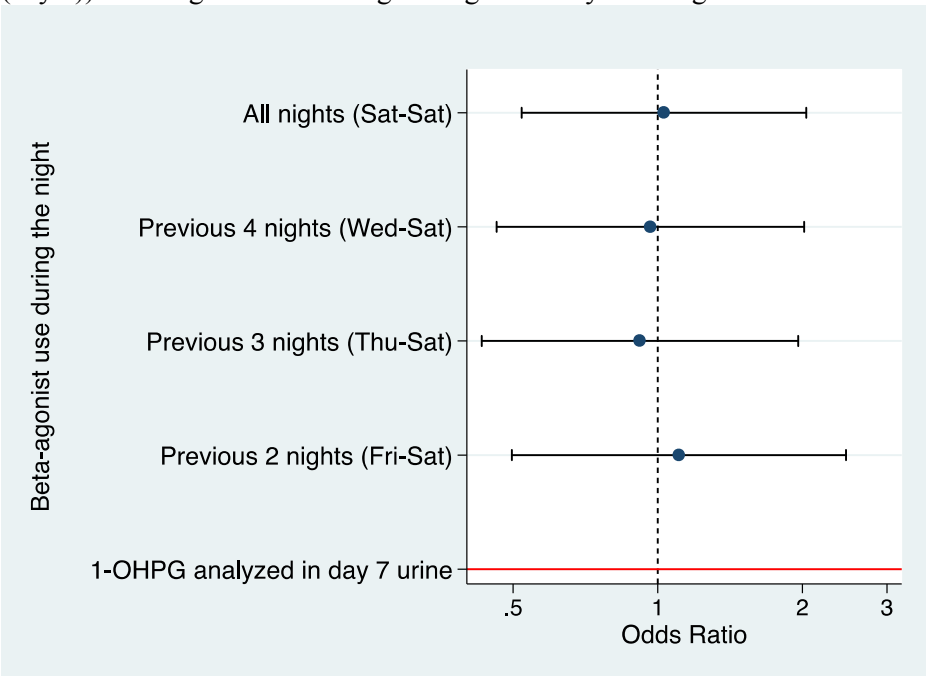
*Unadjusted
**Adjusted for air nicotine (continuous), age, gender, atopic status, season, and caregiver's education

Figure 3-9. Associations between 1-OHPG concentration (from urine collected on Tuesday (day 3)) and ß-agonist use during the night or early morning



Urinary 1-OHPG dichotomized at the median (0.11 µmol/mol Cr)

Figure 3-10. Associations between 1-OHPG concentration (from urine collected on Saturday (day 7)) and ß-agonist use during the night or early morning



Urinary 1-OHPG dichotomized at the median (0.11 µmol/mol Cr)

CHAPTER 4: Polycyclic aromatic hydrocarbon internal dose, oxidative stress, and inflammation in adults with atopic asthma in Baltimore

ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAH) are products of incomplete combustion of organic materials (oil, tobacco, fuel, etc.). PAHs have been associated with increased oxidative stress, inflammation and asthma exacerbation. The *GSTM1*-null polymorphism, which results in the inability to produce the GSTM1 enzyme, has been associated with increased risk of asthma and increased susceptibility to asthma attacks. We examined whether PAH internal dose was associated with systemic oxidative stress, induction of local antioxidant genes, and local and systemic inflammatory immune responses in atopic asthmatic adults in Baltimore. We also evaluated whether the *GSTM1* genotype modified associations between urinary 1-OHPG and biomarkers of oxidative stress, gene induction, and inflammation.

Methods: The Nasal Challenge to Indoor Particulate Matter study enrolled 32 non-smoking atopic asthmatic adults ages 18-50 to measure inflammatory and oxidative stress biomarkers and gene expression, after being challenged intranasally with particulate dust suspended in saline. We used the baseline samples only, making our study design cross-sectional with 2 visits per person. Urine specimens were collected and analyzed for 1-OHPG and isoprostane; and blood, nasal lavage fluid and nasal epithelium cells were also collected and analyzed for induction of antioxidant genes and inflammatory biomarkers.

Results: Most of the participants were women (75%), African American (84%), between 18 and 50 years of age (mean: 30 years of age), and 31% of the participants had the *GSTM1*-null polymorphism. Urinary 1-OHPG was independently associated with

significantly increased nasal lavage white blood cell count; increased serum inflammatory chemokines and mediators: GRO, GRO-α, IL-8, RANTES, and ECP; increased peripheral blood inflammatory biomarkers: lymphocytes, and monocytes (both activated with PMA), and decreased peripheral blood polymorphonuclear leukocytes (PMN) in *GSTM1*-null participants. *GSTM1* genotype modified the associations between 1-OHPG and urinary isoprostane, serum GRO and GRO-α, peripheral blood lymphocytes, with stronger positive associations among *GSTM1*-null participants compared to *GSTM1*-present participants (interaction p-value <0.1). Among *GSTM1*-present participants, urinary 1-OHPG was associated with an up-regulation of *GCLC* mRNA in nasal epithelium cells; increased nasal lavage eosinophil percent; and decreased peripheral blood PBMC and monocytes, and PMN (with PMA); and decreased serum IL-6 and CCL20.

Conclusion: PAH exposures were associated with increased local and systemic inflammation in adults with atopic asthma, with different responses among *GSTM1*-null and *GSTM1*-present participants. *GSTM1* genotype modulates these associations with greater inflammatory responses in participants with the *GSTM1*-null polymorphism compared to participants with *GSTM1*-present genotype. Our results suggest that individuals who are unable to produce the GSTM1 enzyme may be more susceptible to inflammation associated with PAH exposures.

INTRODUCTION

Asthma

Asthma is a complex chronic disease of the airways characterized by reversible airway obstruction, sensitivity of nerve endings, and an influx of inflammatory cells and cellular elements (i.e. eosinophils, neutrophils, macrophages, mast cells and T-lymphocytes) (Delfino et al. 2002; Dozor et al. 2010). Asthma prevalence in the United States was 8.4% in 2010, with a higher prevalence among children ages 0-17 (9.5%) compared to adults (7.7%) (Akinbami et al. 2012). Asthma rates also differ by racial group and socioeconomic status (measured by poverty-to-income ratio), with African Americans (11.2%) and people living below the poverty level (11.2%) experiencing disproportionately higher asthma burden than national average (Akinbami et al. 2012). Atopy, an allergic phenotype characterized by increased production of IgE in response to specific allergens, is associated with asthma pathogenesis and severity (Pearce et al. 1999; Suh and Koh 2013). Most asthmatics have an atopic constitution and are vulnerable to inflammatory responses from multiple environmental stimuli (Nelson et al. 1999; Eggleston 2000; Lau et al. 2000). Co-exposures to allergens and environmental air pollutants (such as second hand smoke (SHS), particulate matter (PM), ozone (O_3) and nitrogen dioxide (NO_2)) may lead to increased frequency of asthma exacerbations in atopic asthmatics (D'Amato et al. 2002; Diaz-Sanchez et al. 2006). Living in an urban environment (such as inner city Baltimore) may also increase asthma morbidity. Residing in an urban environment has also been associated with increased exposures to substances associated with asthma exacerbation and severity, including SHS, indoor and outdoor PM, indoor allergens (e.g. mouse, cockroach, and dust mite allergens), endotoxin, mold,

and fungus (Wallace et al. 2003; Breysse et al. 2005; Simons et al. 2007; Matsui et al. 2008; McCormack et al. 2009).

Oxidative Stress

Inflammation due to reactive oxygen species (ROS) has been associated with the development of asthma and asthma exacerbations (Cho and Moon 2010; Dozor et al. 2010). ROS, which include superoxide radicals (O_2^-), hydroxyl radicals (OH^-), hydrogen peroxides (H_2O_2), peroxynitrites ($ONOO^-$), are formed through the addition of electrons to oxygen groups on organic molecules. ROS are formed both endogenously (through oxidative phosphorylation, metabolic activation of organic compounds, and production by macrophages and other inflammatory mediators), and through exposures to exogenous oxidants (such as inhalation of ozone, nitric oxide and cigarette smoke) (Nel et al. 2001; Bowler and Crapo 2002). ROS are associated with lipid peroxidation, cell apoptosis, damage to cellular structures, tissue damage, DNA and protein adducts, and increased inflammation (Li et al. 2004; Nel et al 2001; Wan and Diaz-Sanchez 2007). Oxidative stress is the result of an imbalance between ROS and antioxidants, under which antioxidant responses cannot adequately counteract an abundance of ROS (Nadeem et al. 2003). The mechanism for oxidative stress by organic compounds has been well characterized. Polycyclic aromatic hydrocarbons (PAH) and other organic air pollutants can bind to cytosolic aryl hydrocarbon receptors (Ahr) in the lung and induce expression of phase I enzymes, especially cytochrome P450 1A1 (CYP1A1). CYP1A1 and CYP enzymes (such as CYP1B1 and CYP1A2) add oxidative groups to PAHs resulting in reactive intermediates in the form of quinones, semiquinones and epoxides. These oxidative intermediates can actively participate in redox cycling leading to increased

ROS and subsequently oxidative stress (Nel et al. 2001; Li et al. 2003; Wan and Diaz-Sanchez 2007). In this study, we measured urinary isoprostane (8-iso-$PGF_{2\alpha}$), a by-product of non-enzymatic lipid peroxidation, as an internal dose biomarker of oxidative stress.

PAHs and 1-OHPG

PAHs are formed from the incomplete combustion or pyrolysis of organic materials (e.g. coal, wood, fuel and oil) and are commonly found on fine and ultrafine particulates, $PM_{2.5}$ (aerodynamic diameter <2.5 μm) and $PM_{0.1}$ (aerodynamic diameter <100 ng), respectively (Rosa et al. 2011). Sources of airborne PAH exposures include motor vehicle emissions (combustion products from diesel and conventional gasoline engines), burning fossil fuels (e.g. coal and oil), wood burning, tobacco smoking, cooking with gas stoves, heating appliances (e.g. kerosene space heaters), and incense burning (ATSDR 1995; Larsen et al. 2003). Diesel exhaust particulates (DEP) are present in fine ($PM_{2.5}$) and ultrafine particulates ($PM_{0.1}$), and have high concentrations of volatile and semi-volatile PAHs (e.g. naphthalene, fluorine, phenanthrene, and pyrene) on the surface of particulates. DEP are potent inducers of allergic and non-allergic inflammatory responses due to high oxidation potential of activated PAHs, formed through conversion by CYPs to reactive intermediates (quinones, epoxides, etc.) (Nel et al. 2001; Reidl et al. 2008). PAHs are primarily know for their carcinogenicity, as PAHs and PAH mixtures have been categorized as human carcinogens (e.g. benzo[a]pyrene and coal tar pitch), probable carcinogens (e.g. dibenzo[a,l]pyrene and dibenz[a,h]anthracene) and possible carcinogens (e.g. benz[a]anthracene, benzo[b]fluoranthene and indeno[1,2,3-cd]pyrene) by the International Agency for Research on Cancer (IARC), and the Agency for Toxic

Substances and Disease Registry (ATSDR) (ATSDR 1995; IARC 2010). PAHs have also been associated with development of asthma (Miller et al. 2004; Jung et al. 2012), most likely through non-mutagenic (i.e. inflammatory) mechanisms.

Urinary 1-hydroxypyrene-glucuronide (1-OHPG) is an accepted biological indicator of PAH exposure, as its parent compound, pyrene, is present in virtually all environmental PAH mixtures and is a reliable biomarker of low-level exposure (Buchet et al. 1992; Jongeneelen 2001). Urinary 1-OHPG concentrations reflect exposures through inhalation, ingestion, and dermal absorption (from contaminated air and soil) (Jongeneelen 2001). 1-hydroxypyrene (1-OHP) and 1-OHPG are commonly used urinary biomarkers, however 1-OHPG is more sensitive, as the glucuronide molecule confers 3-5 times more fluorescence than 1-OHP (Singh et al. 1995; Strickland et al. 1996; Kang et al. 2005). 1-OHPG levels have been associated with increased levels of oxidative stress biomarkers (i.e. urinary MDA, isoprostane and 8-OHdG), decreased lung function, and having asthma (Kim et al. 2005; Leem et al. 2005; Bae et al. 2010; Hong et al. 2009; Lai et al. 2013). The molecular pathway for oxidative stress and allergic inflammation mediated asthma-related responses to PAH exposures (e.g. DEP) has been explored in *in vitro*, animal and human studies. The proposed mechanism for PAH-asthma exacerbation involves high concentrations of quinones, semiquinones and epoxides derived from biotransformed PAHs overriding protective pathways (i.e. antioxidants and phase II enzymes), leading to increased oxidative damage in cells and the surrounding tissues, and subsequent biomolecular, immunological and physiological responses (e.g. airway inflammation and airway hyperreactivity) associated with asthma exacerbations (Nel et al. 2001; Park et al. 2006; Cho et al. 2010). PAHs also target eosinophils, basophils,

neutrophils, and antigen presenting cells, and act as adjuvants for inflammatory responses with allergic sensitization (Nel et al. 1998; Delfino et al. 2002). In epidemiologic studies, adjuvant activities by PAHs have been shown to increase allergic inflammatory responses among atopic individuals exposed to PAH-rich DEP and allergens in randomized crossover nasal provocation studies (Diaz-Sanchez et al. 1997; Diaz-Sanchez et al. 1999). Urinary 1-OHP(G) has been associated with decreased lung function, and increased levels of oxidative stress biomarkers (i.e. urinary isoprostane and MDA) (Leem et al. 2005; Bae et al. 2010; Hong et al. 2009); and PAHs have been associated with increases in pro-inflammatory (e.g. interleukin (IL)-6), and anti-inflammatory cytokines and chemokines (i.e. IL-4, IL-5, IL-10 and IL-13, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1α (MIP-1α)) (Diaz-Sanchez et al. 1997; Leem et al. 2005), and inflammatory mediators in nasal lavage fluid (e.g. eosinophils, monocytes, and lymphocytes) (Diaz-Sanchez 1997b; Casillas et al. 1999).

GSTM1

Glutathione-S-transferases (GST) are a superfamily of phase II antioxidant enzymes that catalyze the conjugation of ROS and reactive oxidative and xenobiotic compounds to more stable compounds that can be excreted in urine (Hayes and Pulford 1995). GSTs are categorized into three major families, cytosolic GSTs, mitochondrial GSTs and microsomal GSTs (Hayes et al. 2005). Cytosolic GSTs are coded at five loci, alpha (A), mu (M), pi (P) and theta (T) and zeta (Z), of which *GSTM1*, *GSTP1* and *GSTT1* are commonly associated with chronic lung diseases (e.g. asthma and COPD) (Geisler and Olshan 2001; Tamer et al. 2004). Our study will focus on *GSTM1* gene, as the cytosolic GSTM1 enzyme is found in lung epithelial cells and *GSTM1* polymorphisms have been

associated with susceptibility to lung inflammation and asthma (Brasch-Anderson et al. 2004; Joubert et al. 2011; Wu et al. 2012a). The *GSTM1* gene is located on chromosome 1p13.3 and has two variants, the wild type-*GSTM1*1* allele, which expresses GSTM1 protein, and *GSTM1*0* allele which fails to express the GSTM1 protein (Hayes et al. 2005). *GSTM1*-null genotype is found in about 50% of the population, with differences in polymorphism frequencies by race (To-Figueras et al. 1997). *GSTM1*-null genotype frequencies were 23-46.5% in African Americans, and range from 13.1-54.5% in Caucasians, 35-53% people of Hispanic descent, and 40-53% in people of Asian descent (Cotton et al. 2000; Geisler and Olshan 2001; Carlsten et al. 2008; Mo et al. 2009). The *GSTM1*-null polymorphism has been associated increased susceptibility and aggravation of lung diseases, including an increased risk of developing asthma in children (Gilliland et al. 2002; Tamer et al. 2004; Li et al. 2013), increased risk of allergic sensitization (Perzanowski et al. 2013), lower lung function (Tamer et al. 2004), and increased airway inflammation from exposures to air pollutants (such as DEP, SHS and ozone) (Gilliland et al. 2004; Gilliland et al. 2006; Alexis et al. 2009). The *GSTM1*-null polymorphism, has also been associated with an increased risk of lung cancer (Quiñonez et al. 2001; Matakova et al. 2009).

Previous studies examining associations between *GSTM1* genotype and urinary 1-OHP(G) have reported inconsistent results. Some studies have found associations between urinary 1-OHP(G) and *GSTM1* genotype (Gabbani et al. 1996; Pan et al. 1998; Nan et al. 2001; Alexandrie et al. 2000; Lee et al. 2009; Islami et al. 2012), while others have reported no difference in 1-OHPG by *GSTM1* genotype (Hemminki et al. 1997; Merlo et al. 1998; Nerukhar et al. 2000; Adonis et al. 2003; Apostoli et al. 2003; Yang et

al. 2003; Wu et al. 2004; Bosso et al. 2006; Moretti et al. 2007). The reported mechanism for increases in urinary 1-OHP(G) in *GSTM1*-null individuals involves an accumulation of reactive PAH derivatives (such as quinones, semiquinones and epoxides) due to lack of GSTM1 enzyme, thereby reducing antioxidant capacity, and a concomitant induction of CYP1A1 through PAH-Ahr activity (Alexandrie et al. 2000). The reactive PAH intermediates are readily conjugated by the phase II enzyme, uridine diphosphoglucuronosyltransferase (UGT) resulting in increased urinary 1-OHPG output. The glutathione GSH conjugation pathway (which is catalyzed by GST) competes with the glucuronide pathway for phase II detoxification of activated PAH intermediates. Therefore, a lack of GSTM1 enzyme activity (in individuals with *GSTM1*-null genotype) may be compensated through the induction of other phase II enzymes (including UGT, GSTP1 and GSTT1) (Rihs et al. 2005). Inter-individual differences in PAH metabolism may therefore be partially explained by polymorphisms in genes encoding for phase II enzymes, such as *GSTM1*.

We evaluated associations between internal dose PAHs (measured by urinary 1-OHPG) and biomarkers of oxidative stress (urinary isoprostane), gene expression of antioxidants, and peripheral blood, serum and nasal lavage biomarkers of inflammation in an observational study of atopic asthmatic adults in Baltimore City. We also evaluated whether *GSTM1* genotype was an effect modifier of the associations between internal dose PAHs and oxidative stress and inflammatory biomarkers. We hypothesized that PAH internal dose would be associated with increased oxidative stress, increased induction of antioxidant genes (*HO-1, NQO1, GCLC* and *GCLM*), increased serum mediators (eosinophil cationic proteins (ECP), MIP-1α, eotaxin, CCL20 and RANTES),

and increased local and systemic inflammation (whole blood and nasal lavage eosinophils and neutrophils, and nasal lavage cytokines and chemokines). We also hypothesized that *GSTM1* genotype will be a significant effect modifier, with *GSTM1*-null participants having stronger positive associations between 1-OHPG and oxidative stress and inflammatory biomarkers compared to *GSTM1*-present participants, due to less antioxidant capacity in individuals who are unable to produce the GSTM1 protein.

MATERIALS AND METHODS

Study design

Our study was conducted within an ongoing PM nasal exposure study: The Nasal Challenge to Indoor Particulate Matter study. The parent study enrolled 32 non-smoking atopic asthmatic adults ages 18-50 to measure inflammatory biomarkers and gene expression in adults challenged intranasally with particulate dust suspended in saline (Ong et al. 2011; Dr. Elizabeth Matsui, personal communication). The parent study design is a single blind, placebo-controlled crossover with individuals randomized to nasal challenges of PM in saline followed by saline, or saline followed by PM. There was a minimum one-week washout period before returning for the second instillation.

