ABSTRACT

Title of Dissertation: EXPOSURE TO AMBIENT AIR POLLUTION AND CORRELATES OF CARDIOVASCULAR DISEASE AMONG YOUTH WITH TYPE 1 DIABETES

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Air pollution from traffic-related sources is associated with cardiovascular disease (CVD), potentially through changes in systemic inflammatory responses, vascular function and oxidative stress leading to atherosclerosis, thrombosis or endothelial dysfunction. Individuals with type 1 diabetes (T1D) have a greater risk of CVD-related morbidity and mortality than the general population, and they may be more susceptible to the effects of air pollution on CVD. Although these increased risks begin during childhood, very few studies have assessed the impact of air pollution on children and youth with T1D. This dissertation directly addresses gaps in the epidemiologic evidence by: 1) evaluating the relationship of short-term exposures to traffic-related air pollutants with pulse wave velocity (PWV), a measure of arterial stiffness, 2) assessing the effects of changes in air

pollution exposures on changes in inflammatory biomarkers, including C-reactive protein, fibrinogen and interleukin-6 (IL-6), and 3) examining the relationship of long-term exposures to traffic-related air pollution with allostatic load (AL), a measure of cumulative biological risk, among a cohort of youth with T1D.

Data were obtained from the SEARCH for Diabetes in Youth (SEARCH) study. SEARCH was initiated in 2000 and includes a diverse population of US youth diagnosed with diabetes prior to age 20 years. Anthropometric and laboratory measures were taken at SEARCH study visits, and standardized questionnaires were used to collect information on important covariates. Air pollution exposures were estimated using spatio-temporal models and assigned to residential addresses for each participant.

In the first study, we identified a significant association between increased exposure to $PM_{2.5}$ on the day of the examination with higher PWV using generalized linear models adjusted for lifestyle and demographic characteristics. In the second analysis, we found consistent positive effects of increases in $PM_{2.5}$ over the week prior to the examination with IL-6 using longitudinal mixed models. In the third study, no significant associations were observed for monthly and annual $PM_{2.5}$ exposures or proximity to major roadways with AL in fully adjusted linear mixed models. However, effects differed by race/ethnicity and gender. Overall, this research indicates that youth with T1D may be at higher risk for air pollution-related cardiovascular impacts.

EXPOSURE TO AMBIENT AIR POLLUTION AND CORRELATES OF CARDIOVASCULAR DISEASE AMONG YOUTH WITH TYPE 1 DIABETES

by

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Dissertation submitted to the Faculty of the Graduate School of the
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DEDICATION

This dissertation is dedicated to my husband, Lucas López, our children, Caroline, Audrey, Emily and Madison López, and to my mother, Mary Montresor, for all of their love and support. I also dedicate this work to the memory of my grandmother, Bonnie Blue Jones, who inspired me to begin this journey.

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LIST OF ABBREVIATIONS

ADMS-Roads – Atmospheric Dispersion Modeling System

AIx – Augmentation Index

AL – Allostatic Load

ANS – Autonomic Nervous System

AQS – Air Quality System

BC – Black Carbon

BrachD – Brachial Artery Distensibility

BP – Blood Pressure

CAA – Clean Air Act

cm – Centimeters

CO – Carbon Monoxide

CRP – C-reactive Protein

CVD – Cardiovascular Disease

DBP – Diastolic Blood Pressure

EC – Elemental Carbon

EPA – Environmental Protection Agency

GM – Geometric Mean

HbA1c - Glycated Hemoglobin

HDL – High Density Lipoprotein

Hg – Mercury

HIPAA – Health Insurance Portability and Accountability Act

HR – Hazard Ratio

IL-6 – Interleukin-6

IMPROVE – Interagency Monitoring of Protected Visual Environments Network

IRB – Institutional Review Board

Km – Kilometers

LDL – Low Density Lipoprotein

m – Meters

MA – Moving Average

MI – Myocardial Infarction

mm – Millimeters

mmHg – Millimeters of Mercury

NAAQS – National Ambient Air Quality Standards

NHANES – National Health and Nutrition Examination Survey

NIDDK – National Institute of Diabetes and Digestive and Kidney Diseases

NIEHS – National Institute for Environmental Health Sciences

NO_2 – Nitrogen Dioxide

NO_x – Nitrogen Oxides

O_3 – Ozone

Pb - Lead

PM – Particulate Matter

$PM_{2.5}$ – Particulate Matter <2.5 μm in diameter

PM_{10} – Particulate Matter <10 μm in diameter

PWV – Pulse Wave Velocity

SBP – Systolic Blood Pressure

SD – Standard Deviation

SE – Standard Error

SEARCH – Search for Diabetes in Youth Study

SEM – Standard Error of the Mean

SEP – Socioeconomic Position

SO_2 – Sulfur Dioxide

SO_x – Sulfur Oxides

SWAN – Study of Women's Health Across the Nation

TC – Total Cholesterol

TIGER – Topographically Integrated Geographic Encoding and Referencing

TNF-α – Tumor Necrosis Factor Alpha

T1D – Type 1 Diabetes

T2D – Type 2 Diabetes

UMD – University of Maryland

US – United States

VOCs – Volatile Organic Compounds

WC – Waist Circumference

WHP – Waist-to-Hip Ratio

µg – Micrograms

CHAPTER 1

INTRODUCTION

The focus of this dissertation is to evaluate and characterize potential cardiovascular health impacts associated with exposures to traffic-related air pollution along children and youth with type 1 diabetes (T1D) in the United States (US). The work that follows has been divided into six chapters, beginning with a brief introduction and outline (Chapter 1). The specific aims and corresponding research questions that guided this research will be presented in detail in the background section (Chapter 2), along with a comprehensive review of the relevant literature. These concepts will be further expanded in subsequent chapters.

The content of Chapter 3 corresponds to Specific Aim #1 in the form of a manuscript entitled, "Exposures to Traffic-related Air Pollution and Measures of Arterial Stiffness in a Cohort of Youth and Children with T1D". The focus of this chapter is to explore the cross-sectional relationship between measures of arterial stiffness and acute exposures to traffic-related air pollutants using generalized linear models.

In Chapter 4, the manuscript "Are Changes in Air Pollution Exposures Associated with Changes in Inflammation among Children and Youth with T1D?", which corresponds to Specific Aim #2, is presented. This analysis focuses on identifying associations between biomarkers of systemic inflammation and short-term exposures to traffic-related air pollutants in a longitudinal analysis using data at two points in time.

The content of Chapter 5, which coincides with Specific Aim #3, builds upon analyses in the previous two chapters to explore whether long-term exposures to traffic-related air pollutants are associated with an index of cumulative biological risk. The

corresponding manuscript entitled, “The Relationship of Air Pollution Exposures with Allostatic Load among Youth with T1D in the SEARCH Cohort” blends typical methods in environmental epidemiology with the social sciences by incorporating allostatic load – a measure traditionally encountered in social and behavioral research – as an outcome measure.

The final chapter (Chapter 6) includes a brief synopsis of our findings, in addition to a detailed discussion on the strengths and limitations of this work. We also discuss public health implications and suggest directions for future research.

CHAPTER 2

BACKGROUND

A growing body of literature indicates that ambient air pollution is a risk factor for cardiovascular morbidity and mortality (Brook et al. 2004, Brook et al. 2010). Proposed biological mechanisms underlying this relationship include endothelial dysfunction, vasoconstriction, changes in blood pressure (BP), prothrombotic and coagulant changes, systemic inflammatory and oxidative stress responses, and the progression of atherosclerosis (Brook et al. 2010). Furthermore, existing research indicates that individuals with diabetes may be more susceptible to the detrimental effects of air pollution on CVD (O'Neill et al. 2005; Dubowsky et al 2006). For example, diabetics have a greater risk of dying and/or being hospitalized for heart disease during periods of high air pollution, and long-term exposure to fine particulate matter ($PM_{2.5}$) has been associated with a higher relative risk of mortality among people with diabetes (Goldberg et al. 2001; Zanobetti and Schwartz 2002). However, it is poorly understood why individuals with diabetes are particularly vulnerable to these negative health effects, and the underlying physiologic mechanisms have yet to be fully elucidated. It has been hypothesized that chronic inflammation is partially responsible for this enhanced sensitivity; other mechanisms implicated include oxidative distress, endothelial dysfunction, and impaired vascular function (Brook et al. 2010; O'Neill et al. 2005).

To date, the vast majority of existing research on the cardiovascular effects of air pollution involving diabetic individuals has been conducted among adults with Type 2 Diabetes (T2D). T2D is an extremely prevalent metabolic disorder caused by inadequate

secretion of insulin in response to overnutrition, physical inactivity and insulin resistance that primary affects ageing populations (Nolan et al. 2011). In contrast, T1D is an autoimmune disorder with defective insulin production usually presenting in younger ages (Atkinson et al. 2014). T1D is a leading chronic disease among youth, and data from large epidemiologic studies indicate that its incidence is increasing 2-5% worldwide (Maahs et al. 2010). Although CVD events are not generally expected to occur during childhood, youth and adolescents with T1D are at significantly increased long-term risk of CVD-related morbidity and mortality. For instance, CVD is the leading cause of premature death among young adults with diabetes, and the risk of CVD-related mortality before the age of 40 years is increased up to 20-fold in patients with T1D compared to non-diabetics (Dahl-Jorgensen et al 2005; Skrivarhaug et al. 2006; Laing et al 2003). However, very little existing research has focused on the impact of air pollution in children with T1D, an increasing and extremely vulnerable population at high risk of comorbidities and complications likely to be exacerbated by air pollution. In order to address this current research gap, we will examine the effects of exposures to traffic-related air pollutants on subclinical measures of cardiovascular function (i.e., arterial stiffness), inflammatory biomarkers, and an index of cumulative biological risk among a cohort of children and young adults with T1D (SEARCH for Diabetes in Youth). We will address the following aims:

- **Specific Aim #1.** To evaluate the cross-sectional relationship of short-term exposure (≤7 days in duration) to ambient air pollution ($PM_{2.5}$, elemental carbon [EC] and traffic-related particulate matter [PM]) with pulse wave velocity (PWV), a measure of arterial stiffness, among youth with T1D.

- Research Question 1.1 Do youth with T1D who are exposed to higher levels of ambient air pollution in the short-term exhibit increased arterial stiffness independent of known CVD risk factors?

- **Specific Aim #2.** To assess the longitudinal relationship of short-term exposure (≤7 days in duration) to ambient air pollution ($PM_{2.5}$, EC and traffic-related PM) with inflammatory biomarkers (C-reactive protein [CRP], Interleukin-6 [IL-6], fibrinogen) among youth with T1D.
  - Research Question 2.1 Are acute changes in ambient air pollution exposures associated with changes in inflammation among youth with T1D?
- **Specific Aim #3.** To examine the cross-sectional relationship of long-term exposure (1 month and 1 year in duration) to ambient air pollution ($PM_{2.5}$, EC and traffic-related PM) with allostatic load (AL) as a measure of cumulative biological risk among youth with T1D.
  - Research Question 3.1 Do youth with T1D who are chronically exposed to higher levels of ambient air pollution exhibit increased AL?
  - Research question 3.2 Does the association between air pollution and AL differ according to demographic factors (e.g., gender, race/ethnicity, socioeconomic position [SEP])?

This research is innovative because it presents a substantial departure from the existing air pollution-related CVD literature which is primarily focused on adults with T2D, while we will concentrate on youth with T1D, a highly vulnerable and increasing population. Prior studies have not investigated the short-term effects of $PM_{2.5}$ and traffic-

related air pollution on measures of arterial stiffness among youth with T1D, although the existing literature indicates that associations exist among adults, the elderly, and healthy children (Wu et al. 2016; Mehta et al. 2014; Iannuzzi et al. 2010). We anticipate that these associations may be stronger among our study population given the increased risk for CVD among individuals with diabetes and exacerbation of the effects by ambient air pollution. In addition, no existing studies have examined the relationship of AL with outdoor air pollution exposures even though a growing body of evidence suggests that individuals who experience higher levels of AL are at significantly increased risk for adverse health outcomes including CVD (Logan and Barksdale 2008; Saban et al. 2014). In addition, racial/ethnic and SES differences in AL exist among youth and may translate into increased health risks for African Americans and low-income individuals (Rainisch and Upchurch 2013). These populations are also simultaneously overburdened by increased exposure to various environmental risks, including ambient air pollution (Wilson et al. 2014), but differences in the association between outdoor air pollutants and AL by sociodemographic factors (e.g., race/ethnicity, SEP) have not been previously explored.