In our study, we used samples from the two baseline time points only. Our study design was therefore an observational study with two visits per participants. The time between visits ranged from 7-114 days, with a mean of 30 days. Urine, blood, exhaled nitric oxide (eNO), nasal lavage fluid, nasal epithelial cells, and measures of lung function using spirometry (FEV, FVC, and FEF_{25-75}) were collected. The following

biomarkers were available from the parent study: urinary isoprostane (8-iso-$PGF_{2\alpha}$); gene expression (mRNA) from nasal epithelial cells: HO-1, NQO1, GCLC, GCLM, TFF2, and IL-33; whole blood inflammatory markers with and without phorbol 12-myristate 13-acetate (PMA) activation: PBMC, monocytes, PMN, eosinophils, neutrophils and lymphocytes; cytokines and chemokines in serum: IL-6, IL-8, IL-10, IL-13, eotaxin, MIP-1α, growth-regulated oncogene (GRO), RANTES, chemokine (C-C motif) ligand 20 (CCL20), GRO-α, and ECP); and in nasal lavage fluid: IL-6, IL-8, IL-10, IL-13, eotaxin, MIP-1α, GRO, RANTES, CCL20 and GRO-α. Atopy was defined as having allergic responses to at least one of 14 common aeroallergens (dust mite mix, rat and mouse epithelia, dog hair/dander, cat dander, American and German cockroach, three pollens (Eastern Oak mix, grass mix, ragweed mix) and four molds (*Helminthosporium, Alternariam, Penicillium* and *Aspergillus*)) from skin prick tests.

Urinary 1-OHPG

Spot urine samples were analyzed for urinary 1-OHPG concentrations using immunoaffinity chromatography (IAC) and synchronous fluorescence spectroscopy (SFS), as previously described (Strickland et al. 1994). Urine samples (2ml) were treated with 0.1N HCl (90°C, 60 min), neutralized, and loaded onto Sep-pak C18 cartridges (Waters). After washing with 30% methanol, the relatively non-polar metabolites were eluted with 4 ml of 80% methanol and the volume of eluate is reduced to 0.5ml by evaporation on a Speedvac. The concentrated samples were diluted to 4ml with 15mM phosphate buffered saline (PBS) and loaded onto immunoaffinity columns prepared with 0.8ml cyanogen bromide activated sepharose 4B (Sigma) coupled with monoclonal antibody 8E11 that recognizes several PAH-DNA adducts and metabolites (Santella et al.

1986). We have previously shown that 1-OHPG binds to these columns (Strickland et al. 1994). After washing the columns three times with 4 ml 15 mM PBS, bound compounds were eluted with 2 ml of 40% methanol in three fractions. Eluate fractions were analyzed by SFS with a Perkin-Elmer LS50 fluorescence spectrometer. The excitation-emission monochromators are driven synchronously with a wavelength difference of 34 nm. Under these conditions 1-OHPG produces a characteristic fluorescence excitation maximum at 347 nm with emission maximum at 381 nm (Strickland et al. 1994). Fluorescence intensity was used to quantify 1-OHPG; in our laboratory the limit of detection was about 0.03 pmol/ml. This level of sensitivity is sufficient to detect urinary 1-OHPG in >90% of subjects with low-level exposure to PAHs (such as in the U.S.), and in 100% of subjects with high exposure. The coefficient of variation of the assay is typically 6-10% (interbatch) in our laboratory.

Urinary Creatinine

Creatinine concentrations were determined using a modified version of the Jaffe reaction using the Creatinine Assay Kit (Cayman Chemical Company; Ann Arbor, MI). Briefly, 150 μl of alkaline picrate solution was added to wells containing 15 μl creatinine standard or urine samples in duplicate, and the solution was mixed for 10 minutes. The absorbance of the solution (due to the reaction between the alkaline picrate and urinary creatinine metabolites) was read at 450-500 nm using a Biotek ELx800 Absorbance Microplate Reader. 5 μl of acid solution was then added to the assay and the absorbance was read again at 450-500 nm, 20 minutes after mixing. The difference in color intensity before and after acidification is proportional to urinary creatinine concentration. Urinary

creatinine levels were determined using a creatinine standard curve, which was estimated in each batch from analysis of the standard creatinine.

Urinary isoprostane

Isoprostane (8-iso-Prostaglandin F2α) was measured in urine using the OxiSelectTM 8-iso-Prostaglandin F2α ELISA Kit (Cell Biolabs Inc., San Diego, California, USA). Briefly, 100µl of diluted Anti-8-iso-PGF2α was added to the antibody coated plate and incubated for 1 h at 25°C on an orbital shaker. The wells were then washed five times with 300 µl 1X Wash buffer per well to remove the antibody solution. 55 µl of sample and 55 µl of 8-iso-PGF2α-HRP conjugate were mixed in microtubes and approximately 100 µl of the mixture was transferred to the wells. After an hour at 25°C, the wells were washed five times with 300 µl 1X Wash buffer per well. 100 µl of Substrate Solution was added to each well and incubated at room temperature for 10-30 minutes on an orbital shaker. The enzyme reaction was stopped by adding 100 µl of Stop Solution to each well. Absorbance was read at 450nm (primary wave length) using a microplate reader.

Complete blood counts

Peripheral blood total cell counts and differential counts were done using an automated counter.

Chemokines and cytokines in serum and nasal lavage fluid

Cytokine and chemokine (IL-6, IL-8, IL-10, IL-13, eotaxin, MIP-1α, GRO, RANTES, CCL20, GRO-α, and ECP) concentrations in the sample supernatants were determined by enzyme-linked immunosorbent assay (ELISA) using Milliplex^{TM} Multiplex kits (Millipore, Billerica, MA) according to manufacturer's protocol. Briefly,

in a 96 well multiscreen filter plate, 25 μL sample in duplicate was incubated with 25μL antibody coated beads overnight at 4°C on a plate shaker. Plates were then washed two times on a vacuum apparatus and 25 μL of secondary antibody was added and incubated at room temperature for 1 hour on while shaking. Finally, 25 μL of streptavidin-RPE was added directly to the secondary antibody and incubated for 30 minutes at room temperature with shaking. Plates were then washed two more times and 100 μL of sheath fluid was added. Plates were shaken for 5 minutes and then read using luminex technology on the Bio-Plex™ (Bio-Rad, Hercules, CA). Concentrations were calculated from standard curves using recombinant proteins and expressed as pg or ng/ml.

Serum and nasal lavage GRO and GRO-α

Plates were coated with capture antibodies (Ab) overnight at 4°C. Plates were then washed three times with 175 μl wash, and blocked with 1% BSA/PBS for 1 h at room temperature. Samples and standards were loaded and incubated for 2 hours at room temperature. Plates were then washed again. Secondary Ab was added and incubated at room temperature for 1 h. Plates were washed again. Streptavidin-HRP was added and incubated for 30 minutes, in the dark at room temperature. Plates were then washed again and developed with a 1:1 mixture of Color A and Color B. Plates were read at 450nm with a 595nm background subtraction.

Serum and nasal lavage ECP

Samples were loaded onto pre-coated plates and incubated at room temperature for 1 h. Plates were washed four times with 300 μl wash. Conjugate reagent was added and incubated for 1 h at room temperature. Plates were washed again. Substrate solution

was added and incubated at room temperature for 10 minutes and then stopped with H_2SO_4. Plates were then read at 450nm with a 595nm background subtraction.

Nasal epithelial RNA

Nasal epithelial cells were collected by using a Rhinoprobe curette and immediately lysed in 100 mL lysis solution provided with the RNAqueous-micro kit (Ambion, Austin, Tex). Lysed nasal samples were stored at -80°C before RNA isolation. Briefly, 50 µL 100% ethanol was added to 100 µL lysate and vortexed thoroughly. Lysate/ethanol mixture was loaded onto a microfilter cartilage assembly (column) and centrifuged for 30 seconds at 13,000g. The filter column was washed once with 180 µL wash and twice with 180 µL wash II. Finally, the filter column was spun at 13,000g without adding any wash buffer. RNA was eluted in 20 µL (10 µL x 2) elution buffer and quantified by using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Rockford, Ill).

cDNA synthesis and real-time PCR

Total RNA was extracted from cells by using the RNeasy kit (QIAGEN). RNA samples were reverse-transcribed by using a high-capacity cDNA reverse-transcription kit following the manufacturer's instructions. A 50-ng cDNA template was used for each real-time reaction with ABI 7000. Primer and probes used for this study are inventoried by Applied Biosystems, span the exon junction, and do not detect genomic DNA. The sequences for these primers and probes are proprietary of Applied Biosystems. An aliquot of diluted cDNA was used to measure human *NQO1* (Hs00168547_m1), *HMOX1* (Hs1110250_m1), *GCLM* (Hs00157694_m1), *GCLC* (Hs00155249_m1), *TFF2 (*Hs00193719_m1*),* and *IL33* (Hs00369211_m1) gene expression with TaqMan primers

and probe mixes from Applied Biosystems. β-Actin (Hs99999903_m1) was used for normalization. The sequences for these primers and probes are proprietary to Applied Biosystems. The assays were performed using the ABI 7000 TaqMan system (Applied Biosystems).

Statistical analysis

Summary statistics were reported for urinary 1-OHPG concentrations. 1-OHPG concentrations and all continuous biomarker values (except MIP-1α) were log-transformed to adjust for skewed distributions. Multivariate linear regressions with generalized estimating equations (GEE) were used to assess associations between the primary predictor (e.g. urinary 1-OHPG) and outcome (dependent) variables (i.e. urinary isoprostane; and nasal lavage, serum, and whole blood mediators, chemokines and cytokines) while adjusting for repeated measurements (i.e. visit) and possible confounders. Explanatory variables were considered possible confounders if the ß-coefficient changed by more than 10% after inclusion of the exposure variable (e.g. 1-OHPG) in the model or if the variable was significantly associated with the exposure or outcome. Our final models for multivariate linear regression with GEE were adjusted for age, race, gender, season, caregiver educational attainment and *GSTM1* genotype). Urinary 1-OHPG was adjusted for urinary creatinine by including creatinine concentrations in the model as an independent variable (Barr et al. 2005). Age, 1-OHPG, urinary isoprostane, serum biomarkers, whole blood biomarkers, mRNA, and nasal lavage eosinophil, neutrophil, white blood cell count, IL-8 and GRO were measured as continuous variables; race, gender, and nasal lavage IL-6, IL-13, ECP, CCL20, eotaxin and MIP-1α were binary variables; season was categorical variable; and caregiver

education was measured as an ordinal variables. Two-tailed p-value<0.05 was used to determine statistical significance. Effect modification was assessed using pairwise interaction terms for independent variables (e.g. the product of 1-OHPG concentrations and *GSTM1* genotype) and interaction terms were considered statistically significant at p-value<0.1. We used only the two baseline values for each study subject in our analyses. 1-OHPG concentrations were reported as μmol/mol creatinine. Missing values were not included in analyses. All data were analyzed using STATA 11.1 (College Station, TX).

Sensitivity analyses

Multivariate linear regression (MLR) was performed without GEE analysis to test whether adjusting for repeated measurements resulted in over-correction (Supplemental Tables 4-2 to 4-4). There were fewer significant associations using MLR without GEE, suggesting that accounting for intra-individual variation was important for these analyses. We therefore reported MLR with GEE as our primary analysis. In addition, we included number of days between visit in the MLR with GEE model as an independent variable (Supplemental Table 4-6). Multivariate linear regression with GEE analyses with the number of days between visits included in the model as an independent variable yielded similar results to crude analyses (excluding the number of days between visits variable), suggesting that the washout period (minimum 7 days) was sufficient and intra-individual differences based on time between visits were not influential in associations between biomarkers of PAH internal dose and early biological effects (Figure 4-4).

RESULTS

Demographics

Most of the participants in the Nasal Challenge to Indoor Pollutants study were women (75%), African American (84%), between 18 and 50 years of age (mean: 30 years of age), had at least a high school diploma (66%) and resided in households with annual incomes of less than $25,000 (70%) (Table 4-1). All participants were atopic asthmatics, and most experienced asthma symptoms (66%) and used rescue medication (72%) during the two weeks prior the baseline visit. Over two thirds of the participants (69%) were able to express the GSTM1 protein (*GSTM1*-present genotype), and about a third (31%) were unable to express the GSTM1 protein (*GSTM1*-null genotype).

Predictors of 1-OHPG

1-OHPG concentrations were analyzed in 60 spot urine samples, and urinary creatinine (Cr) was measured to adjust for differences in urine dilutions (Table 4-2, Figure 4-1). Urinary 1-OHPG had an arithmetic mean (standard deviation (SD)) of 2.54 (4.51) pmol/ml, geometric mean (GM) of 0.78 pmol/ml, median (interquartile range (IQR)) of 0.87 (0.20-2.71) pmol/ml, 95th percentile of 11.3 pmol/ml, and range of 0.05-22.76 pmol/ml. Six samples (10%) were below the limit of detection (LOD) of 0.05 pmol/ml. Urinary 1-OHPG concentration (pmol/ml) was divided by urinary creatinine (µmol/ml) to adjust for urine dilution differences, and were reported as µmoles 1-OHPG per mole creatinine (µmol/mol creatinine). Urinary 1-OHPG was positively associated with urinary creatinine (ß=0.478, p=0.001; 95% CI [0.198-0.757]), age (ß=0.051, p=0.024; 95% CI [0.007-0.095]), but not associated with gender, race, education or *GSTM1* genotype using bivariate analysis (Table 4-3). Using multivariate linear

regression analysis, urinary 1-OHPG was associated with age (ß=0.031, p=0.036; 95% CI [0.002-0.060]), and having a HS diploma (compared to some HS education) (ß=1.082, p=0.003; 95% CI [0.361-1.803]) (Table 4-3). Seasonally, 1-OHPG was lowest in spring (which was used as the reference), and significantly higher in the summer (ß=1.398, p=0.017; 95% CI [0.252-2.545]) and the winter (ß=1.832, p<0.001; 95% CI [0.992-2.671]), compared to spring. *GSTM1* genotype also predicted urinary 1-OHPG. Participants with the *GSTM1*-present genotype (able to produce the GSTM1 enzyme) had significantly higher 1-OHPG concentrations (ß=0.795, p=0.031; 95% CI [0.071-1.519]) than participants with *GSTM1*-null genotype (cannot produce the GSTM1 enzyme), using multivariate analysis (Table 4-3, Figure 4-1).

1-OHPG and inflammatory effects

Overall, urinary 1-OHPG was associated with significant decreased serum IL-10 (ß=-0.052, p=0.027; 95% CI [-0.098-(-0.008)]), and CCL-20 (ß=-0.128, p=0.010; 95% CI [-0.225-(-0.031)]), decreased PBMC (ß=-0.128, p=0.010; 95% CI [-0.225-(-0.031)]) and decreased IL-33 mRNA from nasal epithelium (ß=-0.101, p=0.024; 95% CI [-0.142-(-0.010)]) in atopic asthmatic adults (Table 4-4). Urinary 1-OHPG was also associated with significant increases in nasal lavage eosinophil percent (ß=0.013, p=0.008; 95% CI [0.003-0.023]). However, urinary 1-OHPG was not associated with oxidative stress (urinary isoprostane), induction of antioxidant and cytokine mRNA (NQO1, HO-1, GCLM, GCLC, TFF2, and IL-33) in nasal epithelium cells, serum inflammatory cytokines (IL-6, IL-8 and IL-13), serum inflammatory chemokines (eotaxin, GRO, GRO-α, MIP-1α, and RANTES), serum ECP, and nasal lavage cytokines and chemokines (Table 4-4).

GSTM1-null genotype

Urinary 1-OHPG was independently associated with increased oxidative stress, as measured by urinary isoprostane in *GSTM1*-null participants (ß=0.237, p=0.015; 95% CI [0.046-0.428]) using multivariate linear regression analysis with GEE and adjusting for possible confounders (Table 4-5 and Supplemental Table 4-5 (full table)). Urinary 1-OHPG was also associated with significant increases in serum biomarkers of inflammation, including ECP, GRO, GRO-α, IL-8 and RANTES, in *GSTM1*-null participants. Local inflammation, as measured by total white blood cells in nasal lavage fluid, was significantly associated with elevated urinary 1-OHPG in *GSTM1*-null participants. 1-OHPG was also significantly associated with both increased peripheral blood inflammatory biomarkers: lymphocytes, and monocytes (both activated with PMA), and decreased peripheral blood polymorphonuclear leukocytes (PMN) in *GSTM1*-null participants (Table 4-5 and Supplemental Table 4-5).

GSTM1-present genotype

Among *GSTM1*-present participants, urinary 1-OHPG was associated with an up-regulation of nasal epithelium NQO1 and GCLC mRNA (Table 4-5 and Supplemental Table 4-5).1-OHPG was associated with local inflammation, specifically significantly increased nasal lavage eosinophil percent in *GSTM1*-present participants. Urine 1-OHPG was also associated with significant decreases in peripheral blood mononuclear cells (PBMC), PMA activated polymorphonuclear leukocytes (PMN) and monocytes, and significant decreases in serum IL-6 and CCL20 in *GSTM1*-present individuals (Table 4-5 and Supplemental Table 4-5).

GSTM1 effect modification

Overall, biomarkers of oxidative stress, inflammation and antioxidant responses did not differ by *GSTM1* genotype, except with nasal lavage ECP, which was significantly higher in *GSTM1*-null individuals compared to *GSTM1*-present individuals (p=0.060) (Supplemental Table 4-1). *GSTM1* genotype modified the associations between 1-OHPG and urinary isoprostane, with stronger associations in *GSTM1*-null subjects compared to *GSTM1*-present subjects (p-interaction=0.002) (Table 4-5; Figures 4-2 and 4-3). Associations between 1-OHPG and the up-regulation of GCLC mRNA were also significantly stronger in participants with *GSTM1*-null genotype than with GSTM1-present genotype (p-interaction=0.024). *GSTM1* genotype also modified associations between urinary 1-OHPG and IL-33 mRNA in nasal epithelial cells, with associations in opposite directions (p-interaction<0.001). Urinary 1-OHPG was inversely associated with IL-33 mRNA in *GSTM1*-null participants, whereas 1-OHPG was positively associated with IL-33 mRNA induction in *GSTM1*-present participants. *GSTM1* genotype also modified associations between urinary 1-OHPG and serum chemokines. *GSTM1*-null participants had significantly stronger positive associations between 1-OHPG and serum GRO and GRO-α than *GSTM1*-present participants (p-interaction=0.075 and 0.004, respectfully). *GSTM1* genotype also modified associations between urinary 1-OHPG and peripheral blood biomarkers. *GSTM1*-null participants had significantly stronger positive associations between 1-OHPG and peripheral blood lymphocytes than *GSTM1*-present participants (p-interaction<0.001). *GSTM1* genotype also modified the associations between 1-OHPG and PBMC (p-interaction=0.002) and PMA activated peripheral blood monocytes (p-interaction<0.001) and PMN (p-

interaction=0.002). These 1-OHPG-peripheral blood biomarker associations were in opposite directions depending on the *GSTM1* genotype. 1-OHPG was positively associated with PBMC, monocytes and PMN in *GSTM1*-null participants, whereas 1-OHPG was inversely associated with these peripheral blood biomarkers in *GSTM1*-present participants.

DISCUSSION

1-OHPG levels

Participants in our study (non-smoking atopic adults with active asthma) had twice the geometric mean, and about 2-4-fold higher median urinary 1-OHPG concentrations than the US average for adults (Table 4-6). The GM of 1-OHPG concentration in our study (0.07 μmol/mol Cr) was also similar to a cross-sectional study of the South Korean population in 2008 (0.07 μmol/mol Cr) (Sul et al. 2012), but over two-fold higher than non-smoking Swedish adults (median: 0.03 μmol/mol Cr) (Levin et al. 1995), and South Korean university students (GM: 0.03 μmol/mol Cr) (Kim H et al. 2001). The high levels of PAH internal dose in our study may be attributed to elevated PAH exposures from living in an urban environment, including exposures to indoor sources (e.g. SHS and heating) and ambient sources (e.g. vehicular exhaust, industrial activity, and by-products from the burning of fossil fuels).