Overall, our results have the potential to help elucidate pathophysiological responses to air pollution among youth with T1D as well as contribute to the development of clinical and public policy guidelines which could reduce the substantial impacts of air pollution-related CVD risks. Our research will also allow these risks to be identified at an earlier age than previous studies which have primarily focused on adults or the elderly, potentially resulting in a lifetime risk reduction. In addition, our results will inform clinical and behavioral interventions, advance the development of environmental policy, as well as influence the direction of future research.

2.1 A Brief History of Air Pollution in the United States

In 1948, the small industrial town of Donora, Pennsylvania experienced an air inversion that resulted in a dense covering of smog that lasted for several days and caused nearly half of the town's 14,000 residents to experience respiratory and cardiovascular problems (EPA 2014a). Although this situation was extreme, it reflected a general trend in the US: air pollution was an unintended consequence of industrial growth. As these crises became more widely publicized, the public began to demand legislative action. In 1955, the US government passed the Air Pollution Control Act of 1955, which provided federal funds for air pollution research and enabled scientists to begin investigating links between air pollutants and health (EPA 2015). Individual states also began to pass legislation aimed at reducing air pollution.

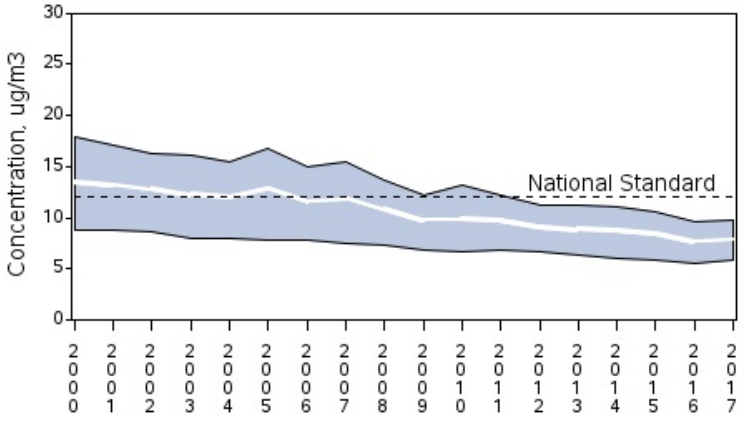
In 1970, the Clean Air Act (CAA) was enacted. This landmark legislation authorized the development of comprehensive federal and state regulations to limit air pollution emissions from stationary and mobile sources and also initiated National Ambient Air Quality Standards (NAAQS) for six criteria air pollutants (Table 2.1), including carbon monoxide (CO), lead (Pb), nitrogen dioxide (NO_2), ozone (O_3), particle pollution (PM_{10} and $PM_{2.5}$), and sulfur dioxide (SO_2). Primary standards were intended to protect public health, including protections for sensitive subpopulations such as children and the elderly (EPA 2014b). Secondary standards were meant to protect public welfare, including protections against decreased visibility and damage to vegetation. The US Environmental Protection Agency (EPA) was established in 1970 in part to implement requirements of the CAA. To date, the CAA has been amended twice (in 1977 and 1990) in order to increase enforcement authority, expand research programs, and modify and

expand the NAAQS. Enforcement of these laws and regulations has resulted in a major reduction in air pollution levels over time. For example, annual average $PM_{2.5}$ levels in the US decreased by approximately 41% from 2000 to 2017 (Figure 1) (EPA 2016).

Table 1. Current National Ambient Air Quality Standards (EPA 2014b)

Pollutant	**Primary Standards**	**Secondary Standards**
CO	9 ppm (1 hr) 35 ppm (8 hr)	n/a
Pb	0.15 µg/m^3 (rolling 3 month average)	Same as primary
NO_2	100 ppb (1 hr) 53 ppb (annual)	53 ppb (annual)
O_3	0.070 ppb (8 hr)	Same as primary
PM_{10}	150 µg/m^3 (24 hr)	Same as primary
$PM_{2.5}$	35 µg/m^3 (24 hr) 12 µg/m^3 (annual)	35 µg/m^3 (24 hr) 15.0 µg/m^3 (annual)
SO_2	75 ppb (1 hr)	0.5 ppm (3 hr)

Figure 1. Trends in Annual Average $PM_{2.5}$ Air Quality in the US, 2000-2017 (EPA 2016)



2.1.1 Overview of Criteria Air Pollutants

Carbon monoxide is a colorless, odorless gas that is primarily released into the air during the combustion of fossil fuels (EPA 2016a). The greatest sources of CO in outdoor air are cars, trucks and other vehicles and machinery. Lead is a naturally occurring element that is used in a variety of household products, including paint, ceramics, pipes, plumbing materials, batteries and cosmetics (EPA 2013). It may enter the environment through these sources, and it is also emitted from industrial sources. Certain activities like mining and smelting may cause substantial increases in lead levels. When released into the air, lead can travel long distances. NO_2 is part of a group of highly reactive gases known as nitrogen oxides (NO_x) (EPA 2016b). It is primarily emitted into the air from the burning of fuel, and major sources include emissions from vehicles, power plants and off-road equipment. NO_2 and NO_x react with other chemicals in the air to form particulate matter (PM) and ozone. Ground-level ozone is not emitted directly into the air, but is created by chemical reactions that occur between NOx and volatile organic compounds (VOCs) in the presence of heat and sunlight (EPA 2015). O_3 can be transported long distances by the wind. SO_2 is an indicator of a larger group of gaseous sulfur dioxides (SO_x) (EPA 2016c). Major sources of SO_2 emissions are from the combustion of fossil fuel at power plants and other industrial facilities.

Particulate air pollution is a complex mixture of solid and liquid particles that vary in size, origin and composition that are suspended in the air (Pope 2000). Coarse particles, often referred to as PM_{10} (or particles with aerodynamic diameter <10 μm), tend to be naturally occurring and are primarily derived from dust, sand and soil. Fine particles ($PM_{2.5}$ or particles with aerodynamic diameter <2.5 μm) are mainly derived from the combustion

of fossil fuels in processes such as manufacturing, power generation and transportation, and may include metals, acids, sulfates, nitrates and various carbon molecules. Evidence from physiologic and toxicologic studies indicates that the size fraction of $PM_{2.5}$ allows these particles to be inhaled deeply into the lungs. Fine particles also more readily penetrate indoor spaces and may be transported over longer distances. Carbon that is present in particulate matter is typically classified into three basic forms: carbonate carbon, organic compounds and elemental carbon (EC) (Schauer 2003). EC is operationally defined as the carbon that is neither carbonate or organic carbon. EC is often used as a surrogate measure of diesel particulate matter in occupational exposure investigations, and more recently, in environmental health and air quality studies.

2.2.1 Determinants of Exposure to Ambient Air Pollution

Many factors contribute to population-level exposures to ambient air pollution. Concentrations of air pollutants vary based on emission rates, weather patterns – including air temperature, humidity, wind speed, and frequency of precipitation – as well as seasonal temperature and solar radiation cycles (Brook et al. 2010; Jhun et al. 2015). Important temporal aspects include atmospheric formation and decay rates. Among the criteria air pollutants, $PM_{2.5}$ and O_3 have the longest atmospheric lifetime and their concentrations can gradually increase over the course of several days, in addition to spreading geographically with wind patterns. There are substantial differences in the characteristics of pollution episodes within different regions of the US.

Personal microenvironments may also considerably affect air pollution exposures, including factors such as time spent in traffic, residing near major roadways, exercising

outdoors, indoor sources such as environmental tobacco smoke, occupational exposure and the degree of indoor penetration of air pollutants into residences (Brook et al. 2010; Mueller et al. 2011; Hankey et al. 2017). The vast majority of epidemiological studies use exposure assessment techniques such as land-use regression modeling, spatio-temporal modeling or proximity to the nearest air pollution monitor to estimate individual-level exposures – often at the place of residence – rather than using monitoring devices to quantify personal exposure, which would account for individual time-activity patterns. However, the use of personal monitoring devices is often time and cost prohibitive, particularly in larger studies.

2.2 Cardiovascular Health Effects Associated with Ambient Air Pollution

Although extreme events like the 1948 Donora Smog provided ample evidence that exposure to air pollution caused adverse cardiopulmonary health effects, many epidemiologic reports have indicated that exposure to lower levels of ambient air pollution below current standards – especially particulate pollution – is associated with increased cardiovascular morbidity and mortality. Since the early 2000s, PM exposure has been specifically associated with cardiac events, including arrhythmia, heart failure and myocardial infarction (MI), as well as increases in cardiovascular mortality, cardiovascular hospital admissions and emergency department visits, and decreased life expectancy (Schwartz 1999; Zanobetti et al. 2000; Pope 2000, Samet et al. 2000; Wellenius et al. 2005; Pun et al. 2017; Atkinson et al. 2014; Pope et al. 2015; Dominici et al. 2006). Several reports have also indicated that PM can adversely affect plasma viscosity, heart rate (HR), cardiac rhythm and biomarkers of systemic inflammation (Pope et al. 2000; Dominici et

al. 2006; Brook et al. 2010). Acute studies indicate that for each increase of 5 $\mu g/m^3$ in $PM_{2.5}$, there is a 1.3% increase in cardiovascular mortality (Pope 2000). Evidence from longitudinal cohort studies demonstrates a 10% increase in all-cause mortality per 10 $\mu g/m^3$ elevation in long-term average $PM_{2.5}$ exposure, while the specific CVD-related mortality risk ranges from 3-76% (Brook et al. 2010).

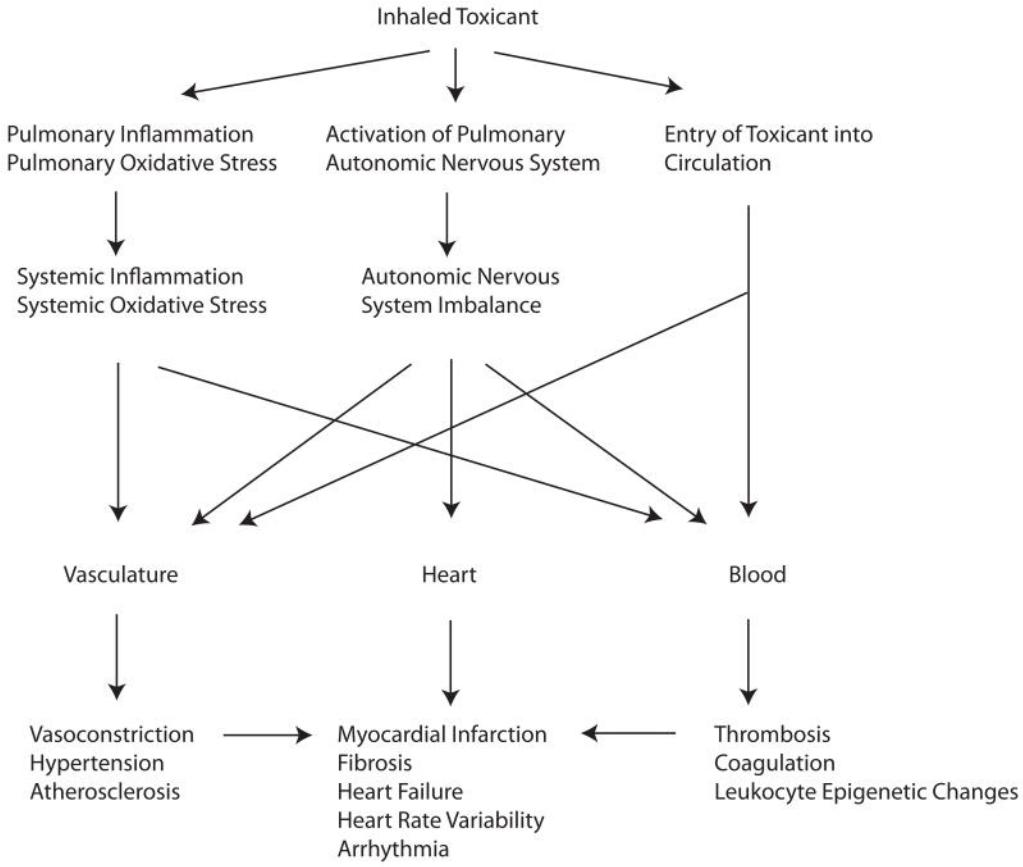
Evidence of relationships between other traffic-related air pollutants and cardiovascular morbidity and mortality is more limited. Some studies have also suggested that CVD-related deaths are associated with NO_2, SO_2, O_3 and CO exposures (Samoli et al. 2006; Samoli et al. 2007; Pope et al. 2002; Yin et al. 2017; Bero Bedada et al. 2016; Cakmak et al. 2016). In addition, short-term exposures to NO_2 have been linked with increased cardiovascular hospital admissions and higher risk of death due to MI, arrhythmia, atrial fibrillation and heart failure (Chiusolo et al. 2011; Milojevic et al. 2015; Collart et al. 2018; Wellenius et al. 2005). Acute exposure to O_3 air pollution has additionally been associated with changes in platelet activation, which is a risk factor for clotting, and elevated blood pressure, which increases the overall risk of CVD (Day et al. 2017).

2.2.1 Biological Mechanisms

Many epidemiologic reports have demonstrated associations between short and long-term exposure to ambient air pollutants and increased cardiovascular morbidity and mortality, with the strongest evidence reported for PM exposures (Utell et al. 2002; Brook et al. 2010; Fiordelisi et al. 2017). To date, several biological mechanisms which could be

responsible for air pollution-dependent adverse cardiovascular outcomes have been identified (Figure 2).

Figure 2. Effects of Air Pollution on the Development of Cardiovascular Diseases (Chin 2017)



The primary hypothesis, which is best supported in the existing literature, suggests that PM entering the lungs provokes a local inflammatory response, which in turn promotes oxidative stress and leads to systemic oxidative stress and inflammation (Chin 2017; Brook et al. 2010). The resulting pro-inflammatory state is then thought to promote multiple pathological processes related to the development of CVD, including increased thrombosis,

hypercoagulability, endothelial dysfunction and the progression of atherosclerosis (Chin 2017; Fiordelisi et al. 2017; Utell et al. 2002; Brook et al. 2010). In particular, it has become clear that atherosclerosis is an inflammatory disease and development of the arterial plaque involves the classic mediators of inflammation (Utell et al. 2002).

Another prominent hypothesis implicates autonomic nervous system (ANS) imbalance following pulmonary PM exposure, which then leads to pathological alterations in vasoconstriction, endothelial dysfunction, hypertension, platelet aggregation, tachycardia, increased heart rate variability and increased potential for arrhythmias (Chin 2017; Utell et al. 2002; Brook et al. 2010). In addition, PM inhaled through the lungs may directly enter the circulatory system where it interacts with receptors in various tissues to promote vasoconstriction, endothelial dysfunction, atherosclerosis, hypertension, platelet aggregation, systemic oxidative stress and inflammation.

Although several biologically plausible mechanisms underlying associations between ambient air pollution exposures and adverse cardiovascular outcomes have been suggested, this area requires further investigation. Current knowledge remains limited and the majority of existing work has specifically focused on PM. Additional studies are needed to more fully elucidate the pathophysiologic pathways between air pollution exposure and CVD.

2.2.2 Diabetes as a Susceptibility Factor

Individuals with diabetes may be particularly susceptible to the cardiovascular health effects of traffic-related air pollution. Zanobetti and Schwartz (2002) observed that patients with diabetes had twice the risk of a PM_{10}-associated cardiovascular hospital

admission compared with non-diabetics. In another study, 2-day moving average SO_2 exposure was associated with a 20.0% increase (95% CI: 5.0, 44.0) in CVD-related emergency department visits among individuals with diabetes compared to a 7.0% increase (95% CI: 4.0, 11.0) among non-diabetic individuals (Pereira Filho et al. 2008). In addition, Pinault and colleagues (2018) reported that co-mention of diabetes on a death certificate increased the magnitude of association between CVD-related mortality and $PM_{2.5}$ exposure (hazard ratio [HR] =1.51; 95% CI: 1.39, 1.65) per 10 $\mu g/m^3$ increase. In that study, long-term exposure to $PM_{2.5}$ was also associated with an increased risk of diabetes deaths (HR=1.49; 95% CI: 1.38, 1.60).

The exact biological mechanisms underlying these relationships remain unclear. Certain dimensions of cardiovascular vulnerability, including increased systemic inflammation and reduced heart rate variability are affected by both ambient air pollution and diabetes (Pope et al. 1999; Vora et al. 2014; Benichou et al. 2018; Lontchi-Yimagou et al. 2013). Additional research is indicated given that diabetes is a highly prevalent chronic illness and exposure to traffic-related air pollution is ubiquitous; thus, the resulting public health implications of these associations are considerable. In addition, very little existing research has focused specifically on individuals with T1D.

2.3 Overview of Type 1 Diabetes

T1D is a lifelong, chronic condition characterized by defective insulin production that is thought to be precipitated by an immune-associated or immune-mediated destruction of insulin-producing pancreatic β-cells (Atkinson et al. 2014; Regnell and Lernmark 2017). This gradually leads to diminishing insulin production and subsequent loss of blood sugar

control. Disease presentation has three classic symptoms: frequent urination, increased thirst and increased appetite. Although T1D can be diagnosed at any age, it is typically diagnosed during childhood and is slightly more common among males than females (Atkinson et al. 2014). The causes of and typology of T1D remain unclear, although many patients show features of an immunological contribution to disease pathogenesis, such as the development of autoantibodies. Genetic predisposition and environmental influences are thought to play a role in the disease etiology (Atkinson et al. 2014; Regnell and Lernmark 2017). Management of T1D typically involves exogenous insulin replacement and frequent monitoring of blood glucose levels.

Recent data indicate that the annual incidence rate of T1D is approximately 34.4 per 100,000 persons among US children and young adults 0-19 years of age (Rogers et al. 2017). Moreover, the incidence rate of T1D in this age group increased by 1.9% annually from 2001 to 2015, with the number of new cases estimated at 64,000 per year. Health care costs associated with T1D in the US have been estimated to be between $14.4-14.9 billion annually (Atkinson et al. 2014).

2.3.1 Type 1 Diabetes and Cardiovascular Disease

Individuals with T1D have a high lifetime risk of developing CVD and CVD-related complications (Atkinson et al. 2014). For example, the risk of adverse cardiovascular events is estimated to be 10 times higher among type 1 diabetics than age-matched non-diabetic populations. The main reason for this increased CVD risk is that individuals with diabetes are more likely to have conditions which increase the risk of CVD, such as hypertension, abnormal cholesterol levels, high triglycerides, obesity, lack

of physical activity, and poorly controlled blood glucose (AHA 2015). Managing or controlling these risk factors may help patients with T1D avoid or delay the development of heart and blood vessel disease.

The overall risk of developing CVD for individuals with T1D compared to those without diabetes is two to three times higher in men and three to five times higher in women (Schnell et al. 2013). The presence of CVD in patients with T1D also largely impairs life expectancy. CVD is the leading cause of premature death among young adults with diabetes, and the risk of CVD-related mortality prior to age 40 years is increased up to 20-fold in individuals with T1D compared to non-diabetics (Dahl-Jorgensen et al 2005; Skrivarhaug et al. 2006; Laing et al 2003).

Among individuals with type 1 diabetes, the increased risk for CVD begins in childhood, although major cardiovascular events do not typically manifest at a young age (Gourgari et al. 2017). Children and young adults with T1D show evidence of subclinical CVD early in life, which may present as increased carotid intima-thickness, increased arterial stiffness, and endothelial and myocardial dysfunction.

2.3.2 The SEARCH for Diabetes in Youth Study

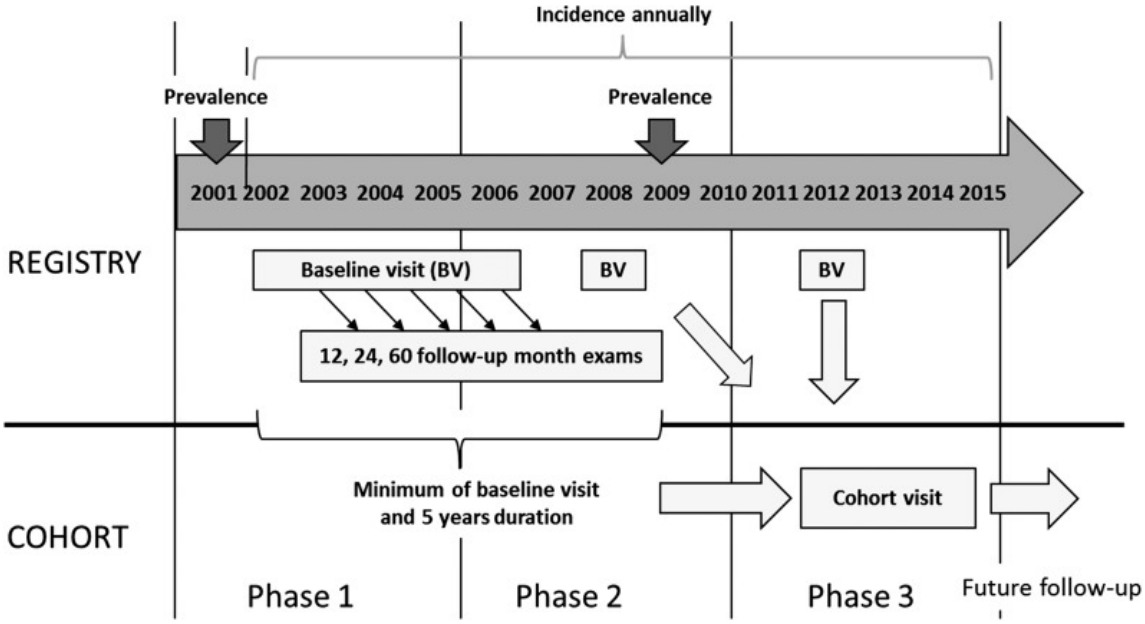
SEARCH for Diabetes in Youth (SEARCH) is a national multi-center study aimed at understanding more about diabetes among US children and young adults (Hamman et al. 2014). SEARCH is funded by the Centers for Disease Control and Prevention (CDC) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The study was initiated in 2000 and will continue through at least 2020. SEARCH centers are currently located in five states, including: South Carolina (statewide), Ohio (eight counties

surrounding Cincinnati, Ohio including Butler, Clermont, Hamilton, Warren counties in Ohio, Boone, Campbell, and Kenton counties in Kentucky, and Dearborn, Indiana), Colorado (statewide), Washington (five counties surrounding Seattle, King, Kitsap, Pierce, Snohomish and Thurston) and health plan membership in five counties of Southern California (Los Angeles, Orange, Riverside, San Bernardino, Ventura, Imperial, Kern). The first two phases of recruitment (2000-2010) also included a study center located in Hawaii.

SEARCH has two main components: a registry and a cohort study (Figure 3). The registry study has identified incident cases of diabetes annually each year from 2002-present and includes approximately 5.5 million children and young adults diagnosed with diabetes prior to the age of 20 years. Using Health Insurance Portability and Accountability Act (HIPAA) waivers of consent, centers conduct active surveillance using networks of health care providers, hospitals, community health centers, electronic medical records, as well as clinical and administrative data systems. Case validation is confirmed through medical record review or by contacting the referring physician, and eligibility is determined based on age, residence, non-military, non-institutionalized, and health plan membership at diagnosis. Duplicate entries are removed at local centers and then cases are registered anonymously with the SEARCH Coordinating Center located at Wake Forest University in North Carolina. Case ascertainment is estimated to be >90% for both T1D and T2D. Youth diagnosed in 2002-2006, 2008 and 2012 had a baseline in-person visit that included questionnaires on demographics and medical history, dietary intake and physical activity patterns, as well as a brief clinical exam. The active registry component allows for the assessment of prevalence (in 2001 and 2009), annual incidence (since 2002), as well as

trends by age, race/ethnicity, sex and diabetes type. The SEARCH registry population is similar to the US youth population with regard to age, race/ethnicity, household income and parental education level.

Figure 3. Summary of the SEARCH study design (Hamman et al. 2014)



The SEARCH cohort component was developed by recruiting incident cases in 2002-2006, 2008 and 2012 that had a baseline visit near the date of diagnosis and a minimum of five years of diabetes duration at the cohort visit assessment in 2012-2015. The cohort data are used to estimate prevalence and incidence of acute and chronic diabetes-related complications. In addition, a repository of biological specimens has been developed and is available for use in ancillary studies. For this research, data were obtained from two ancillary studies of SEARCH: SEARCH CVD and SEARCH Air.