GSTM1 and 1-OHPG

In our study, urinary 1-OHPG was significantly higher in *GSTM1*-present participants compared to *GSTM1*-null participants. Some studies have shown similar

associations, with higher urinary 1-OHP(G) concentrations in *GSTM1*-present individuals (Gabbani et al. 1996; Schoket et al. 2001; Lee et al. 2002; Petchpoung et al. 2011), while other studies have reported higher urinary 1-OHP(G) concentrations in *GSTM1*-null individuals (Pan et al. 1998; Nan et al. 2001; Alexandrie et al. 2000; Lee et al. 2003; Chuang et al. 2007; Lee et al. 2009; Islami et al. 2012) or no associations between 1-OHP(G) and *GSTM1* genotype (Hemminki et al. 1997; Merlo et al. 1998; Nerukhar et al. 2000; Adonis et al. 2003; Apostoli et al. 2003; Yang et al. 2003; Wu et al. 2004; Bosso et al. 2006; Moretti et al. 2007). The *GSTM1*-present genotype was associated with higher 1-OHPG in multivariate regression analysis only, suggesting an independent relationship between 1-OHPG and *GSTM1*-present genotype–that is observable only after adjustment for possible confounding variables. Similarly, Lee et al. (2002) reported significantly higher 1-OHPG levels in multivariate analyses of *GSTM1*-present participants who were smokers, suggesting that gene-environment interactions may modulate PAH internal dose analysis.

Urinary 1-OHPG concentrations also may be influenced by phase I enzymes (CYP1A1, CYP1A2, CYP1B1), which hydrolyze PAHs to epoxides, quinones and semi-quinones, prior to detoxification by glutathione (GSH), glucuronide and sulfate conjugation (Alexandrie et al. 2000). Cytochrome P450 (CYP) enzyme activity, which can be modified due to polymorphisms may also be associated with phase II enzyme (e.g. GSTM1 and UDP glucuronosyl transferase (UGT)) activity. *CYP1A1* polymorphisms, such as *CYP1A1 MspI* T6235C (TC (*1/*2) and CC (*2/*2)) variant alleles, or *CYP1A1* exon 7 Ile462Val (Ile/Val (*1/*3) and Val/Val (*3/*3)) variant alleles, confer increased CYP1A1 activity compared to wild type monozygotic (*1/*1) alleles. Increased CYP

activity may lead to increases in activated oxidative PAH intermediates which are subsequently conjugated through glucuronidation and excreted in urine (as 1-OHPG). (Nerurkar et al. 2000; Petchpoung et al. 2011). A study of Thai bus drivers reported higher 1-OHPG in workers with *CYP1A1*2* and *CYP1A1*3* variant alleles and higher 1-OHP in workers with *GSTM1*-present genotype compared to *GSTM1*-null genotype, in workers exposed to DEP (Petchpoung et al. 2011). In a Swedish aluminum plant, workers with *CYP1A1* Ile/Val and *GSTM1*-null variant alleles had significantly higher 1-OHP concentrations than workers with other *CYP1A1/GSTM1* allele combinations, suggesting gene-environment interaction in workers with dual polymorphisms (Alexandrie et al. 2000). Adonis et al. (2003) also reported similar increases in urinary 1-OHP in Chilean diesel revision plant workers with *CYP1A1*2/ GSTM1*-null variant alleles compared to those with *CYP1A1/GSTM1* wild type alleles. The mechanism for increased 1-OHPG in *GSTM1*-present individuals is unknown. Based on the reported metabolic pathways for PAHs, phase II conjugation by UGT and GSTs are not in the same pathway, and glucuronide conjugation does not directly involve GSTs (ATSDR 1995; Rihs et al. 2005; IARC 2010). Based on this metabolic pathway, the role of GSTM1 in directly conjugating PAH metabolites to urinary glucuronide metabolites may therefore be limited, and the absence of the GSTM1 enzyme (as with *GSTM1*-null individuals) would therefore be expected to make little difference in 1-OHPG excretion. UGT and GST may, however, compete with each other for conjugating reactive PAH intermediates. In addition, gene interactions between GST and UGT may modulate phase II PAH metabolism (such as induction of *UGT* by *GST* to adapt to elevated levels of PAH oxidative intermediates, ROS or other reactive agents), and the results of these

interactions (e.g. increased glucuronidation) may be reflected in urinary internal dose glucuronide biomarkers. Further studies investigating interactions between phase I (*CYP1A1, CYP1B1, CYP1A2*), phase II (*GSTM1, GSTP1, GSTT1, UGT, NQO1*), and other xenobiotic metabolism enzyme genes, and gene-environment interactions are therefore necessary to examine the influences of genetic polymorphisms on susceptibility, biological effects, and the body burden of PAHs and other xenobiotics.

1-OHPG and inflammatory markers

In our study of atopic asthmatic adults, urinary 1-OHPG was independently associated with increased nasal lavage eosinophil percent. Local eosinophilic inflammation is a hallmark of allergic inflammatory responses. Similar eosinophilic inflammatory responses to PAH exposures have been reported in epidemiological studies, including increased ECP, RANTES, monocyte chemoattactant protein-3 (MCP-3), and MIP-1α in nasal lavage fluids after nasal instillation with DEP, in mostly atopic adults (Diaz-Sanchez 1998; Diaz-Sanchez 2000). Increased allergic inflammation in participants with high PAH exposures may also suggest synergism between inhaled PAHs and aeroallergens. Animal and human studies have shown adjuvant effects by PAHs on allergic inflammation when co-exposed to aeroallergens (such as OVA, ragweed, and keyhole limpet hemocyanin (KLH)), including increases in allergen specific immunoglobulin E (IgE), IL-8 levels and a skewing toward Th2-type responses (Takano et al. 1995; Miyabara et al. 1998; Diaz-Sanchez et al. 1999;). IgE, IL-8, and Th2-related cytokines and chemokines promote eosinophil production, development and survival, and are integral to allergen mediated inflammatory pathways (Pandya et al. 2002).

In our study, 1-OHPG was also independently associated with increased whole blood eosinophils and neutrophils activated by PMA, suggesting that PAHs are associated with systemic eosinophilic and neutrophilic inflammation. Serum cytokines (IL-10 and IL-33) and chemokines (CCL20), however, were inversely associated with urinary 1-OHPG, suggesting that PAHs may also suppress some systemic Th2-type inflammatory responses. Specifically, PAH exposures may be related to decreased Th2 lymphocyte proliferation and differentiation (IL-10) (Pandya et al. 2002; Matsumoto et al. 2004), decreased Th2 maintenance and Th2-type cytokine production (IL-33) (Finkelman et al. 2010; Smith 2010), and decreased dendritic cell development (CCL20) (Reibman et al. 2002). Indeed, PAHs have shown immunosuppressive properties. *In vitro* and animal studies have reported associations between PAHs and decreased B-cell and T-cell production and function, and decreased PBMCs (ATSDR 1995; IARC 2010; Zaccaria and McClure 2013). PAHs may therefore be associated with local eosinophilic inflammation, however, associations with systemic inflammatory pathways may be more complicated, with concurrent associations between PAHs and immunotoxicity, and Th1 or Th2-related inflammatory responses. Systemic inflammation may therefore reflect heterogeneous inflammatory immune responses to exposures to various exogenous substances, including air pollutants (such as PAH, PM, DEP, ozone, NO_2), allergens, and biological pathogens (such as viruses, endotoxin and fungi). In observational studies such as our study, inflammatory immune responses to myriad exogenous exposures may not produce clear patterns of systemic inflammation associated with Th1 or Th2-specific cytokine profiles or adaptive immune (eosinophil mediated) or innate immune (neutrophil mediated) inflammation. Rather, complex interactions between these immune response

pathways, and to interactions with many xenobiotics (e.g. PAHs and aeroallergens) will likely be reflected in varying degrees of associations between internal dose biomarkers and pluripotent cytokines, chemokines and inflammatory mediators measured in serum and whole blood.

***GSTM1* genotype**

In our study, PAH internal dose was associated with increased allergic inflammation among *GSTM1*-null participants. Urinary 1-OHPG was independently associated with increased systemic oxidative stress in *GSTM1*-null individuals only. These positive associations between elevated PAH exposures and increased oxidative stress in *GSTM1*-null individuals, and effect modification by *GSTM1* genotype (with a more pronounced PAH-systemic oxidative stress association in *GSTM1*-null individuals) support the concept of inflammation involving increased oxidative stress in persons with less antioxidant capacity. PAH toxicity through oxidative stress and inflammation-mediated pathways have been well characterized (Casillas et al. 1999; Fahy et al. 1999; Diaz-Sanchez et al. 2000; Nel et al. 2001; Pandya et al. 2002; Li et al. 2009; Cho and Moon 2010; Dozor et al. 2010).

Elevated PAH internal dose was associated with significant increases in systemic eosinophil-mediated inflammation (i.e. increases in serum RANTES, ECP, GRO, GRO-α and IL-8), increased peripheral blood monocytes and lymphocytes, and increased local inflammation (nasal lavage white blood cell count (WBCC)) in *GSTM1*-null participants this study. Studies of nasal provocations with combinations of PAH rich pollutants (i.e. DEP and SHS) and allergens showed similar modifications by *GSTM1* genotype, with increased susceptibility to allergic inflammation and larger increases in IgE and

histamine (related to Th2-type responses) among *GSTM1*-null subjects (Gilliland et al. 2004). Similarly, individuals exposed to SHS and challenged with ragweed showed significantly higher IgE in *GSTM1*-null compared to *GSTM1*-present individuals (Gilliland et al. 2006). The chemokine, RANTES is integral to eosinophilic inflammation and is associated with Th2-related cytokine proliferation, and histamine release from basophils. It can activate eosinophils to release ECP, a granular protein that causes epithelial shedding (Turner and Foreman 1999; Diaz-Sanchez et al. 2000). IL-8 and GRO-α are pro-inflammatory CXC chemokines that are associated with neutrophil chemotaxis and degranulation, and leukocyte activation and migration (Fahy et al. 1999; Salvi et al. 2000). *In vitro* studies have also shown PAHs to be potent inducers of pro-inflammatory IL-8 mRNA and protein production (Fahy et al. 1999; Bommel et al. 2000), and have shown that *GSTM1* genotype modulates DEP induced IL-8 expression, such that cells that were GSTM1 deficient (null) showed increased IL-8 expression compared to cells with GSTM1 protein production (Wu et al. 2012b). Therefore, our study, an observational study with atopic asthmatic adults, supports the PAH exposure-allergic inflammatory response mechanism and suggests increased susceptibility for individuals who lack GSTM1 enzyme activity.

***GSTM1*-present**

PAH internal dose was independently associated with antioxidant mRNA induction (*NQO1* and *GCLC*) in nasal epithelium cells among *GSTM1*-present individuals. Glutamate cysteine ligase (GCL), an antioxidant enzyme, is the rate-limiting enzyme in GSH synthesis (Dickenson et al. 2004). Induction of *GCLC* as part of the antioxidant pathway is likely directly associated with GST enzymes, as GST catalyzes

the conjugation of reactive oxidative PAH intermediates by GSH to a stable, more excretable PAH metabolite. The absence of GSTM1 enzymes may therefore directly influence GSH levels, by suppressing GCLC expression. Our study, however found associations between 1-OHPG and induction of GCLC mRNA in both GSTM1-null individuals (although not significant) and significantly in *GSTM1*-present individuals. Our findings therefore suggest an up-regulation of *GCLC* in response to increased oxidative stress associated with PAH exposures, with possible modulations by gene interactions between *GSTM1* and *GCLC* conferring further up-regulation. We also hypothesized that PAH internal dose would be associated with induction of nuclear factor (erythroid-derived 2)–like 2 (Nrf2) related antioxidant enzymes (such as HO-1 and NQO-1) in *GSTM1*-null individuals, because lack of a GSTM1 enzyme may lead to increased ROS and trigger induction of other antioxidants. However, in our study, elevated PAH internal dose was independently associated induction of nasal epithelium NQO1 mRNA in *GSTM1*-present participants, suggesting that exposures to PAHs resulting in increased local oxidative stress and reactive oxidant intermediates (e.g. quinones and epoxides) may be sufficient to elicit increased antioxidant and phase II metabolism responses. Indeed, the responses to increased ROS, involves induction of Nrf2 mediated antioxidant genes (e.g. *NQO1* and *SOD1*), phase II (*GST)* genes, and the transcription factor, antioxidant response element (ARE). Under this mechanism, antioxidant responses to exogenous electrophilic compounds are regulated by the Keap1/Nrf2 pathway, under which Nrf2 translocates to the nucleus, binds to the ARE, and facilitates the transcription of antioxidant enzymes (e.g. NQO1, HO-1 and SOD-1) as well as phase II enzymes (Hayes et al. 2005; Xia et al. 2006). The associations between PAH internal dose and

induction of the antioxidant NQO1 in nasal epithelial cells of *GSTM1*-present individuals may therefore suggest a sufficient response, locally, to oxidative stress.

PAH internal dose was also independently associated with decreased systemic eosinophilic inflammation (decreased whole blood monocytes and PMN with PMA activation, and serum CCL20 and IL-6) in *GSTM1*-present individuals. These inverse associations may reflect sufficient antioxidant responses, including increased induction of Nrf2 related antioxidants, and increased phase II conjugation, in *GSTM1*-present subjects with elevated PAH exposures. Antioxidant and anti-inflammatory responses in *GSTM1*-present participants are supported by associations between PAH internal dose and these protective responses that are in opposite directions depending on *GSTM1* genotype. Overall, our study shows inverse associations between PAHs and inflammatory biomarkers in *GSTM1*-present individuals, and significantly higher inflammation in individuals who cannot produce the GSTM1 enzyme (*GSTM1*-null participants) compared to *GSTM1*-present participants. Our findings are therefore consistent with increased ROS in *GSTM1*-null individuals compared to *GSTM1*-present individuals, specifically that the inability to produce the GSTM1 enzyme may reduce antioxidant capacity. Mechanisms for reduced antioxidant capacity in *GSTM1*-null individuals are also supported by positive PAH internal dose-anti-inflammation associations in *GSTM1*-present individuals, reflecting sufficient anti-inflammatory responses in *GSTM1*-present individuals. *GSTM1*-null asthmatics may therefore be more susceptible to the deleterious effects of oxidative stress, inflammation and asthma exacerbation due to reduced antioxidant capacity. Recent studies using exogenous antioxidant supplementation (Vitamin C and E) (Romieu et al. 2004; Moreno-Macías et al. 2013), and oral

administration of sulforaphane, a potent antioxidant found in broccoli sprouts (Reidl et al. 2009), have shown promise in abrogating the oxidative stress-inflammation pathways by increasing antioxidant and phase II enzymes through the Keap1/Nrf2 pathway. Further studies focusing on countering oxidative stress may therefore be important to reducing inflammation related to asthma pathogenesis and exacerbation.

Strengths

To our knowledge, this is the only study that examines associations between internal dose of PAHs (measured by urinary 1-OHPG concentrations) and oxidative stress, antioxidant gene induction, and inflammatory biomarkers in various biospecimens in atopic asthmatic adults in Baltimore City. The participants were examined at two visits, which allowed us to assess repeated measurements and account for intra-individual differences. Assessment of *GSTM1* genotype allowed us to examine genetic inter-individual differences in internal dose of PAHs and oxidative stress and inflammatory response outcomes. This study also adds to the growing literature on effects of PAHs on oxidative stress and inflammation in atopic asthmatics. This study also focuses on a vulnerable population of predominantly African American adults of low SES in Baltimore with concomitantly high air pollutant exposures and prevalent asthma.

Limitations

One drawback to our study is small sample size (32 participants), and only 9 of the participants had the *GSTM1*-null polymorphism. We also did not apportion routes of exposure (e.g. inhalation and ingestion) for PAHs. Dietary intake of PAHs from eating roasted, charbroiled or smoked foods may account for a significant portion of total PAH exposure (Fiala et al. 2000; Vyskocil et al. 2000). In addition, measurement of urinary

monohydroxylated metabolites of low molecular weight PAHs (predominantly found in gas phase), including 2-naphthol (naphthalene), 3-hydroxyphenanthrene (phenanthrene) and 9-hydroxyflurorene (fluorine) may give a better assessment of internal dose from inhaled PAHs. We also did not measure other air pollutants and biological inhalants (such as ozone, NO_2, endotoxin and aeroallergen) or assess viral infections. These possible co-exposures are associated with inflammation and asthma exacerbation, and may confound the relationships found in our analyses. We did not measure anti-allergen immunoglobulin (e.g. total serum IgE, anti-mouse IgE, anti-cockroach IgE, anti-dust mite IgE, etc.). Exposure assessments and early effect biomarker analyses would allow us to better assess the influence of allergens and allergic immune responses to inflammation and asthma exacerbations. Not all gene-environment interactions, with polymorphic genes, were assessed in this study. Polymorphisms in phase I (e.g. CYP1A1, CYP1A2) and phase II enzymes (e.g. GSTP1, GSTT1, UGT and NAT2) may be influential in explaining inter-individual differences in PAH metabolism, and subsequent 1-OHPG excretion and local and systemic inflammation. The study cohort was comprised of atopic asthmatic adults, mostly African American descent, of mostly low socioeconomic status in Baltimore, and may therefore not be generalizable to asthmatic populations or people not living in urban environments of low socioeconomic status.

Conclusion

In conclusion, PAH internal dose in our study of non-smoking atopic asthmatic adults was higher than the US national average and international studies of adults. *GSTM1* genotype modulated oxidative stress and inflammatory responses with increased inflammation in *GSTM1*-null participants. These results support our hypothesis of

increased ROS in *GSTM1*-null participants (due to decreased antioxidant capacity, and possible gene-gene and gene-environment interactions). Internal dose PAHs are also associated with increased oxidative stress and increased systemic inflammation in *GSTM1*-null participants. The inflammatory responses have a predominantly eosinophilic and Th2-related cytokine profile. However, immune responses to many exogenous exposures (including PAHs, SHS, ozone, NO_2, PM, aeroallergens, endotoxin and viruses) are complex and may include concurrent associations with eosinophilic, neutrophilic, and Th1 and Th2-related inflammatory immune responses. PAH exposures were associated with oxidative stress and inflammatory responses, with implications for deleterious outcomes (e.g. asthma exacerbation) due to increased ROS and reactive PAH intermediates. Future studies may reduce asthma morbidity through interventions focused on reducing exposures and early biological effects. These strategies may include behavioral (e.g. smoking cessation), engineering (e.g. air filtration), policy (e.g. reduction in vehicular and industrial emissions) interventions, thereby reducing exposures to PAHs; and abrogation of oxidative stress and reactive oxidative compounds through supplementation with exogenous antioxidants (such as, Vitamin C and Vitamin E), or oral administration of sulforaphane to stimulate antioxidant pathways and phase II enzymes.