2.3.3 SEARCH CVD

SEARCH CVD is an ancillary study of SEARCH that was conducted in two of the six SEARCH sites, Colorado and Ohio (Urbina et al. 2010). Participants were eligible for SEARCH CVD if they had physician-diagnosed T1D and completed a baseline SEARCH visit between 2004 and 2005 when they were 10 years of age or older. A follow-up in-clinic visit was conducted in 2009-2011 to assess cardiovascular structure and function, which included measures of arterial stiffness. The SEARCH CVD visit also included administration of questionnaires to obtain updated information on medical history, family history, and health behaviors such as smoking and physical activity.

2.3.4 SEARCH Air

SEARCH Air is an ancillary study that was funded by the National Institute for Environmental Health Sciences (NIEHS) to explore associations between air pollution exposures and premature CVD risk factors among youth with T1D using existing data from the SEARCH study (PI: Puett, 5R01ES019168-03). Five SEARCH sites were included in SEARCH Air: South Carolina, Ohio, Colorado, California and Washington. The Hawaii site was not included primarily due to the uniqueness of air pollution resulting from volcanic emissions. Additionally, the number of participants in the Hawaii SEARCH site was too small to allow for site-specific analyses.

Eligible participants had physician-diagnosed T1D (prevalent cases in 2001 and incident cases from 2002 to 2005) and had completed a baseline in-person visit. Additional data were obtained from 12, 24, and 60 month follow-up visits to allow for longitudinal (e.g., repeat measures) investigations. Funding from SEARCH Air allowed for the analysis

of plasma samples from the SEARCH repository, increasing the number of participants with available data for the study by approximately 1600. SEARCH Air also provided funding for the development of detailed air pollution exposure models.

2.3.5 SEARCH Air Exposure Modeling

Spatio-temporal generalized additive mixed models (GAMMs) were developed to estimate 24-hour average $PM_{2.5}$ and EC levels across the conterminous US from 1999 to 2011 with high resolution (Yanosky et al. 2014; Yanosky et al. 2018). In addition, Atmospheric Dispersion Modeling System (ADMS-)Roads v2.3 software (CERC, Cambridge, UK) was used to approximate micro-, middle- and neighborhood-scale primary traffic-related PM levels (Yanosky et al. 2018). ADMS-Roads uses a line-source Gaussian plume dispersion model that allows for advanced treatment of atmospheric stability.

Inputs for the exposure models included 24-hour average $PM_{2.5}$ measurements from the US EPA's Air Quality System (AQS), the Interagency Monitoring of Protected Visual Environments (IMPROVE) network and the Southern Aerosol Research and Characterization Study network. Measurements of EC were from the AQS and IMPROVE networks. Meteorological inputs were obtained from the MERRA project (Rienecker et al. 2011) and included air temperature, total precipitation, total snowfall, wind speed and direction, sensible heat flux and planetary boundary layer height. Annual-average daily traffic counts were obtained from Geographic Data Technology, Inc. (Lebanon, NH) Dynamap Traffic Counts v4.2 and spatially joined to the ESRI StreetMap Pro 2007 network of road segments to obtain the US Census Feature Class Code (US Census Bureau

2013) road class of each road segment. These data were then spatially smoothed for each individual road class (A1 [primary highway with limited access], A2 [primary road without limited access], A3 [secondary and connecting roads], A4 [local, neighborhood and rural roads] and A6 [roads with special characteristics, such as cul-de-sacs or traffic circles]). Additionally, data on county-level population density from the 2000 US Census (US Census Bureau 2013) were obtained from ESRI Data and Maps v10.1, and elevation data originated from the USGS National Elevation Dataset (USGS 2013).

The spatio-temporal GAMMs were of the following generic form (Yanosky et al. 2014):

$$y_{i,t} = \alpha + \alpha_t + \sum_q d_q(X_{i,q}) + \sum_p f_p(Z_{i,t,p}) + g_t(s_i) + g(s_i) + b_i + e_{i,t};$$

$$b_i \sim N(0, \sigma^2{}_b);\ e_{i,t} \sim N(0, \sigma^2{}_{e\,t})$$

where $y_{i,t}$ are monitor measurements for i=1…I sites and t=1…T 24-hr time periods, s_i is the projected spatial coordinate pair for the ith location. $X_{i,q}$ are GIS-based time-invariant spatial covariates for q=1…Q, $Z_{i,t,p}$ are spatio-temporal covariates (including ADMS-Roads traffic-related PM levels) for p=1…P, and α_t is an intercept representing the adjusted mean across all sites on a given day. $d_q$are penalized spline smooth functions of Q GIS-based time-invariant spatial covariates, f_p are penalized spline smooth functions of P spatio-temporal covariates $g_t(s_i)$ accounts for residual spatial variability in the 24-hr average values, and $g(s_i)$ accounts for time-invariant spatial variability across the conterminous US. The site-specific random effect b_i represents unexplained site-specific variability. Predictive accuracy of the spatio-temporal model was moderate for 24-hour averages and higher for longer averaging times (CV R^2 = 0.532, 0.707 and 0.795 for daily, monthly and annual averages, respectively).

2.4 Measures of Subclinical Cardiovascular Disease

Children and youth with T1D have an increased risk of CVD throughout their lifetime (Atkinson et al. 2014; Gourgari et al. 2017). Individuals with diabetes may also be a susceptible subpopulation with regard to the cardiovascular effects of air pollution on CVD. Although overt CVD rarely presents during childhood, subclinical effects such as increased arterial stiffness and systemic pro-inflammatory responses can be assessed from an early age. More research addressing modifiable risk factors early in the progression of CVD is needed and may decrease future CVD-related morbidity and mortality.

2.4.1 Arterial Stiffness

Arterial stiffness refers to the reduced capacity of an artery to expand and contract in response to blood pressure changes (Cecelja and Chowienczyk 2012). Stiffer arteries require greater force to expand and accommodate blood flow, which can lead to an increased workload for the heart (Savant et al. 2014). Stiffening occurs due to changes in structural and cellular components of the vessel wall through a complex interaction of factors (e.g. collagen, elastin, sodium intake, glucose regulation, etc.) and has multiple contributing factors, such as systemic inflammation, endothelial dysfunction and malnutrition. Arterial stiffness is a natural consequence of the aging process, and it may also be accelerated by certain chronic health conditions such as diabetes. Increased arterial stiffness has been demonstrated to be an independent risk factor for the development of CVD (Savant et al. 2014).

There are several different indices of arterial stiffness, including augmentation index (AIx), brachial artery distensibility (BrachD), and pulse wave velocity (PWV). AIx

is defined as the proportion of the central (i.e., aortic) pulse pressure that is due to arterial wave reflection (Van Trijp et al 2006). It provides information on the workload of the heart and reflects systemic arterial stiffness. A higher AIx indicates increased stiffness. AIx is typically derived non-invasively via applanation tonometry of the radial artery in which a small probe is used to capture arterial pressure waves that are subsequently analyzed to calculate a central aortic pressure wave and calibrated with a blood pressure measurement from the brachial artery (Urbina et al. 2010; Van Trijp et al. 2006). AIx is an indicator of cardiovascular risk and has been linked with the development of coronary heart disease among adults (Van Trijp et al. 2016).

BrachD reflects compliance of the brachial artery, which is located in the upper arm, and is considered to be a measure of peripheral arterial stiffness (Urbina et al. 2010). Distensibility typically decreases with age, and a lower BrachD indicates increased arterial stiffness. It is a non-invasive measure that is captured using a hemodynamic monitoring instrument that conducts waveform analysis of arterial pressure signs obtained from a standard blood pressure cuff (Urbina et al. 2010). In adults, BrachD is an independent risk factor for the development of CVD (Budoff et al. 2003).

PWV refers to the speed of the pressure pulse made by the heart as it circulates through the blood vessels, and may be calculated by dividing the distance travelled (e.g., from the carotid to femoral arteries) by the amount of time that it takes to travel that distance (Savant et al. 2014). Stiffer blood vessels result in a faster travel time, and therefore, a higher PWV measurement. Carotid-femoral PWV is considered to be the gold standard method for assessment of central arterial stiffness, although PWV may also be assessed in other areas of the body, such as brachial-ankle PWV. It is typically measured

non-invasively via applanation tonometry, in which a pencil-type probe that captures arterial pressure waves is applied to the skin, although other methods have also been validated. PWV is a powerful independent predictor of cardiovascular risk among individuals with diabetes and the general population (Cruickshank et al. 2002).

In sum, these measures of arterial stiffness are markers of sub-clinical CVD as well as predictors of future cardiovascular events, although there is less direct evidence of the latter among children as compared to general adult populations. Identifying strategies that reduce or prevent arterial stiffness could be important in the prevention of CVD, particularly among individuals who are at higher risk for developing CVD over their lifetime, such as youth with T1D. In the research that follows, we will focus on PWV as the main measure of arterial stiffness in our analyses; however, we have also included AIx and BrachD in the literature review due to the limited number of studies that have been conducted.

2.4.2 Arterial Stiffness and Traffic-Related Air Pollution

To date, a limited number of epidemiological studies have examined whether indicators of arterial stiffness are associated with short-term exposures to traffic-related air pollutants. The majority have specifically focused on exposure to fine particulates. A recent analysis using data from the Framingham Heart Study (n=5842, mean age 51 years) found that living in close proximity to a major roadway was associated with higher stiffness (0.11 m/s higher carotid-femoral PWV [95% CI: 0.01, 0.22]); however, short-term exposures to $PM_{2.5}$ and black carbon (BC) were not associated with increased PWV (Ljungman et al. 2018). In a longitudinal, repeated measured analysis, Mehta et al. (2014) investigated the

association between acute changes in $PM_{2.5}$ and AIx in a community-based cohort of elderly men (mean age 78 years) in the Boston, Massachusetts, metropolitan area (n=445). An interquartile range (IQR) increase in 3-day average exposure was associated with a 0.8% higher AIx (95% confidence interval (CI): 0.2, 1.4) for a 3.6 $\mu g/m^3$ increase in $PM_{2.5}$. Associations averaged over 7 and 14 days were of similar magnitudes. Another study conducted in Shanghai, China, which included 371 participants aged 45-75 years, investigated the association between exposure to $PM_{2.5}$ during typical work hours and AIx (Jiang et al. 2016). Individual exposure to $PM_{2.5}$ was measured from 8:00 AM to 6:00 PM on one calendar day using a personal monitoring device (SidePakTM AM510). Participants were divided into exposure categories based upon residential distance to the nearest major road (≤50, 51-100, 101-200 and >200 m). The authors reported that subjects living within 50 m of a major road had AIx values that were 4.30 times higher than participants residing 200 m or more away. In addition, a panel study of 89 healthy adults (mean age 43.7 years) in Taipei, Taiwan evaluated whether acute exposures to $PM_{2.5}$ were associated with brachial-ankle PWV (Wu et al. 2016). In this study, a 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentrations at a 0-day lag was significantly associated with a 2.1% (95% CI: 0.7, 3.6) increase in PWV. A similar association was also reported at a 1-day lag (2.1%, 95% CI: 0.5, 3.6). One other study assessed the association between 24-hour personal household exposure to $PM_{2.5}$ and arterial stiffness among 25 women (mean age 59 years, range 38-85) living in rural Sichuan, China (Shan et al. 2014). Participants were divided into two exposure groups (high versus low). Women in the high exposure group had increased AIx values compared to lesser exposed women, although the differences were not statistically

significant (e.g., 2.8% increase, 95% CI: -1.6, 7.2). Exposure to $PM_{2.5}$ was not associated with carotid-femoral PWV (e.g., -0.1% decrease, 95% CI: -0.9, 0.7).

Similar studies have focused on acute exposures to other traffic-related air pollutants. In a randomized double-blind crossover study, Lundback et al. (2009) investigated the effect of short-term diesel exhaust exposure on arterial compliance. Twelve participants (mean age 26 years, range 21-30) were exposed to diluted diesel fumes at a particulate concentration of 350 $\mu g/m^3$ or filtered air for one hour during moderate exercise on two separate occasions at least one week apart. AIx was measured 10, 20, and 30 minutes after exposure, and carotid-femoral PWV was assessed 40 minutes post-exposure. AIx increased immediately after diesel exhaust exposure compared to filtered air and then normalized after 30 minutes (-7.0% ± 2.3% [mean ± SEM] for air versus 0.8% ± 3.2% for diesel exhaust at 10 minutes post-exposure, -7.4% ± 2.6% versus -3.8% ± 3.5% at 20 minutes post-exposure, and -7.9% ± 2.2% versus -5.8% ± 2.7% at 30 minutes post-exposure). However, PWV was not significantly different between exposures (5.1 ± 0.2 m/s versus 4.6 ± 0.1 m/s). In addition, another study investigated the effect of short-term exposure to coarse particulate matter (PM_{10} or particles with aerodynamic diameter <10 μm) on arterial stiffness (Adamopolous et al. 2010). In a large, cross-sectional study of 1222 adults (mean age 51 years) who sought medical consultation in hypertension clinics affiliated with two hospitals in Athens, Greece, the authors reported a non-significant 0.5% increase in AIx (95% CI: -1.34, 2.47) corresponding to mean PM_{10} levels (43.4 μg/m3) over a 5-day period. Provost et al. (2016) also investigated associations between PWV and personal short-term exposure to BC among 54 nurses (mean age 40.7 years) from two hospitals in Belgium. Increases in BC exposure 1 to 8 hours prior to the assessment of

arterial stiffness were significantly associated with increased PWV, ranging from 0.51% (95% CI: 0.19, 0.83) to 1.18% (95% CI: 0.51, 1.88).

Comparable studies in youth and children have focused on long-term air pollution exposures. A cross-sectional study conducted among healthy children (n=52) in a coastal Italian town with only one highly trafficked road reported a statistically significant difference in carotid arterial stiffness between children living closer to the main street and other children, both for those living between 330–730 m (mean: 3.26 [standard error (SE): 0.35]) and more than 750 m (mean: 3.32 [SE: 0.33]) from the main street (Iannuzzi et al. 2010). Exposure was assessed over a 1-year time span. Lenters et al. (2010) investigated the association between exposure to ambient and traffic-related air pollution and early vascular damage among a cohort of heathy young adults; outcomes included aortic PWV (n=524) and AIx (n=729). A 5 $\mu g/m^3$ contrast in $PM_{2.5}$ was associated with increases of 0.65% (95% CI: -4.71, 6.01) and 10.17% (95% CI: -37.82, 58.17) in PWV and AIx, respectively, after full adjustment for covariates. Neither association reached the level of statistical significance. Adjusted associations between traffic indicators and vascular damage outcomes were inconsistent in the direction of effect. For example, living near a major road was negatively associated with PWV and AIx (% change -1.33 [95% CI: -5.52, 2.85]; % change -6.82 [95% CI: 43.61, 29.98], respectively) while traffic intensity on the nearest major road equal to 140,000 motor vehicles in 24 hours was associated with weak positive associations in these measures (% change in PWV 2.04 (95% CI: -3.37, 7.45); % change in AIx 11.59 (95% CI: -36.66, 59.85).

The majority of prior studies have been conducted among adults and the elderly. Although some research has been conducted in healthy children, we were unable to identify

existing studies that focused on the association between traffic related air pollution exposures and measures of arterial stiffness among youth with T1D. Children and young adults with diabetes are expected to be at significantly higher risk for the CVD-related effects of air pollution than their non-diabetic counterparts. A prior analysis of SEARCH data indicated increased arterial stiffness in youth with T1D (n=535), including lower BrachD (% change/mmHg 6.11 [SD 1.2] vs. 7.0 [SD 1.2]) and higher AIx (2.07% [SD 10.9] vs. -0.52% [SD 10.8]) measurements compared to healthy control subjects (n=241) even after adjusting for traditional cardiovascular risk factors such as hypertension and obesity (Urbina et al. 2010). These findings provide additional support for investigating the determinants of arterial stiffness among youth with T1D.

2.4.3 Inflammatory Biomarkers

Ambient air pollution is associated with CVD and potential biologic mechanisms underlying this relationship include systemic inflammatory responses (Brook et al. 2010; Utell et al. 2002; Chin 2015). Advances in vascular biology indicate that inflammation plays a central role in the development of CVD (Ridker 2004). Inflammation has been implicated to play a central role in every step of the atherosclerotic process, from initiation to progression and plaque rupture (Hackam and Anand 2003). In addition, inflammatory biomarkers may affect the coagulation cascade and cause pro-thrombotic changes (Ridker 2004). CRP, IL-6 and fibrinogen are inflammatory biomarkers that have been associated with cardiovascular risk in previous work (Hackam and Anand 2003; Ernst and Ludwig 1993).

IL-6 is a procoagulant cytokine that is produced at the site of inflammation where it acts as a central mediator of the acute-phase inflammatory response (Gabay 2006). It is a primary determinant of hepatic production of CRP, and may directly or indirectly impact the pathogenesis of atherosclerosis and hypertension (Sesso et al. 2007). Circulating IL-6 also stimulates the hypothalamic-pituitary-adrenal axis, which is associated with the development of hypertension when activated. In addition, IL-6 plays an important role in the transition from acute to chronic inflammation (Gabay 2006). IL-6 is also involved in cross-talk between systemic inflammation and the coagulation protease cascade (Ridker 2004). Levels of IL-6 predict future vascular risk even in apparently healthy populations and have also been shown to correlate with endothelial dysfunction, arterial stiffness and sub-clinical atherosclerosis (Ridker 2016).

C-reactive protein is a circulating acute-phase protein that is primarily synthesized in the liver, which in turn can lead to the release of glycoproteins that impact coagulation (Hackam and Anand 2003; Bind et al. 2012; Ridker 2004). CRP levels are increased during an inflammatory response, and its release is stimulated by IL-6 as well as other pro-inflammatory cytokines. In addition, CRP may play an active role in proinflammatory and prothrombotic effects (Ridker 2004). Even small increases in CRP within the normal range seem to be predictive of future vascular events in healthy individuals and patients with pre-existing vascular disease; however, it remains unclear whether CRP is a marker or mediator of inflammation. In clinical settings, CRP is often used as a marker of risk for atherothrombosis and vascular dysfunction (Ridker 2016).

Fibrinogen is a plasma glycoprotein produced in the liver that is essential to the clotting cascade, where its conversion to fibrin stabilizes blood clots after injuries (Bind et

al. 2012). Fibrinogen has pro-coagulant and pro-inflammatory properties, and it is thought to promote atherosclerosis and thrombosis. A growing body of evidence supports a prominent role for fibrinogen in regulating the inflammatory response in several target tissues (Davalos and Akassoglou 2012). For example, increased circulating blood levels of fibrinogen is indicator for developing vascular inflammatory diseases such as hypertension and atherosclerosis. Numerous studies have linked fibrinogen with CVD, dating back to the 1950s when levels of fibrinogen were shown to be increased among patients with ischemic heart disease (Ernst and Ludwig 1993). Elevated fibrinogen levels are associated with an increased risk of incident cardiovascular events and with a broad category of cardiovascular deaths, even after adjustment for CVD risk factors (Wolberg 2016).

2.4.4 Inflammation and Traffic-Related Air Pollution

The literature is mixed regarding whether short-term (daily to weekly) exposure to ambient air pollutants is associated with biomarkers of systemic inflammation. To date, the majority of existing studies have been cross-sectional in nature, although several recent investigations have conducted longitudinal analyses. In a cohort study of 704 elderly men with repeat measurements of fibrinogen and CRP, exposure to $PM_{2.5}$ was associated with higher fibrinogen levels at 3, 7, 14, 21 and 28 day moving averages (Bind et al. 2012). However, these associations did not reach the level of statistical significance. $PM_{2.5}$ was also positively but non-significantly associated with CRP levels at a 3-day moving average. Hajat et al. (2015) conducted a longitudinal cohort analysis to assess the long and short-term associations between ambient air pollutants and markers of coagulation and inflammation using participants from the Multi-Ethnic Study of Atherosclerosis (MESA)

from 2000-2012 with repeat biomarker measurements (CRP n=6889; IL-6 n=6663; fibrinogen n=7037). A 5 μg/m^3 increase in $PM_{2.5}$ on the day of blood draw was suggestive of a positive association with CRP (1% difference [95% CI: 0, 3]) and fibrinogen (1.16 mg/dl [95% CI: −0.28, 2.61]). Reported associations with IL-6 were largely null (e.g., 0% difference [95% CI -1, 1] on the day of the blood draw). In a repeat measures analysis of 45 adults, Thompson and Colleagues (2010) found weak positive effects of $PM_{2.5}$ on IL-6 for an IQR increase in exposure on lag days 1-5, but none of the associations reached the level of statistical significance. Another longitudinal study conducted among 2,086 midlife women using data from the Study of Women's Health Across the Nation (SWAN) examined associations of short and long-term $PM_{2.5}$ exposures with several markers of cardiovascular risk (Green et al. 2016). $PM_{2.5}$ exposure 1 day prior to the clinic visit was positively but non-significantly associated with CRP (% change 0.1 [95% CI: -1.7, 1.9]) and fibrinogen (β x 10 1.4 [95% CI: -0.3, 3.1]) levels. Li et al. (2017) investigated the longitudinal effects of short-term exposure to ambient air pollution exposures on repeat measures of inflammation using data from the Framingham Heart Study (n=3996; mean age 53.6 years). The authors reported that 3-7 day moving averages of fine particulates were positively associated with CRP concentrations (e.g., 4.2% increase in CRP corresponding to a 5 μg/m^3 increase in $PM_{2.5}$ over a 5-day moving average). In addition, a 0.5 μg/m^3 higher 5-day moving average of BC was associated with 5.8% higher CRP levels (95% CI: 0.5, 11.4). However, BC was negatively associated with fibrinogen, and associations of $PM_{2.5}$ and BC with IL-6 were either null or weakly positive. Many of the reported associations were stronger among participants with diabetes. In addition, Wang et al. (2015) conducted a longitudinal panel study among 35 patients with diabetes (mean age

66 years) in Shanghai, China to explore associations between particulate matter and circulating biomarkers of inflammation. The authors reported increases in CRP levels corresponding with exposure to $PM_{2.5}$ at different time periods prior to exposure, including 2, 12, 24, and 48 hours. Positive associations were also indicated for fibrinogen at 2, 12 and 24 hours prior to $PM_{2.5}$ exposure.

Additional evidence from cross-sectional and panel studies indicates that individuals with diabetes may be more sensitive the effects of air pollution on markers of systemic inflammation. Dabass et al. (2016) examined the association of exposure to ambient air pollutants with CRP (n=16160) and fibrinogen (n=2461) levels among a representative sample of the US population using data from the National Health and Nutrition Examination Survey (NHANES) from 2001-2008. In the complete study group, none of the $PM_{2.5}$ lags (short or long-term) were significantly positively associated with any of the biomarkers considered. However, in subgroup analyses, participants with diabetes showed consistent increased responses across all biomarkers compared to participants without diabetes. For example, for every 10 $\mu g/m^3$ increase in annual average $PM_{2.5}$, there was a significant increase of 36.9% (95% CI: 0.1%, 87.2%) in CRP. In a panel study conducted among potentially susceptible populations, including individuals with diabetes (n=83) and impaired glucose intolerance (n=104), $PM_{2.5}$ was positively associated with fibrinogen (% change 0.8 [95% CI: 0.0, 1.6], $p < 0.1$) at a lag of 0-119 hours while CRP levels decreased over the same time period (e.g., % change -1.7 [95% CI: -8.1, 5.0) (Rückerl et al. 2014). Associations followed similar patterns for lags of 0-23, 24-47, 48-71, 72-95, and 96-119 hours. There was no consistent pattern for IL-6. At lags of 0-23 and 24-47 hours, IL-6 decreased in response to $PM_{2.5}$ (% change -2.1 [95% CI: -5.4, 1.4) and -

2.3 [95% CI: -5.8, 1.4], respectively); however, IL-6 increased in response to $PM_{2.5}$ exposure at lags of 48-71 (% change 0.7 [95% CI: -3.0, 4.6]), 72-95 (% change 3.3 [95% CI: -0.3,7.1]), 96-119 (% change 2.8 [95% CI: -0.7, 6.5]), and 0-119 hours (% change 0.9 [95% CI: -3.4, 5.3]). In addition, a cross-sectional study of youth with T1D (n=2566; average age 12.3 years) using SEARCH data indicated that EC over the week prior to the biomarker assessment was positively associated with IL-6 and CRP (Puett et al. 2018). Comparing the 25th and 75th percentiles of average week EC exposures resulted in 8.3% higher IL-6 (95% CI: 2.7, 14.3) and 9.8% higher CRP (95% CI: 2.4, 17.7). Some evidence of effect modification by gender and race/ethnicity was also reported. For example, higher CRP levels were positively associated with increased exposures to $PM_{2.5}$ on lag days 4-7 and the weekly average among females while a weak inverse relationship was observed among males (e.g., for lag day 7 comparing exposure in the 25th to the 75th percentile was associated with an 8.8% higher CRP level [95% CI: 1.0, 17.2] compared to a 6.2% lower level of CRP [95% CI: -12.9, 1.0]).