TABLES AND FIGURES

Table 4-1. Descriptive characteristics of the Nasal Challenge to Indoor Particulate Matter Study

Participant characteristics, total (n=32)	No. (%)
Female	24 (75)
Age, years	30 (18-50)*
Race	
African American	27 (84)
White	5 (17)
Education attainment	
Less than high school	11 (34)
HS diploma	8 (25)
College or higher	13 (41)
Income (n=27)	
≤$25,000	19 (70)
>$25,000	8 (30)
GSTM1 genotype (n=29)	
GSTM1-null	9 (31)
GSTM1-present	20 (69)
Asthma symptoms 2 weeks prior to baseline visit	21 (66)
Rescue Medication use in 2 weeks prior to baseline visit	23 (72)
Time between visits (days)	30 (7-114)*

* Mean (range)

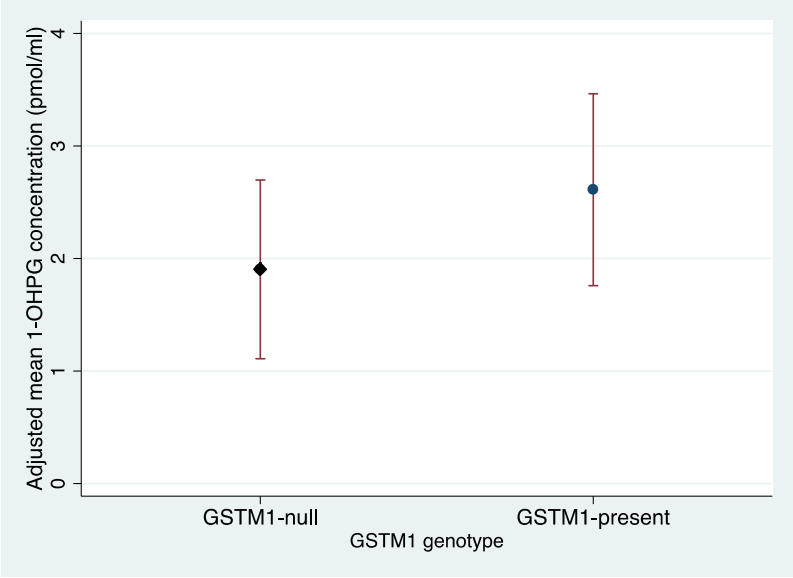
Table 4-2. Descriptive statistics for urinary 1-OHPG

	1-OHPG (pmol/ml)	1-OHPG (µmol/mol Cr)	*GSTM1*-null 1-OHPG (pmol/ml)	*GSTM1*-present 1-OHPG (pmol/ml)
Number of samples	60	60	17	39
Arithmetic Mean (SD)	2.54 (4.51)	0.18 (0.26)	1.22 (1.26)	2.47 (4.18)
Geometric Mean	0.78	0.07	0.58	0.80
Median (IQR)	0.87 (0.20-2.71)	0.10 (0.02-0.21)	0.60 (0.21-1.99)	0.80 (0.19-3.12)
95%ile	11.3	0.65	4.28	10.13
Range	0.06-22.76	0.003-1.49	0.06-4.29	0.06-22.76
% Below LOD	10%	10%		

Table 4-3. Predictors of urinary 1-OHPG concentrations

Bivariate analyses	**β**	**Std. Err.**	**z**	**P-value**	**[95% Conf.**	**Interval]**
Urinary creatinine	0.478	0.143	3.35	**0.001**	0.198	0.757
Age	0.051	0.022	2.25	**0.024**	0.007	0.095
Gender	-0.395	0.592	-0.67	0.504	-1.555	0.765
Race	0.264	0.689	0.38	0.702	-1.086	1.614
Education	0.317	0.293	1.08	0.280	-0.258	0.892
Season	0.402	0.166	2.42	**0.016**	0.076	0.729
GSTM1 genotype	0.319	0.497	0.64	0.521	-0.655	1.293
Multivariate analysis						
Urinary creatinine	0.235	0.139	1.68	0.090	-0.039	0.508
Age	0.031	0.015	2.09	**0.036**	0.002	0.060
Gender	-0.233	0.528	-0.44	0.659	-1.268	0.802
Race	0.105	0.436	0.24	0.810	-0.750	0.960
Visit	0.381	0.323	1.18	0.237	-0.251	1.014
Educational attainment						
Less than HS (reference)	-	-	-	**-**	-	-
HS diploma	1.082	0.368	2.94	**0.003**	0.361	1.803
College or higher	0.626	0.485	1.29	0.196	-0.324	1.576
Season						
Spring (reference)	-	-	-	**-**	-	-
Summer	1.398	0.585	2.39	**0.017**	0.252	2.545
Fall	0.631	0.614	1.03	0.304	-0.572	1.835
Winter	1.832	0.428	4.27	**<0.001**	0.992	2.671
GSTM1 genotype						
GSTM1-null (reference)	-	-	-	**-**	-	-
GSTM1-present	0.795	0.369	2.15	**0.031**	0.071	1.519
Constant	-4.448	0.715	-6.22	**<0.001**	-5.850	-3.046

Figure 4-1. Adjusted mean of urinary 1-OHPG concentration, by *GSTM1* genotype



Adjusted for urinary creatinine, age, race, gender, season and visit

Table 4-4. Associations between urinary 1-OHPG and biomarkers of oxidative stress, gene induction, and inflammation

	Crude[a]				Adjusted[b]			
Urinary oxidative stress biomarker	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Log Urinary isoprostane	0.025	0.104	-0.005	0.055	0.026	0.181	-0.012	0.063
Nasal epithelium mRNA								
Log NQO1	0.008	0.818	-0.059	0.074	0.051	0.084	-0.007	0.109
Log HO-1	0.035	0.423	-0.050	0.120	0.017	0.576	-0.044	0.078
Log GCLM	-0.010	0.566	-0.046	0.025	0.009	0.690	-0.034	0.052
Log GCLC	-0.017	0.512	-0.066	0.033	0.035	0.435	-0.053	0.124
Log TFF2	-0.014	0.626	-0.068	0.041	0.024	0.589	-0.064	0.112
Log IL-33	-0.061	0.111	-0.136	0.014	-0.101	**0.031**	-0.194	-0.009
Serum cytokines and chemokines								
Log Serum IL-6	-0.017	0.199	-0.042	0.009	-0.027	0.157	-0.064	0.010
Log Serum IL-8	-0.014	0.438	-0.050	0.022	-0.009	0.654	-0.050	0.031
Log Serum IL-10	-0.026	0.111	-0.058	0.006	-0.052	**0.027**	-0.098	-0.006
Log Serum IL-13	-0.001	0.839	-0.012	0.010	0.006	0.665	-0.023	0.036
Log Serum CCL20	-0.102	0.066	-0.210	0.007	-0.128	**0.010**	-0.225	-0.031
Log Serum ECP	0.002	0.928	-0.048	0.052	0.006	0.879	-0.068	0.080
Log Serum eotaxin	0.022	0.115	-0.005	0.050	0.011	0.546	-0.026	0.048
Log Serum GRO	0.002	0.919	-0.028	0.031	0.015	0.384	-0.019	0.049
Log Serum GRO-α	0.020	0.378	-0.024	0.064	0.038	0.068	-0.003	0.079
Log Serum MIP-1α	-0.016	0.356	-0.050	0.018	-0.017	0.249	-0.046	0.012
Log Serum RANTES	0.006	0.242	-0.004	0.015	0.008	0.194	-0.004	0.021

Nasal lavage biomarkers								
Log Nasal lavage IL-6	0.119	0.198	-0.062	0.299	*			
Log Nasal lavage IL-8	-0.012	0.784	-0.099	0.075	-0.019	0.532	-0.079	0.041
Log Nasal lavage IL-10	-0.011	0.369	-0.034	0.013	-0.017	0.486	-0.064	0.030
Log Nasal lavage IL-13	0.031	0.651	-0.102	0.164	0.037	0.721	-0.167	0.241
Log Nasal lavage ECP	-0.028	0.795	-0.240	0.184	0.148	0.323	-0.145	0.441
Log Nasal lavage CCL20	-0.061	0.598	-0.286	0.165	-0.041	0.660	-0.224	0.142
Log Nasal lavage eotaxin	0.017	0.842	-0.151	0.185	*			
Log Nasal lavage GRO	0.004	0.916	-0.076	0.085	0.020	0.518	-0.041	0.082
Log Nasal lavage GRO-α	0.018	0.611	-0.050	0.085	0.037	0.114	-0.009	0.083
Log Nasal lavage MIP-1α	-0.007	0.929	-0.162	0.148	*			
Log Nasal lavage RANTES	0.023	0.218	-0.014	0.061	*			
Log Nasal lavage neutrophil count	-0.026	0.223	-0.068	0.016	0.010	0.834	-0.085	0.105
Log Nasal lavage neutrophil percent	0.018	0.115	-0.004	0.041	0.043	0.121	-0.011	0.097
Log Nasal lavage eosinophil percent	-0.002	0.688	-0.011	0.008	0.013	**0.008**	0.003	0.023
Log Nasal lavage white blood cell count	-0.031	0.090	-0.066	0.005	-0.002	0.946	-0.070	0.066
Whole blood biomarkers								
Log PBMC no PMA	0.049	0.307	-0.044	0.142	-0.076	**0.024**	-0.142	-0.010
Log PBMC with PMA	0.005	0.795	-0.032	0.042	-0.034	0.383	-0.111	0.043
Log Monocytes no PMA	0.061	0.096	-0.011	0.134	-0.037	0.276	-0.104	0.030
Log Monocytes with PMA	-0.009	0.624	-0.047	0.028	-0.057	0.092	-0.124	0.009
Log Lymphocytes no PMA	0.041	0.463	-0.068	0.150	-0.009	0.832	-0.089	0.071
Log Lymphocytes with PMA	0.016	0.784	-0.100	0.133	-0.035	0.612	-0.169	0.100
Log PMN no PMA	0.018	0.540	-0.040	0.076	0.039	0.432	-0.058	0.136
Log PMN with PMA	-0.012	0.376	-0.039	0.015	-0.034	0.217	-0.087	0.020

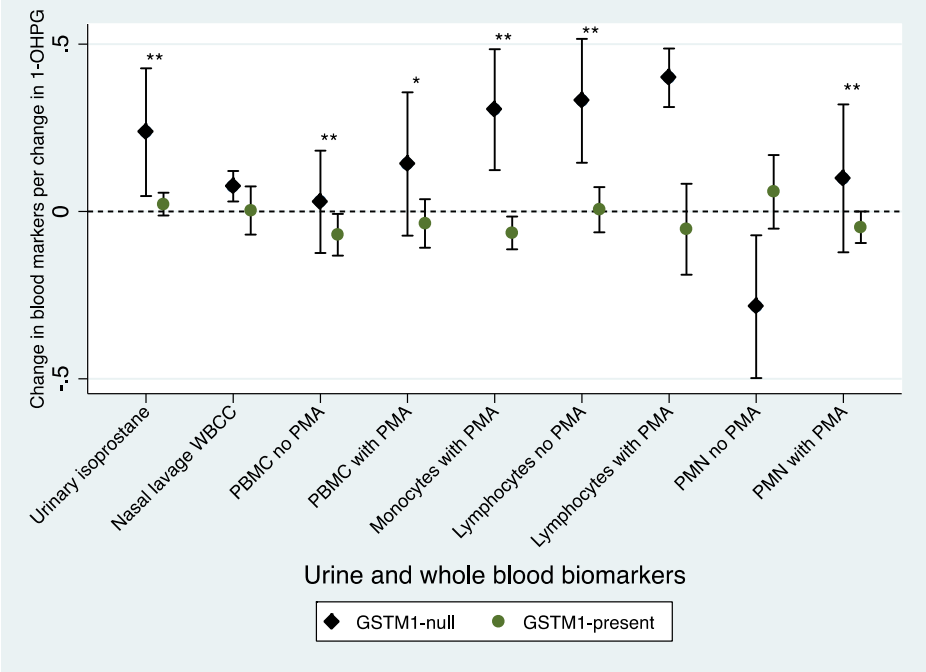
[a]Adjusted for urinary creatinine
[b]Adjusted for urinary creatinine, age, race, gender, *GSTM1* genotype, and educational attainment
*Bivariate regression model did not reach convergence

Table 4-5. Associations between urinary 1-OHPG and biomarkers of oxidative stress, gene induction, and inflammation, stratified by *GSTM1* genotype

	Crude model[a]							Adjusted Model[b]						
	GSTM1-null			*GSTM1*-present				*GSTM1*-null			*GSTM1*-present			
Urinary biomarkers	**β**	**[95%**	**CI]**	**β**	**[95%**	**CI]**	**p-int[a]**	**β**	**[95%**	**CI]**	**β**	**[95%**	**CI]**	**p-int[c]**
Log Isoprostane	0.252	-0.029	0.533	0.01	-0.016	0.035	**0.022**	**0.237**[d]	0.046	0.428	0.022	-0.012	0.056	**0.002**
Nasal epithelium mRNA														
Log HO-1	0.32	-0.063	0.704	0.007	-0.062	0.076	0.148	-0.360	-1.020	0.301	0.017	-0.048	0.082	0.284
Log GCLC	**1.097**[e]	0.507	1.688	-0.017	-0.078	0.045	**0.006**	0.732	-0.156	1.619	**0.101**[d]	0.006	0.195	**0.024**
Log IL-33	*			**-0.088**[d]	-0.146	-0.03	**0.005**	-0.030	-0.115	0.055	0.029	-0.124	0.182	**<0.001**
Serum cytokines and chemokines														
Log IL-6	0.071	-0.38	0.522	-0.025	-0.054	0.004	0.886	0.104	-0.117	0.325	**-0.048**	-0.095	-0.001	0.57
Log IL-8	0.517	-0.157	1.19	-0.028	-0.059	0.002	0.098	**0.584**[e]	0.331	0.837	-0.013	-0.05	0.024	0.134
Log ECP	0.298	-0.377	0.973	-0.003	-0.073	0.067	0.233	**0.236**[e]	0.103	0.368	0.003	-0.082	0.088	0.113
Log Eotaxin	-0.062	-0.313	0.189	0.012	-0.014	0.039	0.712	-0.039	-0.127	0.048	0.009	-0.035	0.052	0.829
Log GRO	*			-0.003	-0.037	0.03	0.159	**0.279**[e]	0.256	0.301	0.005	-0.03	0.04	**0.075**
Log GRO-α	*			0.029	-0.017	0.075	0.25	**0.634**[e]	0.604	0.664	0.034	-0.01	0.078	**0.004**
Log RANTES	0.105	-0.018	0.227	0.003	-0.006	0.011	0.207	**0.090**[d]	0.001	0.18	0.004	-0.01	0.019	0.693
Nasal lavage biomarkers														
Log White blood cell count	*			-0.01	-0.098	0.078	0.222	**0.075**[d]	0.030	0.121	0.003	-0.069	0.075	0.315
Whole blood biomarkers														
Log PBMC no PMA	0.523	-0.027	1.073	-0.035	-0.09	0.02	**0.029**	0.029	-0.124	0.182	**-0.069**[d]	-0.132	-0.007	**0.002**
Log PBMC with PMA	**0.457**[d]	0.149	0.765	0.001	-0.056	0.059	0.078	0.142	-0.072	0.356	-0.036	-0.108	0.037	**0.08**
Log Monocytes with PMA	**0.473**[d]	0.085	0.861	-0.03	-0.073	0.014	**0.014**	**0.305**[d]	0.124	0.485	**-0.064**[d]	-0.113	-0.015	**<0.001**
Log Lymphocytes no PMA	0.372	0.116	0.628	-0.016	-0.103	0.071	**<0.001**	**0.331**[e]	0.146	0.516	0.006	-0.062	0.073	**<0.001**
Log Lymphocytes w/ PMA	0.29	-0.129	0.708	-0.014	-0.168	0.14	0.423	**0.400**[e]	0.312	0.487	-0.053	-0.189	0.083	0.360
Log PMN no PMA	0.239	-0.202	0.681	0.011	-0.102	0.124	0.09	**-0.284**[d]	-0.498	-0.071	0.059	-0.051	0.169	0.272
Log PMN with PMA	**0.338**[d]	0.034	0.641	-0.023	-0.059	0.013	**0.002**	0.099	-0.122	0.32	-0.047	-0.094	0.000	**0.002**

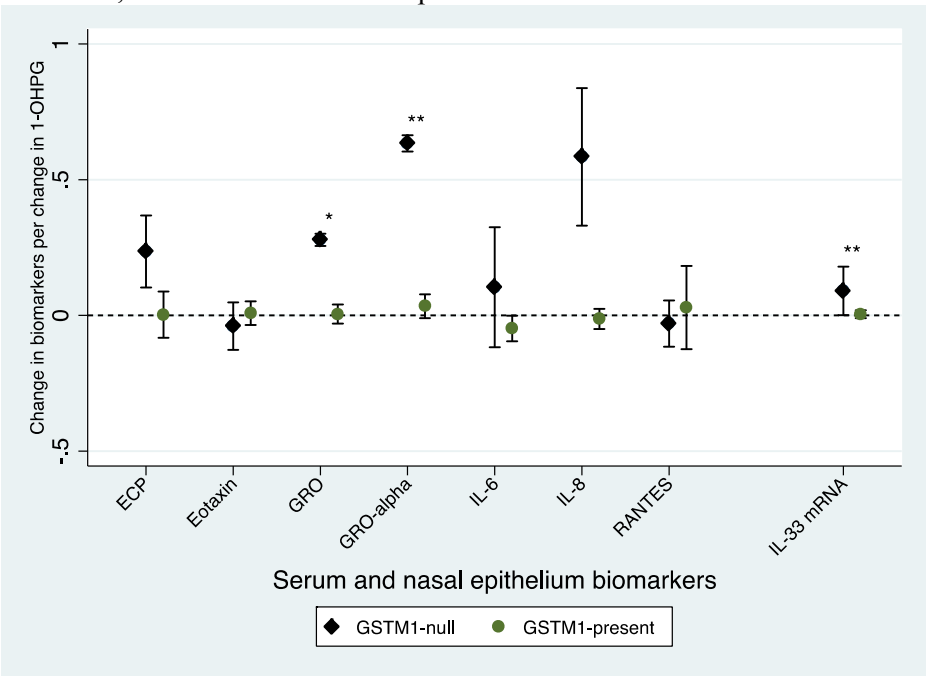
[a]Adjusted for urinary creatinine
[b]Adjusted for urinary creatinine, age, race, gender, GSTM1 genotype, and educational attainment
[c]P-interaction: bolded p-values are <0.1
[d]p<0.05
[e]p<0.001
*Bivariate regression model did not reach convergence

Figure 4-2. Associations between urinary 1-OHPG and urinary, nasal lavage and peripheral blood biomarkers of oxidative stress and inflammation



*p-interaction<0.1
**p-interaction<0.05

Figure 4-3. Associations between urinary 1-OHPG and serum cytokines, chemokines and mediators, and mRNA from nasal epithelium



*p-interaction<0.1
**p-interaction<0.05

Table 4-6. Urinary 1-OHPG (μmol/mol Cr) in adults

Study	Location	Cohort characteristics	Mean	Median (IQR)
Nasal Challenge Study	Baltimore City	Atopic asthmatic adults	AM (SD): 0.18 (0.26) GM: 0.07	0.10 (0.02-0.21)
NHANES II (1999-2000)	US	General population, adults	GM: 0.037	0.036
NHANES III (2001-2002)	US	General population, adults	GM: 0.023	0.021
NHANES IV (2003-2004)	US	General population, adults	GM: 0.041	0.038
Kim et al. 2001	South Korea	Non-smoking university students	GM: 0.03	
Kim et al. 2001	South Korea	Non-smoking shipyard workers	GM: 0.18	
Levin et al. 1995	Sweden	Non-smoking adults	GM: 0.03	
Levin et al. 1995	Sweden	Smoking adults	GM: 0.09	

CHAPTER 5: Conclusions

CONCLUSIONS

We investigated the links between internal dose of PAHs and biological responses associated with asthma using biomarkers of oxidative stress and immune response in cohorts of children and adults living in the inner city of Baltimore. We quantified PAH internal dose in this cohort of children by measuring urinary 1-hydroxypyrene-glucuronide (1-OHPG), and assessed associations between PAH internal dose and early effect biomarkers of oxidative damage, and inflammatory responses in cohorts of both children and adults, and assessed associations between PAH internal dose and asthma related symptoms in the cohort of asthmatic children, accounting for possible confounders.

Urinary 1-OHPG concentrations among asthmatic children and adults in the DISCOVER, Asthma-Diet, and Nasal Challenge to Indoor Particulate Matter studies were higher than the US national average and many international studies, reflecting the disproportionate burden of exposures to environmental toxicants for inner city, predominantly African American communities of low SES. We hypothesized that spending time in the home would be associated with increased urinary 1-OHPG due to exposures to indoor air pollutants (such as PM). Our study, however, found that residing in a non-smoking home conferred a "protective" effect for 1-OHPG concentrations compared to residing in a smoking household. Exposures to SHS may therefore contribute to urinary 1-OHPG concentration. Most children in our study resided in homes with cigarette smoking at some time during the monitoring period, highlighting the importance of indoor air exposures to cigarette smoke. Although internal dose of PAHs

was not associated with biomarkers of oxidative stress in children (urinary MDA), atopic asthmatic adults with the *GSTM1*-null polymorphism showed increased oxidative stress and increased local and systemic inflammatory responses to internal dose of PAHs, suggesting that lack of the GSTM1 enzyme may confer increased susceptibility to early biological effects related to asthma exacerbation. In children, internal dose of PAHs was associated with increased systemic inflammation (e.g. eosinophilia), with atopic asthmatic children reporting significantly higher inflammatory responses than non-atopic asthmatic children. These results suggest an adjuvant type effect of inflammatory responses in children sensitized to allergens, and likely co-exposed to these allergens and PAHs. PAH exposures were also associated with increased nighttime asthma symptoms and ß-agonist use. PAH exposures have shown consistent associations with inflammatory responses in children and adults, and have been associated with increased risk of asthma symptoms in children. These results support the proposed mechanism of activated PAHs causing increased oxidative stress, leading to increased inflammation and subsequently asthma exacerbation. The adjuvant activity of PAHs on allergen-mediated inflammatory responses are also supported by our findings, as atopic asthmatic children had more pronounced inflammatory responses to elevated PAHs than non-atopic asthmatic children.