The existing literature also supports the role of air pollution on systemic inflammation among healthy children. A panel study conducted among young adults (n=76) from a university in Taipei, Taiwan reported increases in markers of inflammation after exposure to $PM_{2.5}$ (Chuang et al. 2007). More specifically, $PM_{2.5}$ was associated with increased CRP at lags of 1, 2 and 3 days (e.g., % change 90.2 [95% CI: -10.2, 190.1] for lag 1; similar effects were reported for the other lag days). Fibrinogen levels also increased in response to $PM_{2.5}$ exposure (e.g., % change 2.6 [95% CI: -2.7, 7.8] for lag 1, 1.5 [95% CI: -4.1, 7.1] for lag 2, and 3.6 [95% CI: -0.8, 8.1] for lag 3.) Another longitudinal study conducted among 52 healthy children (average age 8.6 years) from two different areas in

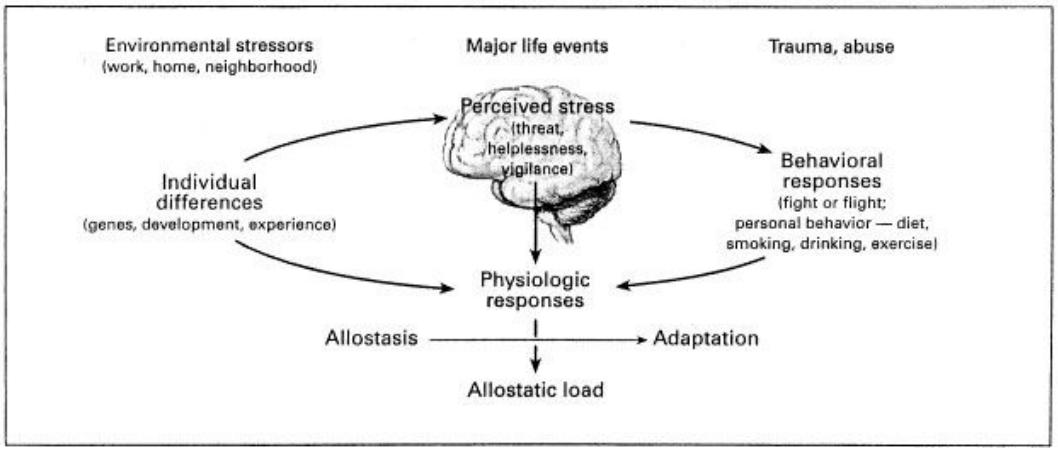
Mexico (n=28 from Mexico City, an urban area with chronically high levels of air pollution, and n=24 controls from Polotitlán, a smaller and less polluted city) reported that urban children who are exposed to $PM_{2.5}$ concentrations above the standards have systemic inflammation as supported by increased circulating levels of inflammatory markers, including CRP (833 ng/ml [SD 257] in Mexico City children vs. 227.8 ng/ml [SD 36] among controls) (Calderon-Garcidueñas et al. 2008).

Additional research is indicated given the variation in the strength and consistency of reported associations from prior analyses. The proposed study will also fill a major gap in the existing literature by examining the longitudinal effects of traffic-related air pollution on markers of systemic inflammation among youth with T1D.

2.5 Allostatic Load Defined

Allostatic load (AL) is a conceptualization of the cumulative biological burden exacted on the body through attempts to adapt to repeated or chronic stress over time (Seeman et al. 2001). Stress may result from major life events, psychosocial and environmental burden, as well as individual factors such as the presence of chronic disease and low physical activity levels (Mattei et al. 2010). Allostasis is a closely related concept which refers to "maintaining homeostasis through change", or more specifically, the ability of the body to successfully adapt to changing environments and stressful challenges (Logan and Barksdale 2008). AL represents the dysregulation of several major biological systems resulting from repeated and cumulative allostasis over time, i.e., if demands from the environment are excessive and/or occur over a prolonged period of time, AL increases, and the system becomes overloaded (Figure 4).

Figure 4. Stress response and the development of allostatic load (McEwen 1998)



The concept of AL is relatively recent, having first been introduced by McEwen and Steller in the early 1990s as a way to explain the relationship between stress and the processes leading to disease (McEwen and Steller 1993). They hypothesized that chronic stress places a strain on the physiologic systems that maintain homeostasis, leading to chronic "wear and tear" that can predispose the organism to disease. The original operational definition of AL included 10 biological parameters that reflected functioning of the hypothalamic-pituitary-adrenal axis, sympathetic nervous system, cardiovascular system and metabolic processes (Seeman et al. 1997). In general, the literature supports AL as a valid measure of cumulative biological burden; however, it has also been criticized because many of the components of AL are also standard indicators of metabolic syndrome, such as systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC) and abdominal obesity. Higher AL load scores have been linked with incident CVD, all-cause mortality, declines in physical and cognitive functioning, diabetes, arthritis and depressive symptoms (Seeman et al. 1997; Karlamangla, Singer and Seeman 2006; Mattei et al. 2010; Kobrosly et al. 2014). There is also evidence that AL is socially

patterned among adults and children, with higher AL associated with lower socioeconomic position (SEP), in addition to potential differences by gender and race/ethnicity (Logan and Barksdale 2008).

2.5.1 Operationalization of Allostatic Load

Summary indices of AL are meant to capture the interrelated dysregulations in multiple physiologic systems that develop over time in response to environmental demands (Bird et al. 2010). Examining the summary index rather than each of the biomarkers individually allows us to better capture the cumulative extent of the interrelated feedback loops among the physiological systems included in the AL model. Typically, AL is assessed using a multi-system framework that includes indicators of physiologic activity across a range of important regulatory systems (e.g., cardiovascular, metabolic, inflammatory). The AL score is then derived by summing the number of parameters that represent the highest risk quartile (e.g. $\geq 75^{th}$ percentile) across indices.

As shown in Table 2, our AL model will be operationalized to include cardiovascular (diastolic blood pressure [DBP], systolic blood pressure [SBP]), metabolic (total cholesterol [TC], high density lipoprotein [HDL], glycated hemoglobin [HbA1c], body mass index [BMI], waist circumference [WC], triglycerides), and inflammatory (CRP, fibrinogen) biomarkers guided by prior research (Jung et al. 2014; Rainisch and Upchurch 2013; Robertson et al. 2015; Bird et al. 2010) and based on SEARCH data availability. For reference, we have also included a table that shows how AL has been operationalized in several prior studies (Table 3).

For each of the indicators, empirical cut-points identifying high risk will be determined based on the sample distribution (e.g., 75th percentile and above for all biomarkers except HDL, for which we will utilize the 25th percentile and below); youth who exhibit high risk levels of biomarkers will receive a score of 1 for that parameter. A composite AL index will then be created by summing the number of parameters identified as high risk. The range of AL scores is 0 to 10, with higher values signifying greater systemic dysregulation. In addition to the composite AL score, we will also create scores for each individual dimension of AL (e.g., cardiovascular, inflammatory and metabolic AL), which will incorporate the biomarkers included in each category, respectively.

Table 2. Operationalization of allostatic load

Biological System Subscales	**Component Biomarkers**
Cardiovascular	Diastolic Blood Pressure Systolic Blood Pressure
Inflammatory	C-reactive Protein Fibrinogen
Metabolic	Body Mass Index Glycated Hemoglobin High Density Lipoprotein Total Cholesterol Triglycerides Waist Circumference

Table 3. Operationalization of allostatic load in prior studies

Study	**Biological System Subscales**	**Component Biomarkers**
Seeman et al. (1997)	Cardiovascular	Diastolic Blood Pressure Systolic Blood Pressure
	Metabolic	Waist-Hip Ratio High Density Lipoprotein Total Cholesterol Glycated Hemoglobin
	Neuroendocrine	Epinephrine Norepinephrine Cortisol Dehydroepiandrosterone Sulfate

Jung et al. (2014)	Cardiovascular and Respiratory	Diastolic Blood Pressure Systolic Blood Pressure Heart Rate
	Anthropometric	Body Mass Index Body Fat
	Neuroendocrine	Epinephrine Norepinephrine
	Metabolic	Creatinine
	Immune	Interleukin-6 Tumor Necrosis Factor Alpha
Rainisch and Upchurch (2013)	Cardiovascular	Diastolic Blood Pressure Systolic Blood Pressure
	Metabolic	Body Mass Index Waist Circumference Total Cholesterol High Density Lipoprotein Glycated Hemoglobin
	Inflammatory	Serum Albumin C-reactive Protein
Robertson et al. (2015)	Cardiovascular	Diastolic Blood Pressure Systolic Blood Pressure Pulse Rate
	Metabolic	Glycated Hemoglobin Total Cholesterol High Density Lipoprotein Waist-to-Hip Ratio
	Inflammatory	C-reactive Protein Serum Albumin
Bird et al. (2010)	Metabolic	Total Cholesterol High Density Lipoprotein Glycated Hemoglobin Waist-to-Hip Ratio
	Cardiac	Diastolic Blood Pressure Systolic Blood Pressure Heart Rate
	Inflammatory	C-reactive Protein Serum Albumin

2.5.2 Allostatic Load and Traffic-Related Air Pollution

To the best of our knowledge, there are no existing studies which have examined the role of outdoor concentrations of $PM_{2.5}$ and/or traffic-related air pollutants on AL. One occupational health study investigated the cross-sectional association between indoor environmental quality and AL level among 115 office workers in Taiwan (Jung et al. 2014).

In this analysis, the AL model was operationalized based on multi-systemic physiological measurements categorized in five systems: (1) cardiovascular and respiratory, including SBP, DBP and HR; (2) anthropometric, including BMI and body fat; (3) neuroendocrine, including epinephrine and norepinephrine in urine; (4) metabolic, including creatinine in urine; and (5) immune, including IL-6 and tumor necrosis factor alpha (TNF-α) in saliva. The authors reported that indoor $PM_{2.5}$ exposures over the work day were not significantly associated with total AL score (β = -0.014) or any of the biological system subscales examined (cardiovascular and respiratory [β = -0.002], anthropometric [β = -0.002], neuroendocrine [β = -0.006], immune [β = -0.005], and metabolic [β = -0.002]). Although outdoor levels of $PM_{2.5}$ were collected, associations with AL score were either not examined or not reported.

However, prior studies have shown that exposure to ambient air pollution may result in alterations to the individual physiologic parameters that quantify the AL score. Existing epidemiological evidence suggests that both short and long-term exposures to ambient air pollutants cause significant increases in BP parameters, with particularly robust evidence for exposures to fine particulate matter (Hoffman et al. 2012; Giorgini et al. 2016; Yang et al 2018). Furthermore, a recent review article suggested that PM-mediated elevations in BP might be an important pathway that could act as a potential triggering factor for acute cardiovascular events (Giorgini et al. 2016). A sizeable body of research has also explored potential effects of ambient air pollutants on inflammatory biomarkers, including CRP, fibrinogen, and IL-6. Although the literature is mixed regarding a direct association between air pollution exposures and inflammation, several

studies have suggested a positive association (Hajat et al. 2015; Green et al. 2016; Bind et al. 2012; Wang et al. 2015).