Public health relevance and implications

The medical and social burden of asthma is considerable, with a disproportionate impact on vulnerable groups including young children, African-Americans and those living in the inner city. Exposure to air particulate matter is associated with asthma symptoms, but the chemical constituents underlying this association have not been well

characterized. It has been proposed that combustion products in PM may exacerbate asthma symptoms in atopic asthmatics. This is a major problem for inner city residents where the indoor environment can be a strong contributor to asthma. We used urinary biomarkers of PAH exposure, a major combustion product constituent of fine PM, to explore the hypothesis that combustion products play a role in childhood asthma exacerbation in the urban environment.

This study helps to determine the importance of PAH exposure in understanding the mechanisms of asthma exacerbation in children and adults living in an urban environment. This information will be useful in designing and implementing preventive interventions to reduce childhood asthma exacerbation and severity.

Based on our findings, future studies to reduce asthma morbidity from PAH exposures could focus on interventions focused on reducing exposures and early biological effects. These strategies may include behavioral (e.g. smoking cessation), engineering (e.g. air filtration), and policy (e.g. reduction in vehicular and industrial emissions) interventions, thereby reducing exposures to PAHs. Another approach could be abrogation of oxidative stress and reactive oxidative compounds through supplementation with exogenous antioxidants (such as, Vitamin C and Vitamin E), or oral administration of compounds, such as sulforaphane, to stimulate antioxidant pathways and phase II enzymes. A multifaceted approach featuring further mechanistic, observational, intervention and clinical studies focused on PAH exposures and the continuum of biological and physiological responses associated with the development of asthma and asthma exacerbation, as well as studies incorporating exposures to other common air pollutants, and integrating multiple factors that influence asthma morbidity

(such as SES, environmental justice, and healthcare utilization) may be necessary to reduce the asthma burden.

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APPENDIX A: Daily Diary Questionnaire

Cigarettes/Tobacco Use:

Did anyone smoke in your home today? Yes No [If yes fill out box below]:

Activity	Night (6 PM to 6 AM)	Morning (6 AM to Noon)	Afternoon (Noon to 6 PM)
Number of people smoking	○ 1 ○ 2-3 ○ 4+	○ 1 ○ 2-3 ○ 4+	○ 1 ○ 2-3 ○ 4+
Number of cigarettes smoked	○ 1-5 ○ 6-10 ○ 11+	○ 1-5 ○ 6-10 ○ 11+	○ 1-5 ○ 6-10 ○ 11+

Activities (Cooking and Cleaning):

Check all that apply:

Activity	Don't Have	Night (6 PM to 6 AM)	Morning (6 AM to Noon)	Afternoon (Noon to 6 PM)
Stove Use	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Burn Anything	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Vacuuming	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Oven Use	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Dusting	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Wet Mopping	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Toaster Use	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Sweeping	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Candles or Incense	☐	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

Windows & Doors:

How many windows and outside doors were open more than 10 min today?

○ None ○ 1-2 ○ 3-4 ○ 5+

Where did your child spend his/her time today?

Location	Night (6 PM to 6 AM)	Morning (6 AM to Noon)	Afternoon (Noon to 6 PM)
INDOORS in this house	_ _	_·_	_·_
OUTDOORS	_ _	_·_	_·_
Somewhere else (vehicle or another building)	_ _	_·_	_·_
TIMES SHOULD TOTAL	**12 HOURS**	**6 HOURS**	**6 HOURS**

APPENDIX B: Daily Symptom Diaries

Daily Symptom Diary: Morning Questions

1. Were you woken by asthma (either during the night or in the morning)?

- ○ No
- ○ Once
- ○ More than once
- ○ Awake all night

2. Number of puffs of Albuterol inhaler used since going to sleep (if you did not wake up for asthma during the night or in the morning, you should not have used any puffs).

_ _# puffs

Daily Symptom Diary: Evening Questions

1. Were you absent from school any part of the day due to asthma?

○ Vacation/Weekend ○ Yes ○ No

2. Did you visit a doctor, emergency room or hospital for asthma (other than a scheduled visit to the doctor) or treated with prednisone during the previous 24 hours?

○ Yes ○ No

3. How much of the time did you have trouble breathing today?

○ None of the time
○ A little of the time
○ Some of the time
○ A good bit of the time
○ Most of the time
○ All of the time

4. How much did your asthma bother you today?

○ Did not bother you
○ Bothered me a little
○ Bothered me somewhat
○ Bothered me a good deal
○ Bothered me very much
○ Bothered me as much as possible

5. How much of the time did your asthma limit your activity today (activities include any sort of physical activity: running, playing, jumping, sports, bike-riding, gym, etc.)?

○ None of the time
○ A little of the time
○ Some of the time
○ A good bit of the time
○ Most of the time
○ All of the time

6. Number of puffs of albuterol inhaler taken since doing peak flow this morning: _ _ # of puffs

APPENDIX C: Supplemental Tables for Chapter 2

Supplemental Table 2-1. Predictors of urinary creatinine (mg/ml)

	Coef. (β)	Std. Err.	z	P-value	[95% Conf.	Interval]
Age	0.123	0.018	6.98	**<0.001**	0.088	0.158
Gender (reference: girls)	-0.013	0.068	-0.19	0.852	-0.145	0.120
Season	-0.036	0.026	-1.41	0.159	-0.086	0.014
Atopic status	-0.074	0.076	-0.97	0.334	-0.224	0.076
BMI percentile (continuous)	0.002	0.001	1.91	0.056	0.000	0.004
Asthmatic status (reference: non-asthmatic)	-0.267	0.073	-3.64	**<0.001**	-0.411	-0.123
Caregiver's education	-0.042	0.035	-1.22	0.223	-0.110	0.026

Comparison: Urinary Creatinine (day 3 vs. day 7)		
	z	**p-value**
Wilcoxon sign-rank test	2.74	**0.006**

Supplemental Table 2-2 Comparisons, correlations and multivariate linear regression analyses of 1-OHPG from urine collected on day 3 and day 7

Comparisons: 1-OHPG/Cr (day 3 vs. day 7)				
	AM (SD)	**GM**	**Median (IQR)**	**n**
1-OHPG/Cr all samples	0.19 (0.22)	0.111	0.13 (0.06-0.25)	359
1-OHPG/Cr day 3	0.16 (0.19)	0.085	0.11 (0.05-0.21)	255
1-OHPG/Cr day 7	0.18 (0.22)	0.092	0.11 (0.05-0.23)	339
Wilcoxon sign-rank test (day 3 vs day 7)	p=0.422			

Correlations: (Log) 1-OHPG/Cr (day 3 vs. day 7)		
	r	**p-value**
Pearson's correlation coefficient	0.20	**0.003**
Pearson's correlation coefficient (Log)	0.27	**<0.001**
Spearman's rho	0.34	**<0.001**

Multivariate Linear Regression: Log 1-OHPG/Cr (day 3 vs day7)				
	β	**P>z**	**[95%**	**CI]**
Crude*	0.190	**0.003**	0.065	0.316
Adjusted**	0.204	**0.001**	0.085	0.323

* Adjusted for urinary creatinine
** Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Supplemental Table 2-3. Geometric means and medians of creatinine adjusted 1-OHPG concentrations (μmol/mol Cr) by season

	Geometric mean	Median (IQR)
Winter	0.088	0.12 (0.04-0.23)
Spring	0.091	0.11 (0.05-0.22)
Summer	0.081	0.10 (0.05-0.23)
Fall	0.096	0.13 (0.05-0.22)

Supplemental Table 2-4. Predictors of urinary 1-OHPG concentrations (continued)

	β	P>z	[95%	CI]
Time with windows open	-0.065	0.485	-0.248	0.118
Gas stove	-0.250	0.205	-0.637	0.137
Types of heating				
Radiator	-0.134	0.374	-0.430	0.162
Forced air	0.004	0.975	-0.254	0.262
Type of curb				
Parking lot	0.220	0.243	-0.149	0.588
Arterial street	0.032	0.850	-0.299	0.363
Side street	-0.175	0.180	-0.432	0.081
Curb distance	0.012	0.820	-0.093	0.117

Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Supplemental Table 2-5. Predictors of indoor $PM_{2.5}$, PM_{10}, $PM_{2.5-10}$ concentrations

Log $PM_{2.5}$

	β	P>z	[95%	CI]
Smoking in the home	0.275	**0.001**	0.119	0.431
Average cigarettes per day	0.037	**<0.001**	0.023	0.051
Time with windows open	0.037	0.522	-0.076	0.151
Stove use	0.106	0.173	-0.047	0.259
Burning candles or incense	0.059	0.580	-0.149	0.267
Burned something on stove	0.097	0.381	-0.120	0.314

Log PM_{10}

	β	P>z	[95%	CI]
Smoking in the home	0.306	**<0.001**	0.162	0.450
Average cigarettes per day	0.030	**<0.001**	0.018	0.041
Time with windows open	-0.008	0.890	-0.117	0.102
Stove use	0.115	0.087	-0.017	0.247
Burning candles or incense	0.033	0.671	-0.120	0.186
Burned something on stove	0.114	0.229	-0.072	0.300

Log $PM_{2.5-10}$	All $PM_{2.5-10}$ samples				Without $PM_{2.5-10}$ outlier*			
	β	P>z	[95%	CI]	β	P>z	[95%	CI]
Smoking in the home	0.148	0.117	-0.037	0.332	0.176	0.064	-0.011	0.362
Average cigarettes per day	0.005	0.563	-0.011	0.020	0.006	0.487	-0.010	0.021
Time with windows open	-0.136	0.071	-0.283	0.011	-0.110	0.146	-0.259	0.038
Stove use	0.182	**0.044**	0.005	0.359	0.158	0.072	-0.014	0.330
Burning candles or incense	0.129	0.227	-0.081	0.340	0.150	0.159	-0.059	0.358
Burned something on stove	0.240	0.084	-0.032	0.512	0.101	0.500	-0.193	0.396

*$PM_{2.5-10}$ outlier: 215.3 μg/m^3

Supplemental Table 2-6. Predictors of indoor air nicotine concentrations

Correlations between indoor air nicotine and PM2.5 and urinary 1-OHPG/Cr		
	Correlation coefficient	p-value
Log air nicotine vs. log $PM_{2.5}$ (Pearson's r)	0.550	**<0.001**
Air nicotine vs. $PM_{2.5}$ (Spearman's rho)	0.530	**<0.001**
Log air nicotine vs. log 1-OHPG/Cr (Pearson's r)	0.107	**0.046**
Air nicotine vs. 1-OHPG/Cr (Spearman's rho)	0.115	**0.031**

Predictors indoor air nicotine concentrations (MLR with GEE)				
	β	P>z	[95%	CI]
Smoking in the home	1.037	**<0.001**	0.703	1.371
Average cigarettes per day	0.083	**<0.001**	0.055	0.110
Log $PM_{2.5}$ (dependent variable)	0.795	**<0.001**	0.467	1.124

Supplemental Table 2-7. Associations between average time spent in the home and urinary 1-OHPG concentrations, adjusting for indoor $PM_{2.5}$, $PM_{2.5-10}$, and PM_{10}

	Crude*				Adjusted**			
Adjusting for indoor $PM_{2.5}$	β	P>z	[95%	CI]	β	P>z	[95%	CI]
Average time in the home, all samples	-0.020	0.238	-0.054	0.013	-0.017	0.360	-0.052	0.019
Average time in the home days 0-2 (urine day 3)	-0.020	0.252	-0.053	0.014	-0.019	0.272	-0.052	0.015
Average time in the home days 4-6 (urine day 7)	-0.047	**0.012**	-0.083	-0.010	-0.054	**0.002**	-0.089	-0.019
Adjusting for indoor $PM_{2.5-10}$								
Average time in the home, all samples	0.140	**0.027**	0.016	0.264	0.148	**0.029**	0.015	0.281
Average time in the home days 0-2 (urine day 3)	0.113	0.297	-0.100	0.326	0.084	0.430	-0.125	0.294
Average time in the home days 4-6 (urine day 7)	0.173	**0.018**	0.030	0.315	0.201	**0.007**	0.055	0.347
Adjusting for indoor $PM_{2.5-10}$ without outlier								
Total urine	0.121	0.057	-0.003	0.245	0.117	0.074	-0.011	0.246
Urine collected on Day 3	0.113	0.297	-0.100	0.326	0.083	0.437	-0.127	0.293
Urine collected on Day 7	0.144	0.055	-0.003	0.291	0.177	**0.021**	0.027	0.326

Adjusting for indoor PM_{10}								
Average time in the home, all samples	-0.019	0.233	-0.051	0.012	-0.012	0.496	-0.046	0.022
Average time in the home days 0-2 (urine day 3)	-0.028	0.097	-0.061	0.005	-0.025	0.142	-0.058	0.008
Average time in the home days 4-6 (urine day 7)	-0.031	0.087	-0.066	0.004	-0.032	0.064	-0.065	0.002

* Adjusted for urinary creatinine
** Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Supplemental Table 2-8. Associations between average time spent in the home and urinary 1-OHPG, stratified by $PM_{2.5}$, and adults smoking in the home

Associations between average time in the home and urinary 1-OHPG, stratified by $PM_{2.5}$ (dichotomized at median: 21.3 $\mu g/m^3$)

	Low $PM_{2.5}$				**High $PM_{2.5}$**				
	β	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-int**
Avg. time in the home all samples	-0.019	0.462	-0.070	0.032	-0.050	**0.040**	-0.099	-0.002	0.809
Avg. time in the home days 0-2 (urine day 3)	-0.013	0.560	-0.056	0.031	-0.067	**0.001**	-0.106	-0.027	0.291
Avg. time in the home days 4-6 (urine day 7)	-0.104	**<0.001**	-0.153	-0.056	-0.018	0.488	-0.070	0.033	**0.003**

Associations between average time in the home and urinary 1-OHPG, stratified by adults smoking in the home

	No smoking				**Smoking**				
	β	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-int**
Avg. time in the home all samples	-0.013	0.612	-0.065	0.039	-0.024	0.261	-0.065	0.018	0.758
Avg. time in the home days 0-2 (urine day 3)	-0.033	0.135	-0.075	0.010	-0.013	0.566	-0.056	0.030	0.628
Avg. time in the home days 4-6 (urine day 7)	-0.092	**<0.001**	-0.144	-0.040	-0.001	0.966	-0.044	0.043	**0.012**

Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Supplemental Table 2-9. Geometric mean and median for time spent in the home and time spent outdoors (hours), by season

	Hours spent in the home		**Hours spent outdoors**	
	Geometric mean	**Median (IQR)**	**Geometric mean**	**Median (IQR)**
Winter	15.0	16.0 (14.2-17.1)	1.2	0.9 (0.6-1.8)
Spring	14.6	15.0 (13.4-16.2)	2.4	2.9 (1.3-4.7)
Summer	16.2	16.8 (14.0-20.2)	2.0	2.4 (0.7-4.6)
Fall	14.6	15.0 (13.5-17.0)	1.6	1.9 (0.7-3.4)
Kruskal-Wallis rank test	**p=0.005**		**p<0.001**	

Supplemental Table 2-10. Comparison of time spent in the home, indoors elsewhere, and outdoors (hours) on weekdays and during the weekend

	Hours spent in the home	Hours spent indoors (vehicles, other buildings, etc.)	Hours spent outdoors
Weekdays (median (IQR))	15.4 (13.6-17.1)	6.2 (2.8-8)	2.0 (0.8-3.9)
Weekends (median (IQR))	18.0 (13.5-21.5)	1.5 (0-7.0)	1.5 (0-4.5.0)
Wilcoxon sign-rank test	**p<0.001**	**p<0.001**	p=0.792

Comparison of time spent outdoors (hours) on weekdays and during the weekend, by gender

	Hours outdoors on weekdays	Hours outdoors on weekends
Boys (median (IQR))	2.0 (0.8-4.3)	1.5 (0-4.0)
Girls (median (IQR))	1.4 (0.6-3.4)	1.5 (0-5.0)
Wilcoxon rank-sum test	**p=0.009**	p=0.301

Supplemental Table 2-11. Associations between average time spent outdoors and urinary 1-OHPG, adjusting for indoor $PM_{2.5}$ concentrations

	Crude*				Adjusted**			
All Samples	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Average time outdoors all samples	-0.012	0.663	-0.064	0.040	-0.005	0.867	-0.060	0.050
Average time outdoors days 0-2 (urine day 3)	-0.006	0.881	-0.081	0.069	0.004	0.915	-0.077	0.086
Average time outdoors days 4-6 (urine day 7)	0.032	0.211	-0.018	0.081	0.044	0.107	-0.009	0.097
By Gender								
Boys								
Average time outdoors all samples	-0.020	0.620	-0.100	0.059	-0.022	0.611	-0.105	0.062
Average time outdoors days 0-2 (urine day 3)	-0.025	0.597	-0.119	0.068	-0.026	0.604	-0.126	0.073
Average time outdoors days 4-6 (urine day 7)	0.086	**0.011**	0.019	0.153	0.081	**0.018**	0.014	0.148
Girls								
Average time outdoors all samples	0.013	0.718	-0.057	0.083	0.016	0.683	-0.062	0.095
Average time outdoors days 0-2 (urine day 3)	0.025	0.695	-0.101	0.151	0.045	0.538	-0.098	0.188
Average time outdoors days 4-6 (urine day 7)	-0.021	0.579	-0.097	0.054	-0.019	0.590	-0.090	0.051

* Adjusted for urinary creatinine
** Adjusted for urinary creatinine, $PM_{2.5}$, gender, age, atopic status, season, caregiver's education

Supplemental Table 2-12. Associations between average time spent outdoors and urinary 1-OHPG, adjusting for indoor air nicotine concentrations

	Crude*				Adjusted**			
All Samples	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Avg. time outdoors all samples	-0.002	0.924	-0.050	0.046	0.013	0.634	-0.041	0.067
Avg. time outdoors days 0-2 (urine day 3)	-0.003	0.920	-0.067	0.061	0.014	0.718	-0.063	0.092
Avg. time outdoors days 4-6 (urine day 7)	0.030	0.197	-0.016	0.075	0.051	**0.043**	0.002	0.100
By Gender								
Boys								
Avg. time outdoors all samples	-0.004	0.917	-0.071	0.064	0.009	0.826	-0.070	0.088
Avg. time outdoors days 0-2 (urine day 3)	-0.024	0.477	-0.091	0.043	-0.022	0.600	-0.105	0.061
Avg. time outdoors days 4-6 (urine day 7)	0.088	0.004	0.028	0.149	0.099	**0.002**	0.037	0.162
Girls								
Avg. time outdoors all samples	0.046	0.046	0.046	0.046	0.025	0.524	-0.051	0.101
Avg. time outdoors days 0-2 (urine day 3)	0.046	0.046	0.046	0.046	0.057	0.432	-0.086	0.200
Avg. time outdoors days 4-6 (urine day 7)	0.046	0.046	0.046	0.046	-0.018	0.574	-0.082	0.045

* Adjusted for urinary creatinine
** Adjusted for urinary creatinine, air nicotine, gender, age, atopic status, season, caregiver's education

Supplemental Table 2-13. Associations between time spent outdoors and urinary 1-OHPG concentrations by season, and effect modification by season

Associations between time spent outdoors and urinary 1-OHPG concentrations by season

All Samples	**β**	**P>z**	**[95%**	**CI]**
Winter	-0.038	0.638	-0.198	0.121
Spring	0.029	0.516	-0.059	0.118
Summer	0.147	**0.003**	0.048	0.246
Fall	-0.067	0.181	-0.164	0.031

Adjusted for urinary creatinine, gender, age, atopic status, season, caregiver's education

Effect modification of seasons on associations between time spent outdoors and urinary 1-OHPG concentrations

	Associations between 1-OHPG and interactions between season and time spent outdoors		
	P-value[a]	**P-value**[b]	**P-value**[c]
Winter x time spent outdoors	0.060	**0.047**	0.078
Spring x time spent outdoors	0.946	0.926	0.925
Summer x time spent outdoors	**0.001**	**<0.001**	**<0.001**
Fall x time spent outdoors	0.500	0.384	0.514

[a] Adjusted for urinary creatinine, gender, age, atopic status, season, caregive
[b] Adjusted for indoor $PM_{2.5}$, urinary creatinine, gender, age, atopic status, season, caregiver's education
[c] Adjusted for air nicotine, urinary creatinine, gender, age, atopic status, season, caregiver's education

Supplemental Table 2-14. Missing data percentages by gender

Total n=359	n	% Missing total	% Missing girls	% Missing boys
Indoor $PM_{2.5}$ concentrations[b]	309	14	14	13
Indoor PM_{10} concentrations[b]	308	14	14	15
Indoor $PM_{2.5-10}$ concentrations[c]	280	22	21	23
Indoor air nicotine concentrations	346	4	3	4
Time spent indoors/outdoors Day 0[a]	282	21	20	23
Time spent indoors/outdoors Day 1	325	9	8	11
Time spent indoors/outdoors Day 2	332	8	8	7
Time spent indoors/outdoors Day 3	341	5	4	6
Time spent indoors/outdoors Day 4	343	4	4	5
Time spent indoors/outdoors Day 5	337	6	6	6
Time spent indoors/outdoors Day 6	340	5	4	6
Average time indoors/outdoors days 0-6[a]	273	24	23	25
Average time indoors/outdoors days 0-2[a]	277	23	22	24
Average time indoors/outdoors days 4-6	331	8	7	8
Smoking in the home days 0-6[a]	288	20	20	20
Smoking in the home days 0-2[a]	296	17	16	19
Smoking in the home days 4-6	332	8	8	7
Playing sports outdoors on days 0-6[a]	258	28	27	30
Playing sports outdoors on days 0-2[a]	272	24	22	26
Playing sports outdoors on days 4-6	315	12	12	13

[a] Less data on first day of monitoring period (day 0, Saturday). Interviews may not have been conducted or children may not have been home.
[b] Due to ripped membrane, equipment malfunction or $PM_{2.5} > PM_{10}$
[c] Obtained by subtracting PM_{10} from $PM_{2.5}$, missing data in each of these would result in no value for $PM_{2.5-10}$.

APPENDIX D: Supplemental Tables for Chapter 3

Supplemental Table 3-1. Associations between urinary 1-OHPG concentrations and ß-agonist use during the day

	Crude*				Adjusted**			
1-OHPG conc. Day 3	**OR**	**P>z**	**[95%**	**CI]**	**OR**	**P>z**	**[95%**	**CI]**
ß-agonist use during previous 4 days (0-3)	1.720	0.027	1.062	2.785	1.811	0.040	1.028	3.190
ß-agonist use during previous 3 days (1-3)	1.184	0.544	0.685	2.047	1.335	0.347	0.731	2.440
ß-agonist use during previous 2 days (2-3)	1.051	0.861	0.600	1.842	1.282	0.453	0.671	2.450
ß-agonist use during next 2 days (4-5)	1.346	0.375	0.698	2.598	1.754	0.146	0.823	3.742
ß-agonist use during next 4 days (4-6)	1.344	0.345	0.727	2.482	1.707	0.140	0.839	3.474
ß-agonist use any day (0-6)	1.646	0.059	0.980	2.764	1.732	0.075	0.946	3.169
1-OHPG conc. day 7								
ß-agonist use during previous 2 days (5-6)	0.853	0.543	0.511	1.424	0.962	0.892	0.546	1.693
ß-agonist use during previous 3 days (4-6)	1.020	0.937	0.626	1.662	1.152	0.624	0.655	2.025
ß-agonist use any day (0-6)	0.880	0.631	0.522	1.483	0.088	0.774	-0.512	0.688
1-OHPG conc. average of samples								
ß-agonist use on days 0-3	1.076	0.755	0.680	1.702	1.119	0.672	0.665	1.883
ß-agonist use on days 4-6	1.136	0.653	0.653	1.976	1.301	0.412	0.694	2.442
ß-agonist use on days 0-6	1.068	0.787	0.662	1.726	1.165	0.580	0.678	2.002

*Adjusted for urinary creatinine
**Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-2. Associations between urinary 1-OHPG concentrations and having trouble breathing during the day

	Crude*				Adjusted**			
1-OHPG conc. day 3	**OR**	**P>z**	**[95%**	**CI]**	**OR**	**P>z**	**[95%**	**CI]**
Trouble breathing during previous 4 days (0-3)	1.500	0.087	0.943	2.387	1.534	0.084	0.944	2.491
Trouble breathing during previous 3 days (1-3)	1.595	0.102	0.912	2.789	1.719	0.108	0.887	3.332
Trouble breathing during previous 2 days (2-3)	1.349	0.304	0.762	2.386	1.615	0.160	0.827	3.153
Trouble breathing during next 2 days (4-5)	1.117	0.645	0.697	1.790	1.074	0.787	0.640	1.802
Trouble breathing during next 4 days (4-6)	1.139	0.597	0.704	1.842	1.055	0.844	0.618	1.803
Trouble breathing any day (0-6)	1.480	0.088	0.943	2.323	1.426	0.146	0.884	2.303
1-OHPG conc. day 7								
Trouble breathing during previous 2 days (5-6)	0.795	0.313	0.509	1.242	0.674	0.165	0.386	1.177
Trouble breathing during previous 3 days (4-6)	0.887	0.602	0.567	1.390	0.776	0.376	0.443	1.360
Trouble breathing any day (0-6)	0.867	0.509	0.568	1.324	-0.156	0.533	-0.647	0.335
1-OHPG conc. average of samples								
Trouble breathing on days 0-3	1.252	0.288	0.828	1.893	1.331	0.224	0.839	2.109
Trouble breathing on days 4-6	0.935	0.782	0.583	1.501	0.902	0.721	0.511	1.591
Trouble breathing on days 0-6	1.148	0.516	0.757	1.739	1.183	0.491	0.733	1.909

*Adjusted for urinary creatinine
**Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-3. Associations between urinary 1-OHPG concentrations and unscheduled doctor's or hospital visit

	Crude*				Adjusted**			
1-OHPG concentrations day 3	**OR**	**P>z**	**[95%**	**CI]**	**OR**	**P>z**	**[95%**	**CI]**
Unscheduled doctor's visit days 0-7	1.785	0.413	0.446	7.150	1.860	0.424	0.407	8.495
1-OHPG concentrations day 7								
Unscheduled doctor's visit days 0-7	0.564	0.401	0.148	2.145	0.284	0.143	0.053	1.528
1-OHPG concentrations average of samples								
Unscheduled doctor's visit days 0-7	0.609	0.455	0.166	2.236	0.327	0.156	0.070	1.529

*Adjusted for urinary creatinine
**Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-4. Associations between urinary 1-OHPG concentrations and being bothered by asthma during the day

	Crude*				Adjusted**			
1-OHPG conc. day 3	**OR**	**P>z**	**[95%**	**CI]**	**OR**	**P>z**	**[95%**	**CI]**
Bothered by asthma during previous 4 days (0-3)	1.358	0.224	0.829	2.225	1.375	0.263	0.787	2.403
Bothered by asthma during previous 3 days (1-3)	1.547	0.128	0.882	2.715	1.696	0.109	0.888	3.237
Bothered by asthma during previous 2 days (2-3)	1.414	0.248	0.785	2.547	1.713	0.121	0.868	3.383
Bothered by asthma during next 2 days (4-5)	1.059	0.814	0.658	1.704	0.966	0.898	0.573	1.631
Bothered by asthma during next 4 days (4-6)	0.977	0.919	0.621	1.537	0.860	0.549	0.524	1.410
Bothered by asthma on days 0-6	1.204	0.464	0.733	1.977	1.071	0.802	0.628	1.826
1-OHPG conc. day 7								
Bothered by asthma during previous 2 days (5-6)	0.753	0.215	0.480	1.180	0.601	0.073	0.344	1.050
Bothered by asthma during previous 3 days (4-6)	0.836	0.464	0.517	1.351	0.707	0.238	0.398	1.257
Bothered by asthma days (0-6)	0.861	0.519	0.545	1.358	-0.236	0.365	-0.747	0.274
1-OHPG conc. average of samples								
Bothered by asthma on days 0-3	1.204	0.386	0.791	1.833	1.244	0.384	0.760	2.037
Bothered by asthma on days 4-6	0.873	0.572	0.546	1.397	0.773	0.378	0.437	1.369
Bothered by asthma on days 0-6	1.053	0.809	0.691	1.606	1.004	0.986	0.616	1.636

*Adjusted for urinary creatinine
**Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-5. Associations between urinary 1-OHPG concentrations and limited activity due to asthma during the day

	Crude*				Adjusted**			
1-OHPG conc. day 3	**OR**	**P>z**	**[95%**	**CI]**	**OR**	**P>z**	**[95%**	**CI]**
Limited activity during previous 4 days (0-3)	1.282	0.371	0.744	2.209	1.341	0.347	0.727	2.476
Limited activity during previous 3 days (1-3)	1.464	0.198	0.819	2.618	1.687	0.127	0.862	3.300
Limited activity during previous 2 days (2-3)	1.379	0.305	0.746	2.549	1.706	0.162	0.807	3.604
Limited activity during next 2 days (4-5)	0.962	0.882	0.580	1.596	0.933	0.816	0.519	1.676
Limited activity during next 4 days (4-6)	0.942	0.811	0.574	1.545	0.907	0.737	0.513	1.603
Limited activity on any day (0-6)	1.077	0.789	0.624	1.859	1.047	0.880	0.578	1.895
1-OHPG conc. day 7								
Limited activity during previous 2 days (5-6)	0.895	0.632	0.570	1.406	0.868	0.613	0.502	1.500
Limited activity during previous 3 days (4-6)	0.920	0.746	0.554	1.526	0.938	0.831	0.518	1.696
Limited activity on any day (0-6)	1.105	0.696	0.669	1.827	0.133	0.647	-0.435	0.701
1-OHPG conc. average of samples								
Limited activity on days 0-3	1.473	0.090	0.941	2.306	1.550	0.109	0.907	2.650
Limited activity on days 4-6	1.039	0.883	0.627	1.719	1.106	0.748	0.597	2.048
Limited activity on days 0-6	1.280	0.273	0.823	1.990	1.296	0.332	0.768	2.186

*Adjusted for urinary creatinine
**Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Effect Modification by atopic status

Supplemental Table 3-6. Atopic status as an effect modifier of associations between urinary 1-OHPG concentrations and waking due to asthma symptoms during the night or in the early morning

	Non-Atopic				Atopic				
1-OHPG conc. day 3	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-int**
Woken by asthma during previous 4 nights (0-3)	0.048	0.283	-0.040	0.136	0.079	0.064	-0.005	0.162	0.789
Woken by asthma during previous 3 nights (1-3)	0.048	0.254	-0.035	0.131	0.066	0.057	-0.002	0.134	0.752
Woken by asthma during previous 2 nights (2-3)	0.008	0.709	-0.035	0.051	0.028	0.168	-0.012	0.068	0.753
Woken by asthma during next 2 nights (4-5)	-0.006	0.486	-0.024	0.011	0.021	0.143	-0.007	0.049	0.908
Woken by asthma during next 4 nights (4-7)	0.038	0.181	-0.018	0.094	0.038	0.135	-0.012	0.089	0.658
Woken by asthma any night (0-7)	0.110	**0.002**	0.040	0.180	0.091	0.094	-0.015	0.197	0.184
1-OHPG conc. day 7									
Woken by asthma during previous 2 nights (6-7)	0.026	0.278	-0.021	0.074	-0.003	0.813	-0.031	0.024	0.152
Woken by asthma during previous 3 nights (5-7)	0.060	0.161	-0.024	0.143	0.003	0.888	-0.039	0.046	0.210
Woken by asthma during previous 4 nights (4-7)	0.075	0.108	-0.017	0.166	0.026	0.403	-0.035	0.088	0.370
Woken by asthma any night (0-7))	0.010	0.793	-0.068	0.089	0.049	0.322	-0.048	0.146	0.939
1-OHPG conc. average of samples									
Woken by asthma on nights 0-3	0.037	0.385	-0.046	0.120	0.052	0.180	-0.024	0.129	0.979
Woken by asthma on nights 4-7	0.075	0.100	-0.014	0.165	0.056	0.210	-0.031	0.143	0.809
Woken by asthma on nights 0-7	0.077	0.216	-0.045	0.198	0.108	0.138	-0.035	0.250	0.881

Adjusted for creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-7. Atopic status as an effect modifier of associations between urinary 1-OHPG concentrations and ß-agonist use during the night or in the morning

	Non-Atopic				Atopic				
1-OHPG conc. day 3	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-interaction**
ß-agonist use during previous 4 days (0-3)	0.082	0.170	-0.035	0.200	0.072	0.124	-0.020	0.164	0.497
ß-agonist use during previous 3 days (1-3)	0.062	0.158	-0.024	0.148	0.069	0.080	-0.008	0.146	0.395
ß-agonist use during previous 2 days (2-3)	0.008	0.709	-0.035	0.051	0.028	0.168	-0.012	0.068	0.753
ß-agonist use during next 2 days (4-5)	-0.006	0.486	-0.024	0.011	0.021	0.143	-0.007	0.049	0.908
ß-agonist use during next 4 days (4-7)	-0.032	0.440	-0.112	0.049	0.074	0.070	-0.006	0.155	0.644
ß-agonist use any day (0-7)	0.171	**0.035**	0.012	0.330	0.139	0.104	-0.028	0.307	0.141
1-OHPG conc. day 7									
ß-agonist use during previous 2 days (6-7)	0.026	0.278	-0.021	0.074	-0.003	0.813	-0.031	0.024	0.152
ß-agonist use during previous 3 days (5-7)	-0.018	0.560	-0.081	0.044	0.008	0.785	-0.051	0.068	0.850
ß-agonist use during previous 4 days (4-7)	-0.002	0.955	-0.067	0.063	0.003	0.948	-0.076	0.081	0.675
ß-agonist use any day (0-7)	-0.050	0.075	-0.197	0.096	0.036	0.608	-0.101	0.172	0.948
1-OHPG conc. average of samples									
ß-agonist use on days 0-3	0.025	0.589	-0.065	0.114	0.069	0.260	-0.051	0.189	0.968
ß-agonist use on days 4-7	-0.010	0.804	-0.087	0.067	0.041	0.471	-0.070	0.151	0.722
ß-agonist use on days 0-7	0.070	0.440	-0.107	0.246	0.140	0.168	-0.059	0.338	0.628

Adjusted for creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-8. Atopic status as an effect modifier of associations between urinary 1-OHPG concentrations and ß-agonist use during the day

	Non-Atopic				Atopic				
1-OHPG conc. day 3	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-interaction**
ß-agonist use during previous 4 days (0-3)	0.021	0.757	-0.113	0.155	0.066	0.302	-0.059	0.192	0.855
ß-agonist use during previous 3 days (1-3)	-0.005	0.900	-0.084	0.074	0.046	0.346	-0.050	0.141	0.720
ß-agonist use during previous 2 days (2-3)	0.003	0.937	-0.068	0.074	0.026	0.425	-0.038	0.090	0.761
ß-agonist use during next 2 days (4-5)	-0.003	0.867	-0.041	0.035	0.029	0.367	-0.034	0.092	0.779
ß-agonist use during next 4 days (4-6)	0.022	0.643	-0.070	0.113	0.021	0.658	-0.071	0.112	0.535
ß-agonist use any day (0-6)	0.033	0.786	-0.204	0.269	0.066	0.553	-0.152	0.284	0.761
1-OHPG conc. day 7									
ß-agonist use during previous 2 days (5-6)	-0.027	0.231	-0.072	0.017	-0.016	0.452	-0.059	0.026	0.955
ß-agonist use during previous 3 days (4-6)	-0.028	0.338	-0.086	0.029	-0.014	0.661	-0.075	0.047	0.896
ß-agonist use any day (0-6)	-0.241	**0.007**	-0.418	-0.065	0.002	0.978	-0.143	0.147	0.520
1-OHPG conc. average of samples									
ß-agonist use on days 0-3	-0.044	0.442	-0.156	0.068	0.084	0.248	-0.059	0.227	0.486
ß-agonist use on days 4-6	-0.022	0.494	-0.084	0.040	-0.011	0.812	-0.106	0.083	0.716
ß-agonist use on days 0-6	-0.113	0.330	-0.340	0.114	0.037	0.755	-0.197	0.272	0.952

Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-9. Atopic status as an effect modifier of associations between urinary 1-OH PG concentrations and having trouble breathing during the day

	Non-Atopic				Atopic				
1-OHPG conc. day 3	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-int**
Trouble breathing during previous 4 days (0-3)	0.033	0.492	-0.061	0.127	0.046	0.386	-0.058	0.149	0.506
Trouble breathing during previous 3 days (1-3)	-0.032	0.316	-0.095	0.031	0.059	0.151	-0.021	0.139	0.147
Trouble breathing during previous 2 days (2-3)	-0.022	0.277	-0.062	0.018	0.042	0.168	-0.018	0.101	0.101
Trouble breathing during next 2 days (4-5)	-0.021	0.326	-0.063	0.021	0.014	0.557	-0.033	0.061	0.400
Trouble breathing during next 4 days (4-6)	-0.051	0.288	-0.146	0.043	0.015	0.665	-0.052	0.081	0.375
Trouble breathing any day (0-6)	-0.012	0.915	-0.225	0.202	0.035	0.656	-0.118	0.187	0.495
1-OHPG conc. day 7									
Trouble breathing during previous 2 days (5-6)	-0.011	0.690	-0.065	0.043	-0.003	0.916	-0.057	0.051	0.591
Trouble breathing during previous 3 days (4-6)	0.000	0.994	-0.076	0.076	0.004	0.910	-0.071	0.080	0.581
Trouble breathing any day (0-6)	-0.094	0.334	-0.283	0.096	0.100	0.347	-0.108	0.307	0.439
1-OHPG conc. average of samples									
Trouble breathing on days 0-3	0.012	0.821	-0.088	0.111	0.123	0.114	-0.030	0.276	0.202
Trouble breathing on days 4-6	-0.019	0.728	-0.128	0.090	0.022	0.705	-0.090	0.133	0.440
Trouble breathing on days 0-6	-0.053	0.699	-0.321	0.216	0.168	0.226	-0.103	0.439	0.199

Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-10. Atopic status as an effect modifier of associations between urinary 1-OHPG concentrations and being bothered by asthma during the day