Fewer studies have focused on potential associations between ambient air pollution exposures and metabolic risk factors such as BMI, waist circumference, triglycerides, measures of cholesterol and HbA1c levels. Evidence is mixed regarding whether air pollution has an impact on body weight status, and reported associations have varied by sex, age group and specific air pollutants (An et al. 2018). Ambient air pollutants including $PM_{2.5}$ and NO_2 have been associated with increased HbA1c levels in adult populations with and without diabetes (Honda et al. 2017; Tamayo et al. 2014; Yitshak Sade et al. 2016). However, several recent studies conducted among children and young adults with T1D reported no adverse effects of air pollutants on HbA1c, including PM_{10}, NO_2, and O_3 (Tamayo et al. 2016; Lanzinger et al. 2018). Exposure to ambient air pollution has also been associated with increased triglyceride and cholesterol levels in a limited number of studies (Yitshak Sade et al. 2016; Yang et al. 2018; Poursafa et al. 2014.

Although alterations in these singular physiological risk factors indicates modest consequences for morbidity and mortality, the combination of biological indicators captured by AL may be associated with marked elevations in cardiovascular risk (Evans et al. 2007). To date, there is an overall lack of research investigating the relationship between ambient air pollution and a summary index of AL.

CHAPTER 3

Short-term Exposures to Traffic-Related Air Pollution and Pulse Wave Velocity in a Cohort of Children and Youth with Type 1 Diabetes

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3.1 Abstract

Background: Changes in vascular stiffness may play a role in the association between short-term exposures to particulate matter (PM) air pollution and cardiovascular events, especially among individuals who are at higher risk for developing cardiovascular diseases (CVD), such as children and young adults with type 1 diabetes (T1D).

Objectives: We examined the relationship of short-term exposures to particulate matter <2.5 μm in diameter ($PM_{2.5}$), elemental carbon (EC), and Atmospheric Dispersion Modeling System (ADMS)-Roads traffic-related PM concentrations near roadways with pulse wave velocity (PWV), a measure of arterial stiffness, using data from the SEARCH for Diabetes in Youth Study (SEARCH).

Methods: PWV, lifestyle and demographic data were collected at a SEARCH study visit among participants enrolled in SEARCH CVD, an ancillary study conducted in 2009-2011. Pollutant exposures were estimated using spatiotemporal models and assigned to the residential address of each participant for 7 days prior to the clinic visit. We estimated associations between air pollutant exposures and PWV using generalized linear models adjusted for demographic and lifestyle variables.

Results: Approximately 44% of the 352 participants were female and 86% were white, with a mean age of 14.7 years (range 10-23). In fully adjusted models, comparing the 25^{th} and 75^{th} percentiles of $PM_{2.5}$ mass exposures on lag day 0 resulted in a 5.8% higher PWV (95% CI: 0.7%, 11.2%). No other significant associations were observed.

Conclusions: In this population of youth with T1D, PWV was associated with estimated $PM_{2.5}$ mass exposures on lag day 0. However, no associations were observed for acute

exposures to other traffic-related air pollutants. In further analysis, we will examine the relationships of $PM_{2.5}$ mass and traffic-related air pollutant exposures with additional measures of cardiovascular structure and function.

3.2 Introduction

A substantial body of evidence indicates that exposure to ambient air pollution from traffic-related sources, including particulate matter <2.5 μm in diameter ($PM_{2.5}$), is a risk factor for cardiovascular morbidity and mortality (Dockery et al. 1993; Samet et al. 2000; Brook et al. 2010; Franklin, Brook and Pope 2015). Several biological mechanisms have been proposed to explain this relationship, including endothelial dysfunction, oxidative stress, systemic inflammatory responses, and vascular dysfunction (Gurgueira et al. 2002; Utell et al. 2002; Brook et al. 2010; Pei et al. 2016).

Individuals with diabetes appear to be more vulnerable to the cardiovascular health effects associated with particulate air pollution as indicated by increased mortality and hospitalizations for heart disease during periods of high air pollution (O'Neill et al. 2005; O'Neill et al 2007; Goldberg et al. 2001; Zanobetti and Schwartz 2002). Although the underlying mechanistic pathways are not fully understood, it has been hypothesized that endothelial dysfunction and/or changes in systemic inflammation and vascular function, which commonly occur with diabetes, may be implicated (Brook et al. 2010, O'Neill et al. 2005).

Arterial stiffness is a vascular biomarker and an independent predictor of CVD risk among adults (Van Bortel et al. 2012). Measurement of carotid-femoral pulse wave velocity (PWV) is considered the gold standard for noninvasive assessment of arterial stiffness. PWV refers to the speed of the pressure wave generated by the heart as it circulates through the arterial tree (higher PWV indicates stiffer arteries). Existing evidence suggests that children and young adults with type 1 diabetes (T1D) have increased PWV compared to healthy control subjects, and traditional cardiovascular risk factors

alone (e.g., hypertension, obesity, smoking) do not fully explain the differences (Gourgari et al. 2017, Urbina et al. 2010). Increased susceptibility to the effects of ambient air pollution among youth with T1D may therefore be an explanatory factor.

To date, only a few studies have examined whether PWV increases in response to acute exposures to traffic-related air pollutants, and findings have been inconsistent (Zanoli et al. 2017, Wu et al. 2016, Shan et al. 2014, Lundback et al. 2009). The majority of prior work has focused on healthy adult populations. However, youth with T1D have increased arterial stiffness from an early age, and individuals with T1D have a higher risk of CVD-related morbidity and mortality which occurs earlier in life than in the general population (Orchard et al. 2006, Maahs et al. 2010, Schnell et al. 2013). Identification of modifiable risk factors, such as ambient air pollution exposures, early in the progression of CVD may help lead to improved cardiovascular outcomes among this vulnerable population.

We examined whether short-term exposures to traffic-related air pollutants ($PM_{2.5}$ mass, elemental carbon [EC], and ADMS Roads traffic-related PM concentrations) were associated with PWV among a cohort of children and young adults with T1D. We also examined effect modification of the associations by sociodemographic factors.

3.3 Methods

Study Population

Participants included in this analysis were enrolled in SEARCH CVD, an ancillary study of the SEARCH for Diabetes in Youth Study (SEARCH). The overall study design and SEARCH CVD ancillary study have been described previously (Hamman et al. 2014; Jaiswal et al. 2013). Briefly, SEARCH is an observational, multicenter, population-based

study funded by the Centers for Disease Control and Prevention (CDC) to address gaps and respond to emerging issues relating to diabetes in youth <20 years of age. SEARCH was initiated in 2000 and is being conducted at six sites across the U.S.; two out of the six SEARCH sites were included in the present analysis: Ohio (Cincinnati and eight surrounding counties, including Hamilton, Butler, Warren, and Clermont in Ohio; Boone, Kenton, and Campbell in Kentucky; and Dearborn, Indiana) and Colorado (statewide). SEARCH centers conduct active surveillance using networks of health care providers, hospitals, community health centers, clinical and administrative data systems, and electronic medical records. Health care providers assign the clinical diabetes type for all registered cases. Case ascertainment is based on verification of a physician diagnosis of diabetes. Cases are then registered anonymously with the SEARCH Coordinating Center at Wake Forest University. Each site identified prevalent cases in 2000 and 2009, and identification of incident cases has been ongoing annually since 2002. SEARCH participants were eligible for SEARCH CVD if they had physician-diagnosed T1D and had completed a baseline SEARCH visit at age 10 years or older in 2004-2005. The baseline visit consisted of a physical examination, laboratory work, and administration of questionnaires to collect information on medical history, family history, quality of life, and health behaviors including diet and physical activity. PWV was measured at a SEARCH CVD study visit in 2009-2011. Participants also completed standardized questionnaires to provide updated information on medical history, medication use, and health behaviors (e.g., smoking).

Exposures

We estimated 24-hour average concentrations of $PM_{2.5}$ mass and EC across the conterminous US from 1999 to 2011 using spatiotemporal generalized additive mixed models (GAMMs). We also estimated traffic-related PM exposures near roadways using Atmospheric Dispersion Modeling System (ADMS)-Roads software (CERC; Cambridge, England). For each participant, residential addresses at the time of the SEARCH CVD study visit were geocoded using ArcGIS 9.3 software (ESRI 2008) and Topologically Integrated Geographic Encoding and Referencing (TIGER) Road Network Files for 2000 and 2006, with a 30 m offset to account for residential distance to roadways. Exposure estimates for $PM_{2.5}$, EC, and traffic-related PM from ADMS-Roads were then assigned based on the geocoded addresses. ADMS-Roads exposures were classified into two categories: all roadways, including US Census Feature Class codes A1-A4 and A6 (ADMS-Roads A1-A6 exposures), and major roadways, including US Census Feature Class codes A1-A3 (ADMS-Roads A1-A3 exposures). For each exposure metric, we calculated moving averages corresponding to the seven days prior to the CVD study visit (weekly averages). In addition, for $PM_{2.5}$ and ADMS-Roads A1-A6, daily exposures were calculated for the day of the CVD study visit (lag 0) through seven days prior to the visit (lag 1-7), as well as moving averages of 2-5 days prior to the CVD study visit.

Inputs for our exposure models included 24-hr average $PM_{2.5}$ concentrations, which were obtained from the US Environmental Protection Agency's Air Quality System (AQS), the Interagency Monitoring of Protected Visual Environments (IMPROVE) network, and the Southern Aerosol Research and Characterization Study (SEARCH) network. In addition, 24-hr average EC measurements were obtained from the IMPROVE and AQS

networks. We incorporated data on meteorological parameters that influence pollutant dispersion including wind speed, wind direction, surface roughness, sensible heat flux, planetary boundary layer height, air temperature, total precipitation, and total snowfall, which were obtained from the MERRA project (Rienecker et al. 2011). Traffic counts were obtained from Geographic Data Technology, Inc. (Lebanon, NH) Dynamap Traffic Counts v4.2 and spatially joined to the ESRI StreetMap Pro 2007 network of road segments to obtain the US Census Feature Class Codes road class: A1 (primary roads or highways with limited access), A2 (primary roads or highways without limited access), A3 (secondary and connecting roads including state and county highways), A4 (local, neighborhood, and rural roads that are typically single lane thorough-fares), and A6 (roads with special characteristics, including access ramps, exits and jug handles); traffic counts were then spatially averaged using the ArcGIS "Point Statistics" tool. Data on county-level population density from the 2000 US Census (US Census Bureau 2013) were obtained from ESRI Data and Maps 10.1 and spatially smoothed. We obtained elevation data from the USGS National Elevation Dataset (USGS 2013).

Assessment of Arterial Stiffness

PWV (m/sec) was measured with a SphygmoCor Vx System (Atcor Medical, Sydney, Australia). Three electrocardiogram leads were applied to the torso, and the average of three distances from the lowest portion of the sternal notch to the carotid and femoral artery sites was obtained. A pressure waveform was obtained for the carotid site and a second was recorded from the femoral artery. Waveforms were gated by the R wave on the simultaneously recorded electrocardiogram. PWV is the difference in the carotid-

to-femoral path length divided by the difference in R wave-to-waveform foot times, using an average of 10 successive measurements to cover a complete respiratory cycle. Measurements were performed in ambulatory research settings after five minutes of rest in the supine position. The average of three recordings was used in our analyses.

Confounders and Effect Modifiers

Covariates were identified a priori through the use of a directed acyclic graph (DAG) (Figure 1), which was developed based on a review of the relevant literature and included various demographic (age, gender, race/ethnicity, body mass index [BMI] and neighborhood affluence) and lifestyle (smoking, physical activity level and sedentary behavior) variables (Quinn et al. 2012; Tanaka and Safar 2005; Urbina et al. 2010; Logan and Kim 2016; Morris et al. 2013; Trudel et al. 2016; Mikati et al. 2018; Park et al. 2005; Giorgini et al. 2016; Pasqua et al. 2018). Race/ethnicity, gender and smoking status were also considered as potential effect modifiers.

Demographic and lifestyle data were collected at a SEARCH CVD study visit. Participants completed standardized questionnaires including medical history, medication inventory, smoking status, physical activity, and sedentary behavior. Race was self-reported, and participants were categorized as white or other racial/ethnic groups (including Hispanic, Black, Asian/Pacific Islander, and multiple/other). Youth who reported smoking cigarettes on one or more of the previous 30 days were considered current smokers. Past smokers were individuals who had previously tried smoking or smoked regularly (defined as at least one cigarette every day for 30 days). Participants who had never smoked a whole cigarette were considered non-smokers. Physical activity level

was assessed by asking participants to report the average number of days in a typical week that they spent exercising or engaging in physical activity for at least 20 minutes that made them sweat or breathe hard (0, 1-4, 5-7). Sedentary behavior was assessed by asking participants to report how many hours of television they watch in a typical day (<2, >2). Height (cm) and weight (kg) were measured using a stadiometer and a standardized scale, respectively. BMI was calculated as weight/height^2 (kg/m^2); age and sex-specific BMI z-scores were then derived using national reference standards developed by the CDC. Neighborhood affluence was assessed by using the residential address at the time of the CVD study visit to obtain the percentage below poverty for the census tract of residence from the 2000 US Census (US Census Bureau 2013).