	Non-Atopic				Atopic				
1-OHPG conc. day 3	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-int**
Bothered by asthma during previous 4 days (0-3)	0.081	0.219	-0.048	0.210	0.038	0.509	-0.075	0.152	0.924
Bothered by asthma during previous 3 days (1-3)	0.044	0.465	-0.073	0.161	0.053	0.237	-0.035	0.141	0.876
Bothered by asthma during previous 2 days (2-3)	0.012	0.700	-0.049	0.073	0.037	0.219	-0.022	0.096	0.818
Bothered by asthma during next 2 days (4-5)	-0.027	0.241	-0.071	0.018	0.016	0.490	-0.030	0.063	0.648
Bothered by asthma during next 4 days (4-6)	-0.053	0.272	-0.148	0.042	0.010	0.782	-0.060	0.080	0.567
Bothered by asthma any day (0-6)	0.060	0.604	-0.167	0.286	0.022	0.794	-0.145	0.190	0.975
1-OHPG conc. day 7									
Bothered by asthma during previous 2 days (5-6)	-0.026	0.348	-0.079	0.028	-0.007	0.780	-0.057	0.043	0.575
Bothered by asthma during previous 3 days (4-6)	-0.027	0.491	-0.103	0.050	-0.002	0.957	-0.072	0.068	0.543
Bothered by asthma any day (0-6)	-0.103	0.329	-0.310	0.104	0.091	0.365	-0.106	0.287	0.474
1-OHPG conc. average of samples									
Bothered by asthma on days 0-3	0.040	0.563	-0.095	0.175	0.117	0.128	-0.034	0.267	0.288
Bothered by asthma on days 4-6	-0.026	0.640	-0.135	0.083	0.008	0.872	-0.092	0.108	0.609
Bothered by asthma on days 0-6	0.020	0.895	-0.275	0.315	0.154	0.237	-0.102	0.411	0.301

Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

Supplemental Table 3-11. Atopic status as an effect modifier of associations between urinary 1-OHPG concentrations and limited activity due to asthma symptoms during the day

	Non-Atopic				Atopic				
1-OHPG conc. day 3	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-interaction**
Limited activity during previous 4 days (0-3)	0.081	0.219	-0.048	0.210	0.038	0.509	-0.075	0.152	0.924
Limited activity during previous 3 days (1-3)	0.044	0.465	-0.073	0.161	0.053	0.237	-0.035	0.141	0.876
Limited activity during previous 2 days (2-3)	0.012	0.700	-0.049	0.073	0.037	0.219	-0.022	0.096	0.818
Limited activity during next 2 days (4-5)	-0.027	0.241	-0.071	0.018	0.016	0.490	-0.030	0.063	0.648
Limited activity during next 4 days (4-6)	-0.053	0.272	-0.148	0.042	0.010	0.782	-0.060	0.080	0.567
Limited activity on any day (0-6)	0.060	0.604	-0.167	0.286	0.022	0.794	-0.145	0.190	0.975
1-OHPG conc. day 7									
Limited activity during previous 2 days (5-6)	-0.026	0.348	-0.079	0.028	-0.007	0.780	-0.057	0.043	0.575
Limited activity during previous 3 days (4-6)	-0.027	0.491	-0.103	0.050	-0.002	0.957	-0.072	0.068	0.543
Limited activity on any day (0-6)	-0.103	0.329	-0.310	0.104	0.091	0.365	-0.106	0.287	0.474
1-OHPG conc. average of samples									
Limited activity on days 0-3	0.040	0.563	-0.095	0.175	0.117	0.128	-0.034	0.267	0.288
Limited activity on days 4-6	-0.026	0.640	-0.135	0.083	0.008	0.872	-0.092	0.108	0.609
Limited activity on days 0-6	0.020	0.895	-0.275	0.315	0.154	0.237	-0.102	0.411	0.301

Adjusted for urinary creatinine, air nicotine (continuous), age, gender, season, and caregiver's education

APPENDIX E: Supplemental Tables for Chapter 4

Supplemental Table 4-1. Descriptive statistics for urine, serum, whole blood, nasal lavage, and nasal epithelium mRNA biomarkers, stratified by *GSTM1* genotype

	Overall	*GSTM1*-null	*GSTM1*-present	
	Median (IQR)	Median (IQR)	Median (IQR)	p-value*
Urinary biomarkers				
1-OHPG (pmol/ml)	0.87 (0.20-2.71)	0.60 (0.21-1.99)	0.80 (0.19-3.12)	0.509
1-OHPG (umol/mol Cr)	0.10 (0.02-0.22)	0.10 (0.03-0.15)	0.11 (0.02-0.24)	0.599
Isoprostane (pg/ml)	3365(3574-5094)	3313 (2541-4937)	3321 (2570-5139)	0.866
Isoprostane (ug/g Cr)	2702 (1964-3393)	2874 (2261-3929)	2493 (1636-3952)	0.742
Nasal epithelium mRNA				
NQO1	50 (28.4-108.3)	50.12 (32.1-108.3)	53.0 (28.8-102.5)	0.959
HO-1	9.04 (5.72-18.03)	8.66 (6.20-18.33)	9.45 (5.06-15.40)	0.938
GCLM	14.39(7.07-26.84)	9.39 (5.25-18.04)	17.35 (7.20-26.84)	0.468
GCLC	82.7 (31.4-978.5)	51.6 (26.3-811.2)	359.5 (48.3-978.5)	0.378
TFF2	238.9 (111.5-1205)	225.7 (54.6-890.0)	252.1 (54.6-1387.5)	0.559
IL-33	6915 (2054-50708)	2789 (1259-63339)	15159 (2489-50048)	0.223
Serum cytokines and chemokines (pg/ml)				
IL-6	0.32 (0.32-1.33)	0.32 (0.32-1.99)	0.32 (0.32-4.33)	0.962
IL-8	8.82 (5.18-12.73)	6.89 (5.84-13.79)	9.34 (4.81-12.73)	0.925
IL-10	0.32 (0.32-0.32)	0.32 (0.32-0.32)	0.32 (0.32-4.97)	0.439
IL-13	0.32 (0.32-0.32)	0.32 (0.32-0.32)	0.32 (0.32-0.32)	0.748
CCL20	10.49 (032-38.28)	6.66 (0.32-49.89)	10.57 (0.32-35.60)	0.921
ECP	7.24 (5.11-14.89)	7.28 (3.76-25.72)	7.24 (5.32-14.19)	0.955
eotaxin	61.93 (41.14-99.96)	55.95 (43.5-86.42)	58.73 (32.01-104.8)	0.910
GRO	1379 (804-2175)	1429 (1002-2215)	1345 (758-2180)	0.460
GRO-α	188.7 (666.6-367.6)	134.6 (87.2-351.2)	120.4 (30.2-322.2)	0.776
MIP-1 α	13.80 (8.10-21.47)	13.87 (5.56-24.32)	14.21 (8.74-20.21)	0.835
Nasal lavage biomarkers (pg/ml)				
IL-6	0.32 (0.32-0.32)	0.32 (0.32-0.32)	0.32 (0.32-0.32)	0.925
IL-8	72.78 (45.74-137.6)	75.72 (45.74-78.9)	76.23 (52.0-212.8)	0.440
IL-10	0.32 (0.32-0.32)	0.32 (0.32-0.32)	0.32 (0.32-0.32)	0.651
IL-13	0.32 (0.32-0.97)	0.32 (0.32-0.82)	0.32 (0.32-1.33)	0.635
ECP	0.32 (0.32-3.28)	3.28 (3.16-3.68)	0.32 (0.32-3.23)	**0.060**
CCL20	0.32 (0.32-3.02)	1.61 (0.32-2.31)	0.78 (0.32-3.75)	0.806
eotaxin	1.6 (1.6-1.6)	1.6 (1.6-1.6)	1.6 (1.6-1.6)	0.308
GRO	343.9 (234.9-541.2)	304.3 (226.6-346)	399.3 (299.2-871.0)	0.123
GRO-α	654.5 (359.2-1054)	472.0 (325.9-1054)	708.4 (600.9-1508)	0.328
MIP-1α	0.32 (0.32-4.21)	0.32 (0.32-2.31)	1.31 (0.32-6.64)	0.202
Neutrophil count	77 (55-96)	80 (71-96)	73 (55-97)	0.817
Neutrophil percent	0.78 (0.58-0.91)	0.78 (0.69-0.92)	0.73 (0.57-0.91)	0.696
Eosinophil count	0 (0-2)	1 (0-3)	0 (0-2)	0.364
Eosinophil percent	0 (0-0.3)	0.1 (0-0.3)	0 (0-0.02)	0.380
White blood cell count	102 (77-112)	101 (36-111)	103 (101-113)	0.240

*Wilcoxon rank-sum test for differences between *GSTM1*-null and *GSTM1*-present values

Supplemental Table 4-2. Associations between urinary 1-OHPG and biomarkers of oxidative stress, gene induction, and inflammation (without GEE analysis)

	Crude[a]				Adjusted[b]			
Urinary biomarkers	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Log Isoprostane	0.025	0.176	-0.012	0.062	0.026	0.328	-0.027	0.078
mRNA for antioxidant genes								
Log NQO1	0.005	0.889	-0.068	0.078	0.040	0.467	-0.071	0.151
Log HO-1	0.002	0.951	-0.075	0.080	0.010	0.833	-0.084	0.103
Log GCLM	-0.011	0.719	-0.071	0.049	0.002	0.968	-0.091	0.095
Log GCLC	-0.022	0.697	-0.137	0.092	0.031	0.712	-0.140	0.203
Log TFF2	-0.016	0.777	-0.132	0.099	0.020	0.827	-0.162	0.201
Log IL-33	-0.062	0.405	-0.211	0.087	-0.086	0.436	-0.309	0.137
Whole blood biomarker								
Log PBMC no PMA	0.055	0.220	-0.034	0.144	-0.004	0.947	-0.136	0.128
Log PBMC with PMA	0.006	0.862	-0.061	0.072	-0.022	0.653	-0.118	0.075
Log Monocytes no PMA	0.062	0.166	-0.027	0.152	-0.003	0.962	-0.134	0.128
Log Monocytes with PMA	-0.010	0.741	-0.071	0.051	-0.052	0.245	-0.140	0.037
Log Lymphocytes no PMA	0.041	0.407	-0.057	0.140	0.007	0.925	-0.139	0.153
Log Lymphocytes with PMA	0.017	0.736	-0.081	0.114	-0.019	0.792	-0.166	0.128
Log PMN no PMA	0.020	0.643	-0.065	0.104	0.040	0.519	-0.084	0.163
Log PMN with PMA	-136.8	0.421	-475.1	201.4	-0.029	0.440	-0.106	0.047
Log Eosinophil no PMA	-0.019	0.869	-0.248	0.211	0.047	0.877	-0.562	0.655
Log Eosinophil with PMA	0.060	0.651	-0.207	0.328	0.146	0.512	-0.304	0.595
Log Neutrophil no PMA	0.015	0.801	-0.103	0.132	0.017	0.916	-0.305	0.338
Log Neutrophil with PMA	-0.005	0.890	-0.081	0.070	0.057	0.527	-0.126	0.240
Serum cytokines and chemokines								
Log CCL20	-0.110	0.203	-0.280	0.061	-0.087	0.470	-0.329	0.154
Log ECP	0.004	0.898	-0.063	0.072	0.000	0.995	-0.102	0.101
Log Eotaxin	0.058	0.012	0.013	0.103	0.018	0.505	-0.035	0.071
Log GRO	-0.006	0.768	-0.046	0.034	0.027	0.285	-0.023	0.078
Log GRO-α	-0.073	0.120	-0.167	0.020	-0.076	0.377	-0.247	0.095
Log IL-6	-0.053	0.327	-0.162	0.055	-0.106	0.193	-0.268	0.056
Log IL-8	-0.025	0.517	-0.103	0.052	-0.016	0.784	-0.130	0.099
Log IL-10	-0.064	0.227	-0.168	0.041	-0.136	**0.048**	-0.271	-0.001
Log IL-13	-0.014	0.790	-0.116	0.089	-0.015	0.852	-0.172	0.143
MIP-1α	-0.035	0.503	-0.139	0.069	-0.024	0.755	-0.175	0.128
Log RANTES	-0.001	0.945	-0.030	0.028	0.006	0.732	-0.028	0.039
Nasal lavage biomarkers								
Log Nasal lavage IL-6	1.126	0.145	0.960	1.321	*			
Log Nasal lavage IL-8	-0.015	0.654	-0.082	0.052	0.003	0.947	-0.089	0.095
Log Nasal lavage IL-10	-0.013	0.587	-0.060	0.035	-0.020	0.602	-0.100	0.060
Log Nasal lavage IL-13	1.029	0.681	0.898	1.180	1.040	0.803	0.762	1.421
Log Nasal lavage ECP	0.941	0.424	0.809	1.093	1.152	0.340	0.861	1.542

Log Nasal lavage CCL20	0.948	0.469	0.820	1.096	0.949	0.663	0.749	1.202
Log Nasal lavage eotaxin	1.016	0.891	0.809	1.276	*			
Log Nasal lavage GRO	-0.001	0.982	-0.059	0.057	0.020	0.596	-0.059	0.100
Log Nasal lavage GRO-α	0.001	0.981	-0.070	0.072	0.030	0.468	-0.054	0.114
Nasal lavage MIP-1α	0.993	0.917	0.867	1.136	1.233	0.464	0.704	2.160
Nasal lavage RANTES	1.032	0.812	0.796	1.338	*			
Log Nasal lavage neutrophil count	-0.026	0.404	-0.088	0.036	0.011	0.859	-0.116	0.139
Nasal lavage neutrophil percent	0.016	0.466	-0.028	0.061	0.040	0.394	-0.053	0.133
Nasal lavage eosinophil percent	-0.001	0.895	-0.017	0.015	-0.001	0.968	-0.037	0.035
Log Nasal lavage WBCC	-0.031	0.177	-0.076	0.014	-0.006	0.905	-0.107	0.095

[a]Adjusted for urinary creatinine
[b]Adjusted for urinary creatinine, age, race, gender, GSTM genotype, and educational attainment
*Multivariate regression models did not reach convergence

Supplemental Table 4-3. Associations between urinary 1-OHPG and biomarkers of oxidative stress, gene induction, and inflammation, stratified by *GSTM1* genotype (without GEE analysis)

	***GSTM1*-null**				***GSTM1*-present**				
Urinary biomarkers	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-interaction**
Log Isoprostane	0.307	**0.008**	0.100	0.514	0.019	0.517	-0.039	0.077	**0.008**
mRNA from antioxidant genes									
Log NQO1	0.146	0.728	-0.807	1.098	0.042	0.519	-0.090	0.174	0.634
Log HO-1	-0.243	0.426	-0.923	0.437	0.008	0.878	-0.097	0.113	0.355
Log GCLM	-0.170	0.674	-1.085	0.745	-0.003	0.954	-0.103	0.098	0.444
Log GCLC	0.863	0.229	-0.685	2.411	0.045	0.556	-0.111	0.201	**0.042**
Log TFF2	1.053	0.348	-1.476	3.581	0.035	0.691	-0.145	0.215	0.358
Log IL-33	-1.244	0.394	-4.480	1.993	-0.051	0.474	-0.196	0.095	0.597
Whole blood biomarkers									
Log PBMC no PMA	0.434	0.335	-0.531	1.399	0.005	0.948	-0.137	0.147	0.166
Log PBMC with PMA	0.299	0.379	-0.432	1.031	-0.025	0.623	-0.127	0.077	0.319
Log Monocytes no PMA	0.257	0.569	-0.717	1.232	-0.001	0.988	-0.137	0.135	0.201
Log Monocytes with PMA	0.256	0.457	-0.481	0.992	-0.056	0.154	-0.134	0.022	**0.041**
Log Lymphocytes no PMA	0.667	0.179	-0.368	1.703	0.005	0.946	-0.157	0.168	0.131
Log Lymphocytes with PMA	0.468	0.233	-0.359	1.294	-0.027	0.748	-0.199	0.145	0.584
Log PMN no PMA	0.089	0.782	-0.617	0.796	0.051	0.471	-0.093	0.196	0.383
Log PMN with PMA	0.230	0.372	-0.325	0.786	-0.038	0.341	-0.119	0.043	0.114
Log Eosinophil no PMA	-2.117	0.375	-8.008	3.775	0.046	0.892	-0.650	0.741	0.675
Log Eosinophil with PMA	-0.283	0.741	-2.768	2.201	0.156	0.575	-0.414	0.726	0.869
Log Neutrophil no PMA	-0.524	0.733	-4.497	3.449	-0.006	0.973	-0.381	0.369	0.952
Log Neutrophil with PMA	-1.077	0.340	-3.838	1.684	0.039	0.658	-0.143	0.222	0.742
Serum cytokines and chemokines									
Log CCL20	1.843	**0.003**	0.785	2.900	-0.103	0.373	-0.337	0.130	**0.011**
Log ECP	0.328	0.153	-0.147	0.802	-0.004	0.940	-0.106	0.099	**0.090**

Log Eotaxin	-0.014	0.878	-0.217	0.189	0.025	0.416	-0.038	0.088	0.594
Log GRO	0.285	**0.016**	0.067	0.504	0.015	0.603	-0.043	0.073	0.111
Log GRO-α	0.511	**0.004**	0.209	0.813	-0.151	0.116	-0.342	0.040	**0.022**
Log IL-6	0.193	0.631	-0.683	1.068	-0.083	0.221	-0.220	0.053	0.302
Log IL-8	0.656	**0.016**	0.157	1.155	-0.001	0.982	-0.122	0.119	0.181
Log IL-10	0.318	0.286	-0.315	0.951	-0.113	0.127	-0.261	0.034	0.601
Log IL-13	0.765	0.158	-0.360	1.890	-0.002	0.979	-0.143	0.139	0.299
MIP-1α	-0.767	0.244	-2.159	0.624	0.050	0.400	-0.070	0.170	0.232
Log RANTES	0.119	**0.014**	0.031	0.207	0.006	0.764	-0.033	0.045	0.830
Nasal lavage biomarkers									
Log Nasal lavage IL-8	0.714	0.298	-1.492	2.920	0.008	0.899	-0.129	0.145	0.843
Log Nasal lavage IL-10	*				-0.022	0.603	-0.112	0.069	
Log Nasal lavage IL-13	*				0.940	0.776	0.612	1.442	
Log Nasal lavage ECP	*				1.074	0.652	0.789	1.462	
Log Nasal lavage CCL20	*				0.391	0.116	0.121	1.261	
Log Nasal lavage GRO	0.553	0.232	-0.850	1.956	0.006	0.912	-0.111	0.123	0.430
Log Nasal lavage GRO-α	0.329	0.480	-1.312	1.969	0.009	0.858	-0.098	0.116	**0.073**
Nasal lavage MIP-1α	*				0.957	0.920	0.407	2.252	0.508
Log Nasal lavage neutrophil count	0.353	0.131	-0.142	0.849	-0.017	0.792	-0.151	0.117	0.282
Nasal lavage neutrophil percent	0.177	0.302	-0.206	0.560	0.024	0.648	-0.084	0.133	0.658
Nasal lavage eosinophil percent	0.021	0.303	-0.025	0.068	-0.002	0.936	-0.048	0.044	0.757
Log Nasal lavage WBCC	0.176	**0.035**	0.018	0.335	-0.015	0.792	-0.132	0.102	0.525

Adjusted for urinary creatinine, age, gender, race, season, and educational attainment
*Bivariate regression models did not reach convergence

Supplemental Table 4-4. Comparison between multivariate linear regression models without and with GEE analysis (model: associations between urinary 1-OHPG and biomarkers of oxidative stress, gene induction, and inflammation)

	MLR without GEE (full model)				MLR with GEE (full model)			
Urinary oxidative stress biomarker	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**
Log isoprostane	0.026	0.328	-0.027	0.078	0.026	0.181	-0.012	0.063
mRNA								
Log NQO-1	0.040	0.467	-0.071	0.151	0.051	0.084	-0.007	0.109
Log HO-1	0.010	0.833	-0.084	0.103	0.017	0.576	-0.044	0.078
Log GCLM	0.002	0.968	-0.091	0.095	0.009	0.690	-0.034	0.052
Log GCLC	0.031	0.712	-0.140	0.203	0.035	0.435	-0.053	0.124
Log TFF2	0.020	0.827	-0.162	0.201	0.024	0.589	-0.064	0.112
Log IL-33	-0.086	0.436	-0.309	0.137	-0.101	**0.031**	-0.194	-0.009
Serum cytokines and chemokines								
Log Serum IL-6	0.659	0.187	0.355	1.223	-0.027	0.157	-0.064	0.010
Log Serum IL-8	-0.016	0.784	-0.130	0.099	-0.009	0.654	-0.050	0.031
Log Serum IL-10	-0.136	**0.048**	-0.271	-0.001	-0.052	**0.027**	-0.098	-0.006
Log Serum IL-13	-0.015	0.852	-0.172	0.143	0.006	0.665	-0.023	0.036
Log Serum CCL20	-0.087	0.470	-0.329	0.154	-0.128	**0.010**	-0.225	-0.031
Log Serum ECP	0.000	0.995	-0.102	0.101	0.006	0.879	-0.068	0.080
Log Serum eotaxin	0.018	0.505	-0.035	0.071	0.011	0.546	-0.026	0.048
Log Serum GRO	0.027	0.285	-0.023	0.078	0.015	0.384	-0.019	0.049
Log Serum GRO-α	-0.076	0.377	-0.247	0.095	0.038	0.068	-0.003	0.079
Log Serum MIP-1α	-0.024	0.755	-0.175	0.128	-0.017	0.249	-0.046	0.012
Log Serum RANTES	0.006	0.732	-0.028	0.039	0.008	0.194	-0.004	0.021
Nasal Lavage biomarkers								
Log Nasal lavage IL-8	0.003	0.947	-0.089	0.095	-0.019	0.532	-0.079	0.041
Log Nasal lavage IL-10	-0.020	0.602	-0.100	0.060	-0.017	0.486	-0.064	0.030
Log Nasal lavage IL-13	1.040	0.803	0.762	1.421	0.037	0.721	-0.167	0.241
Log Nasal lavage ECP	1.152	0.340	0.861	1.542	0.148	0.323	-0.145	0.441
Log Nasal lavage CCL20	0.949	0.663	0.749	1.202	-0.041	0.660	-0.224	0.142
Log Nasal lavage GRO	0.020	0.596	-0.059	0.100	0.020	0.518	-0.041	0.082
Log Nasal lavage GRO-α	0.030	0.468	-0.054	0.114	0.037	0.114	-0.009	0.083
Log Nasal lavage MIP-1α	1.233	0.464	0.704	2.160	*			
Log Neutrophil count	0.011	0.859	-0.116	0.139	0.010	0.834	-0.085	0.105
Neutrophil percent	0.040	0.394	-0.053	0.133	0.043	0.121	-0.011	0.097
Eosinophil percent	-0.001	0.968	-0.037	0.035	0.013	**0.008**	0.003	0.023
Log White blood cell count	-0.006	0.905	-0.107	0.095	-0.002	0.946	-0.070	0.066
Whole blood biomarkers								
Log PBMC no PMA	-0.004	0.947	-0.136	0.128	-0.076	**0.024**	-0.142	-0.010
Log PBMC with PMA	-0.022	0.653	-0.118	0.075	-0.034	0.383	-0.111	0.043
Log Monocytes no PMA	-0.003	0.962	-0.134	0.128	-0.037	0.276	-0.104	0.030
Log Monocytes with PMA	-0.052	0.245	-0.140	0.037	-0.057	0.092	-0.124	0.009
Log Lymphocytes no PMA	0.007	0.925	-0.139	0.153	-0.009	0.832	-0.089	0.071

Log Lymphocytes with PMA	-0.019	0.792	-0.166	0.128	-0.035	0.612	-0.169	0.100
Log PMN no PMA	0.040	0.519	-0.084	0.163	0.039	0.432	-0.058	0.136
Log PMN with PMA	-0.029	0.440	-0.106	0.047	-0.034	0.217	-0.087	0.020
Log Eosinophils no PMA	0.047	0.877	-0.562	0.655	0.012	0.943	-0.319	0.343
Log Eosinophils with PMA	0.146	0.512	-0.304	0.595	0.314	**0.023**	0.043	0.586
Log Neutrophils no PMA	0.017	0.916	-0.305	0.338	-0.079	0.274	-0.219	0.062
Log Neutrophil with PMA	0.057	0.527	-0.126	0.240	0.117	**0.002**	0.041	0.193

Adjusted for urinary creatinine, age, gender, race, season, and educational attainment
*Multivariate regression model did not reach convergence

Supplemental Table 4-5. Associations between urinary 1-OHPG and biomarkers of oxidative stress, gene induction, and inflammation, stratified by *GSTM1* genotype (FULL TABLE)

	Crude model[a]							Adjusted Model[b]						
	GSTM1-null			*GSTM1*-present				*GSTM1*-null			*GSTM1*-present			
Urinary biomarkers	**β**	**[95%**	**CI]**	**β**	**[95%**	**CI]**	**p-int**	**β**	**[95%**	**CI]**	**β**	**[95%**	**CI]**	**p-int**
Log Isoprostane	0.252	-0.029	0.533	0.010	-0.016	0.035	**0.022**	**0.237**[c]	0.046	0.428	0.022	-0.012	0.056	**0.002**
Nasal epithelium mRNA														
Log NQO1	0.135	-0.224	0.495	0.037	-0.002	0.077	0.396	**			**0.092**[c]	0.013	0.172	
Log HO-1	0.320	-0.063	0.704	0.007	-0.062	0.076	0.148	-0.360	-1.020	0.301	0.017	-0.048	0.082	0.284
Log GCLM	0.153	-0.354	0.660	**-0.048**[c]	-0.085	-0.011	0.550	-0.275	-0.995	0.445	**			
Log GCLC	**1.097**[d]	0.507	1.688	-0.017	-0.078	0.045	**0.006**	0.732	-0.156	1.619	**0.101**[c]	0.006	0.195	**0.024**
Log TFF2	**1.265**[c]	0.451	2.078	-0.006	-0.066	0.053	0.203	**			0.033	-0.027	0.094	
Log IL-33	*			**-0.088**[c]	-0.146	-0.030		-0.030	-0.115	0.055	0.029	-0.124	0.182	**<0.001**
Serum cytokines and chemokines														
Log IL-6	0.071	-0.380	0.522	-0.025	-0.054	0.004	0.886	0.104	-0.117	0.325	**-0.048**[c]	-0.095	-0.001	0.570
Log IL-8	0.517	-0.157	1.190	-0.028	-0.059	0.002	0.098	**0.584**[d]	0.331	0.837	-0.013	-0.05	0.024	0.134
Log IL-10	*			-0.048	-0.102	0.005		**			-0.053	-0.111	0.004	
Log IL-13	*			-0.005	-0.019	0.01		**			0.011	-0.019	0.041	
Log CCL20	**1.183**[c]	0.483	1.883	**-0.121**[d]	-0.228	-0.014	**<0.001**	**			**-0.144**[c]	-0.250	-0.037	
Log ECP	0.298	-0.377	0.973	-0.003	-0.073	0.067	0.233	**0.236**[d]	0.103	0.368	0.003	-0.082	0.088	0.113
Log Eotaxin	-0.062	-0.313	0.189	0.012	-0.014	0.039	0.712	-0.039	-0.127	0.048	0.009	-0.035	0.052	0.829
Log GRO	*			-0.003	-0.037	0.030		**0.279**[d]	0.256	0.301	0.005	-0.03	0.04	**0.075**
Log GRO-α	*			0.029	-0.017	0.075		**0.634**[d]	0.604	0.664	0.034	-0.010	0.078	0.250
MIP-1α	-0.141	-0.653	0.372	-0.016	-0.046	0.014	0.698	-0.303	-1.034	0.428	**			
Log RANTES	0.105	-0.018	0.227	0.003	-0.006	0.011	0.207	**0.090**[c]	0.001	0.180	0.004	-0.010	0.019	0.693
Nasal Lavage biomarkers														
Log Nasal lavage IL-8	**0.298**[d]	0.131	0.466	0.006	-0.047	0.059	0.051	**			**			
Log Nasal lavage IL-10	0.353	-0.29	0.996	0.012	-0.017	0.041	0.881	**			-0.026	-0.116	0.064	
Log Nasal lavage IL-13	0.415	-0.104	0.934	-0.072	-0.232	0.087	0.324	**			-0.009	-0.063	0.045	
Log Nasal lavage ECP	*			0.052	-0.118	0.222		**			-0.011	-0.284	0.262	
Log Nasal lavage CCL20	-2.356	-5.108	0.395	0.039	-0.118	0.197	0.806	**			**			
Log Nasal lavage eotaxin	*			0.059	-0.09	0.209		**			**			
Log Nasal lavage GRO	0.213	0.192	0.234	0.021	-0.04	0.082	**0.002**	**			-0.003	-0.083	0.077	
Log Nasal lavage GRO-α	0.340	0.148	0.532	0.034	-0.019	0.087	**<0.001**	**			0.031	-0.024	0.085	
Log Nasal lavage MIP-1α	-0.134	-1.137	0.868	0.027	-0.121	0.175	0.967	**			**			
Eosinophil percent	0.000	-0.021	0.021	-0.01	-0.028	0.007	0.810	**			**0.013**[c]	0.001	0.024	

Log Neutrophil count	*			-0.017	-0.071	0.037		**			-0.022	-0.104	0.060	
Neutrophil percent	0.191	-0.157	0.538	0.031	-0.008	0.070	0.438	**			0.021	-0.032	0.074	
Log White blood cell count	*			-0.010	-0.098	0.078		**0.075**[c]	0.030	0.121	0.003	-0.069	0.075	0.315
Whole blood biomarkers														
Log PBMC no PMA	0.523	-0.027	1.073	-0.035	-0.09	0.020	**0.029**	0.029	-0.124	0.182	-0.069	-0.132	-0.007	**0.002**
Log PBMC with PMA	**0.457**[c]	0.149	0.765	0.001	-0.056	0.059	0.078	0.142	-0.072	0.356	-0.036	-0.108	0.037	**0.080**
Log Monocytes no PMA	0.28	-0.161	0.722	0.002	-0.044	0.047	**0.018**	**			-0.044	-0.112	0.024	
Log Monocytes with PMA	**0.473**[c]	0.085	0.861	-0.030	-0.073	0.014	**0.014**	**0.305**[c]	0.124	0.485	**-0.064**[c]	-0.113	-0.015	**<0.001**
Log Lymphocytes no PMA	0.372	0.116	0.628	-0.016	-0.103	0.071	**<0.001**	**0.331**[d]	0.146	0.516	0.006	-0.062	0.073	**<0.001**
Log Lymphocytes w/ PMA	0.290	-0.129	0.708	-0.014	-0.168	0.140	0.423	**0.400**[d]	0.312	0.487	-0.053	-0.189	0.083	0.360
Log PMN no PMA	0.239	-0.202	0.681	0.011	-0.102	0.124	0.090	**-0.284**[c]	-0.498	-0.071	0.059	-0.051	0.169	0.272
Log PMN with PMA	**0.338**[c]	0.034	0.641	-0.023	-0.059	0.013	**0.002**	0.099	-0.122	0.320	**-0.047**[c]	-0.094	0.000	**0.002**
Log Eosinophil no PMA	**1.626**[d]	0.797	2.456	0.055	-0.280	0.391	0.338	**			-0.042	-0.363	0.279	
Log Eosinophil with PMA	*			0.152	-0.084	0.388		**			0.405	-0.001	0.811	
Log Neutrophil no PMA	0.089	-0.162	0.340	0.055	-0.080	0.190	0.546	**			-0.163	-0.355	0.028	

[a]Adjusted for urinary creatinine
[b]Adjusted for urinary creatinine, age, race, gender, *GSTM1* genotype, and educational attainment
[c]p<0.05
[d]p<0.001
*Bivariate regression model did not reach convergence
**Multivariate regression model did not reach convergence

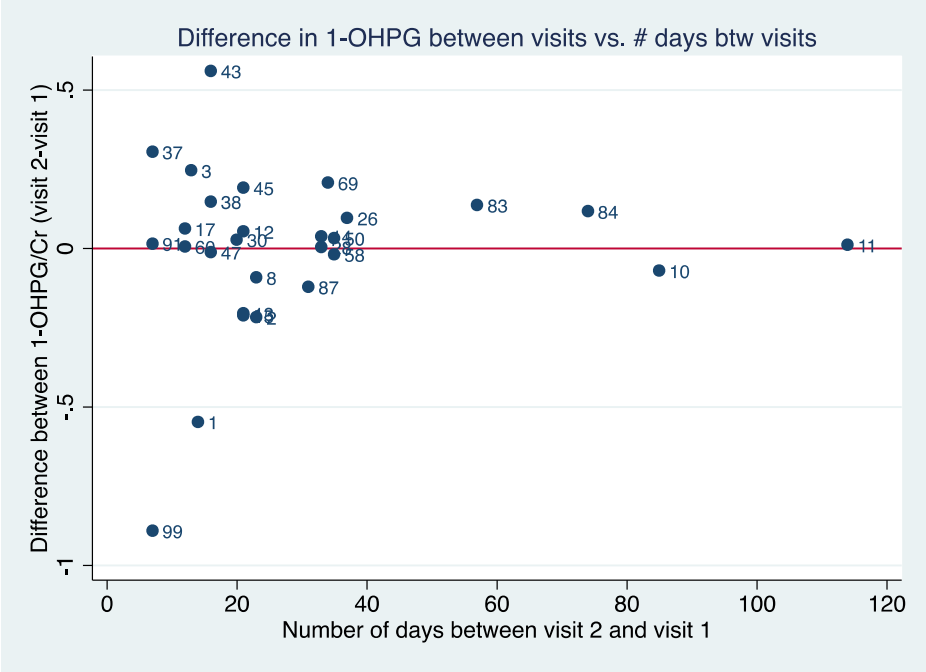
Supplemental Table 4-6. Associations between urinary 1-OHPG and biomarkers of oxidative stress, gene induction, and inflammation with time between visits (days) in the model[a]

	GSTM1-null				*GSTM1*-present				
Urinary biomarkers	**β**	**P>z**	**[95%**	**CI]**	**β**	**P>z**	**[95%**	**CI]**	**P-int**
Log Isoprostane (w/o outlier)	0.306	**0.013**	0.080	0.532	0.019	0.529	-0.041	0.078	**0.010**
mRNA for antioxidant enzymes									
Log *NQO-1*	0.148	0.732	-0.861	1.157	0.047	0.477	-0.088	0.182	0.531
Log *HO-1*	-0.241	0.445	-0.964	0.481	-0.007	0.886	-0.101	0.088	0.750
Log *GCLM*	-0.167	0.675	-1.096	0.762	-0.003	0.950	-0.107	0.101	0.459
Log *GCLC*	0.868	0.226	-0.705	2.441	0.057	0.448	-0.097	0.212	**0.022**
Log *TFF2*	1.019	0.388	-1.754	3.792	0.051	0.550	-0.124	0.227	0.186
Log IL-33	-1.231	0.366	-4.310	1.848	-0.048	0.519	-0.200	0.105	0.544
Whole blood biomarkers									
Log PBMC no PMA	0.484	0.356	-0.656	1.624	0.006	0.936	-0.140	0.152	0.181
Log PBMC with PMA	0.319	0.420	-0.548	1.186	-0.029	0.576	-0.133	0.075	0.399
Log Monocytes no PMA	0.264	0.588	-0.799	1.327	0.003	0.967	-0.137	0.143	0.203
Log Monocytes with PMA	0.254	0.493	-0.550	1.057	-0.057	0.161	-0.137	0.024	**0.055**
Log Lymphocytes no PMA	0.594	0.294	-0.625	1.813	0.014	0.862	-0.151	0.179	0.109
Log Lymphocytes with PMA	0.404	0.366	-0.568	1.376	-0.010	0.907	-0.181	0.161	0.388
Log PMN no PMA	-0.029	0.937	-0.833	0.776	0.043	0.552	-0.103	0.190	0.531
Log PMN with PMA	0.077	0.768	-0.507	0.661	-0.038	0.353	-0.122	0.045	0.143
Log Eosinophil no PMA	-1.609	0.601	-10.399	7.180	0.001	0.997	-0.704	0.706	0.672
Log Eosinophil with PMA	0.245	0.808	-3.573	4.064	0.152	0.595	-0.438	0.743	0.870
Log Neutrophil no PMA	0.077	0.968	-5.562	5.715	-0.014	0.940	-0.402	0.374	0.951
Log Neutrophil with PMA	-0.296	0.779	-3.366	2.773	0.028	0.753	-0.157	0.214	0.749
Serum cytokines and chemokines									
Log CCL20	1.768	**0.006**	0.652	2.884	-0.134	0.233	-0.360	0.091	**0.021**
Log ECP	0.300	0.209	-0.207	0.808	0.006	0.905	-0.096	0.108	**0.067**
Log Eotaxin	0.010	0.909	-0.186	0.206	0.025	0.435	-0.040	0.090	0.597
Log GRO	0.257	**0.020**	0.051	0.463	0.026	0.304	-0.025	0.078	**0.022**
Log GRO-α	0.506	**0.008**	0.175	0.837	-0.132	0.162	-0.320	0.056	**0.022**
Log IL-6	0.340	0.300	-0.368	1.048	-0.076	0.273	-0.215	0.063	0.236
Log IL-8	0.694	**0.016**	0.171	1.217	0.000	1.000	-0.124	0.124	0.173
Log IL-10	0.431	0.074	-0.052	0.915	-0.114	0.135	-0.266	0.038	0.599
Log IL-13	0.910	0.083	-0.149	1.969	0.021	0.745	-0.110	0.153	0.138
MIP-1α	-0.584	0.330	-1.880	0.713	0.040	0.504	-0.080	0.160	0.183
Log RANTES	0.113	**0.024**	0.019	0.206	0.008	0.679	-0.031	0.048	0.673
Nasal lavage biomarkers									
Log Nasal lavage IL-8	0.714	0.298	-1.492	2.920	0.057	0.440	-0.103	0.218	0.824
Log Nasal lavage IL-10	*				-0.024	0.643	-0.140	0.091	0.739
Log Nasal lavage IL-13	*				1.190	0.607	0.613	2.310	0.404
Log Nasal lavage ECP	*				1.041	0.917	0.486	2.229	0.136
Log Nasal lavage CCL20	*				0.426	0.134	0.140	1.299	0.683

Log Nasal lavage GRO	0.553	0.232	-0.850	1.956	0.050	0.431	-0.087	0.186	0.402
Log Nasal lavage GRO-α	0.329	0.480	-1.312	1.969	0.064	0.230	-0.049	0.177	**0.074**
Log Neutrophils	0.364	0.154	-0.194	0.923	-0.030	0.650	-0.166	0.106	0.346
Log Neutrophil percent	0.066	0.425	-0.129	0.261	0.009	0.697	-0.037	0.054	0.841
Log Eosinophil percent	0.021	0.360	-0.032	0.074	0.013	0.469	-0.023	0.048	0.366
Log White blood cell count	0.174	0.057	-0.007	0.355	-0.009	0.885	-0.129	0.112	0.500

[a]jMultivariate linear regression model (without GEE analysis) adjusted for urinary creatinine, age, gender, race, season, and educational attainment
*Bivariate regression model did not reach convergence

Figure 4-4. Difference in urinary 1-OHPG concentration between visits vs. number of days between visits



Curriculum Vitae

Kamau O. Peters, PhD, MPH
Date of birth: December 25, 1977
Birthplace: New York, NY, USA

Contact Information

15 Charles Plaza #1705
Baltimore, MD 21201
(734) 717-0807
email: kpeter27@jhu.edu

Education

2008-2014 **Johns Hopkins University Bloomberg School of Public Health**
Department of Environmental Health Sciences, Baltimore MD
Doctor of Philosophy (PhD) in Environmental Health Sciences

2001-2003 **University of Michigan School of Public Health**
Department of Environmental Health Sciences, Ann Arbor, MI
Master of Public Health (MPH) in Toxicology

1995-2000 **University of Michigan Ann Arbor**
Bachelor of Science (BS) in Biology

Employment

2009 **Public Health Intern**
Baltimore City Health Department, Baltimore, MD

2004-2008 **Associate Toxicologist**
NSF International, Ann Arbor, MI

2003 **Research Assistant**
University of Michigan School of Public Health, Ann Arbor, MI

Honors and awards

2010-2014 NIOSH Education and Research Center (ERC) Training Grant, Predoctoral Fellowship (T42-OH008428; PI: Jacqueline Agnew, PhD)

2008-2010 Awarded Bloomberg School of Public Health Diversity and Health Disparities Fellowship

2010- Member, American Public Health Association (APHA)