Statistical Analysis

Of the 510 SEARCH CVD participants with available data, we excluded 12 with missing values for PWV; 3 missing information on age, gender or percentage of the population living below the poverty line in their census tract of residence; 2 reporting usage of statin medications; and 10 with geocode quality other than a street segment match. An additional 130 subjects with missing or zero values for ADMS Roads A1-A6, EC, and $PM_{2.5}$ exposures were excluded. After examining the distribution of PWV and removing influential outliers (n=1), a total of 352 participants were included in the primary analyses. In our secondary analyses, subjects who had missing or zero values for ADMS Roads A1-A3 exposures because they lived >2 km from a primary or secondary road were also excluded (N=57). Upon examination of the exposure variable distributions, all were log-transformed to achieve normal distributions.

We developed separate generalized linear regression models for each exposure and outcome combination to model PWV as a continuous function of different time windows of estimated exposures to $PM_{2.5}$ mass, EC, and ADMS-Roads A1-A3 and A1-A6: weekly averages for each exposure measure, as well as lags of 0-7 days for $PM_{2.5}$ and ADMS-Roads A1-A6. We estimated the percent difference in PWV per interquartile range (IQR) increase for each pollutant exposure estimate. Base models were unadjusted. Demographic models included age, gender, race/ethnicity, BMI, and percent below poverty in the census tract of residence. Fully adjusted models additionally included smoking, daily time spent watching television and number of days of vigorous exercise in the past week. Effect modification by race/ethnicity, sex and smoking status was assessed through the use of interaction terms in fully adjusted models. In sensitivity analyses, we adjusted for the effects of mean arterial pressure and seasonality. We also included participants with PWV values that were considered to be influential outliers in a sensitivity analysis. All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). Statistical significance was determined by p-values <0.05 corresponding to two-sided tests.

3.4 Results

Characteristics of the study population are presented in Table 1. Approximately 44% of the 352 participants in our primary analyses were female and 86% were white. Subjects ranged in age from 10-23 years with an average of 14.7 years (standard deviation [SD] 3.3). More than two-thirds of participants were from the Ohio site (71%), and the majority were never smokers (72%). Half of study participants reported participating in vigorous exercise between 1-4 days during the past week as well as watching television >2

hours per day. The mean percentage of census tract residents falling below the poverty line was nearly 7% (SD 0.1). The mean value for PWV was 5.4 m/s (SD 0.76). Subjects included in the ADMS Roads A1-A3 analyses (n= 296) had similar characteristics.

Concentrations of ambient air pollutants for SEARCH participants during the study period are displayed in Table 2. Averaged over the week prior to the clinic visit, the geometric mean corresponding to $PM_{2.5}$ mass exposure was 2.5 (SE 0.02), 0.07 for exposures to ADMS-Roads A1-A6 (SE 0.003), 6.6 (SE 0.02) for exposure to EC, and 0.02 (SE 0.001) for exposures to ADMS-Roads A1-A3. The IQR for weekly average pollutant exposures was 7.44 for $PM_{2.5}$ mass, 0.07 for ADMS-Roads A1-A6, 418.7 for EC, and 0.04 for ADMS-Roads A1-A3.

In unadjusted models, we found positive associations for PWV with weekly average $PM_{2.5}$ mass exposures (3.5% higher PWV comparing exposure from the 25^{th} to 75^{th} percentiles of $PM_{2.5}$, 95% Confidence Interval [CI]: -2.3, 9.3) and with $PM_{2.5}$ exposures on lag days 0-7 (Figure 2). However, these associations did not reach nominal statistical significance. In fully adjusted models, a statistically significant positive association was observed for PWV with exposure to $PM_{2.5}$ on lag day 0 (Figure 3). Comparing exposure from the 25^{th} to the 75^{th} percentiles, an interquartile range increase in $PM_{2.5}$ exposure was associated with a 5.7% increase in PWV (95% CI: 0.7, 10.6). Fully adjusted associations between PWV and weekly or daily average $PM_{2.5}$ for lag days 1-7 were also positive in the direction of effect, but did not reach statistical significance.

In contrast, a negative association that did not reach statistical significance was observed between EC exposures averaged over the week prior to the clinic visit and PWV in our unadjusted models (3.5% lower PWV comparing exposure from the 25^{th} to 75^{th}

percentiles of EC, 95% CI: -9.5, 2.5). This effect was somewhat attenuated after adjustment for demographic and lifestyle variables (2.7% lower PWV comparing exposure from the 25th to 75th percentiles of EC, 95% CI: -7.7, 2.4).

Reported associations between PWV and ADMS-Roads exposures were less consistent in the direction of effect. For example, in unadjusted models, PWV was positively but non-significantly associated with the majority of weekly or daily ADMS-Roads A1-3 and A1-6 exposures (Figure 4). However, in fully adjusted models, several of these associations reversed direction. For example, for lag day 1, PWV was 2.0% higher [95% CI: -5.5, 9.6] comparing exposure from the 25th to 75th percentiles in unadjusted analyses, but was 1.1% lower [95% CI: -7.8, 5.5] in models adjusted for demographic and lifestyle variables (Figure 5).

We did not observe differences in the associations by race/ethnicity, sex or smoking status. In sensitivity analyses, adjusting for the effects of mean arterial pressure and seasonality did not significantly alter the effect estimates. The addition of participants with outlying values for PWV did not substantially change the associations.

3.5 Discussion

Our results suggest that short-term exposures to $PM_{2.5}$ air pollution may be associated with increased arterial stiffness among a cohort of children and young adults with T1D. In fully adjusted models, a 5.7% increase in PWV was observed after an IQR increase in $PM_{2.5}$ concentrations on lag day 0. In addition, associations between higher $PM_{2.5}$ exposures on lag days 1-7 and PWV were suggestive of a positive relationship. We did not observe consistent associations for ADMS-Roads exposures. For EC, reported

associations with PWV were negative but not statistically significant. There was no evidence of effect modification by race/ethnicity, sex or smoking status.

To date, only a few studies have investigated the consequences of acute exposure to traffic-related air pollution on PWV, and direct comparisons with our findings are limited due to differences in study design, study population, as well as exposure and outcome metrics. Wu et al. (2016) reported comparable findings for the acute effects of $PM_{2.5}$ on brachial-ankle PWV among a panel study of 89 healthy adults (mean age 43.7 years) in Taipei, Taiwan. A 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentrations at a 0-day lag was associated with a 2.1% (95% CI: 0.7, 3.6) increase in PWV. Similar effects were reported for lag days 1 and 2.

In contrast to our study, Shan et al. (2014) reported that higher personal exposure to $PM_{2.5}$ over a 24 hour period was not associated with increased PWV in a cross-sectional analysis of 25 non-smoking women (mean age 59 years) living in a rural province of China. The authors reported no difference in PWV between high and low exposure groups (in fully adjusted models, -0.1% difference, 95% CI: -0.9, 0.7). However, the personal $PM_{2.5}$ measurements obtained in the study included indoor exposures to dust and biomass fuel used for cooking, while we focused solely on outdoor exposures.

Although we were unable to identify existing studies that considered the effects of short-term exposures to EC or traffic-related PM on PWV, prior work has focused on acute exposures to black carbon (BC) and diesel exhaust. In a panel study of 54 healthy adults (mean age 40.7 years) living in Belgium, Provost et al. (2016) reported that PWV was positively associated with BC exposure 8 hours before the clinical examination (1.18% increase in PWV [95% CI: 0.51, 1.88] for each 100 ng/m^3 increase in BC exposure).

However, Lundback et al. (2009) reported that exposure to diesel exhaust one hour in duration was not associated with increased PWV in a double-blind crossover study conducted among 12 healthy men (mean age 26) in Sweden. PWV measurements did not significantly differ 40 minutes after exposure to diesel fumes compared to filtered air (5.1 m/s [SD 0.2] versus 4.6 m/s [SD 0.1]).

Clearly, additional studies are indicated given the discrepancies in the existing literature. A recent systematic review concluded that the available evidence supports an association between main air pollutants and increased arterial stiffness, with strong evidence for exposures to particulate matter (Zanoli et al. 2017). This work has potentially important implications on cardiovascular risk at a population-based level. Future studies should incorporate larger study samples and compare the effects of acute and chronic exposures to traffic-related air pollution on PWV.

The biological mechanisms underlying the relationship of short-term exposures to traffic-related air pollution with arterial stiffness remain unclear. There is evidence that systemic inflammation plays an important role in the development of arterial stiffness (Mozos et al. 2017, van Bussel et al. 2011), and the negative effects of ambient air pollution on inflammation have been well documented in the existing literature (Bind et al. 2012, Green et al. 2016, Hajat et al. 2015). Arterial stiffness may also be a consequence of endothelial dysfunction, which has been linked with exposures to traffic-related air pollution (van Bussel et al. 2011, Schneider et al. 2008). Additional studies are needed to further explain the role of air pollution in the pathophysiology of vascular dysfunction.

Our study has several notable strengths. To the best of our knowledge, this is the first study to assess the acute effects of traffic-related air pollutants on arterial stiffness

among youth with T1D. In addition, we utilized carotid-femoral PWV measurements, which are the gold standard for assessment of central arterial stiffness. Additionally, we used a validated exposure model to estimate participants' exposures to multiple traffic-related air pollutants. There are also several limitations to our study, including the small sample size and limited generalizability to other populations. In addition, similar to the majority of other observational epidemiologic studies of air pollution using cohort data, there was potential for exposure misclassification as we only assessed exposure at residential locations and had no information on time-activity patterns. We were also unable to account for exposure to secondhand smoke in the household.

In summary, our results are suggestive of an association between short-term exposures to $PM_{2.5}$ mass and PWV in this sample of youth with T1D. Our findings indicate that acute exposures to traffic-related air pollution may affect vascular function, although additional research is indicated.

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3. 7 Tables and Figures

Table 1. Description of the SEARCH CVD study population

Variable	Primary Analyses (N=352) N (%) or Mean (SD)	ADMS Roads A1-A3 (N=296) N (%) or Mean (SD)
Age (years)	14.7 (3.3)	14.6 (3.4)
Female	155 (44.0)	130 (43.9)
Race/Ethnicity		
Non-White	48 (13.6)	43 (14.5)
White	304 (86.4)	253 (85.5)
Clinic		
Ohio	250 (71.0)	209 (70.6)
Colorado	102 (29.0)	87 (29.4)
Smoking		
Current	23 (6.5)	22 (7.4)
Former	34 (9.7)	27 (9.1)
Never	255 (72.4)	212 (71.6)
Missing	40 (11.4)	35 (11.8)
Days of Vigorous Exercise in past week		
0	46 (13.1)	35 (11.9)
1-4	176 (50.0)	144 (48.8)
5-7	90 (25.6)	81 (27.5)
Missing	40 (11.4)	35 (11.9)
Hours of TV watched		
<2	136 (38.6)	107 (36.2)
>2	176 (50.0)	154 (52.0)
Missing	40 (11.4)	35 (11.8)
BMI (kg/m^2)	22.7 (4.6)	22.7 (4.5)
Percent below poverty in tract of residence	6.9 (0.1)	7.3 (0.1)
PWV (m/sec)	5.39 (0.76)	5.39 (0.75)

Table 2. Average concentrations of ambient air pollutants for the SEARCH CVD population the week prior to the CVD study visit

Variable	N	Mean (SD)	Geometric Mean (SE)	Range	IQR
$PM_{2.5}$ ($\mu g/m^3$)	352	13.4 (5.8)	2.6 (0.02)	34.5	7.44
ADMS Roads A1-A6	352	0.1 (0.1)	0.07 (0.003)	0.65	0.07
ADMS Roads A1-A3	296	0.04 (0.07)	0.02 (0.001)	0.56	0.04
EC (ng/m^3)	352	795.3 (329.0)	6.6 (0.02)	1778	418.7

Figure 2. Associations between pulse wave velocity and an interquartile range increase in EC and $PM_{2.5}$ exposures in unadjusted models

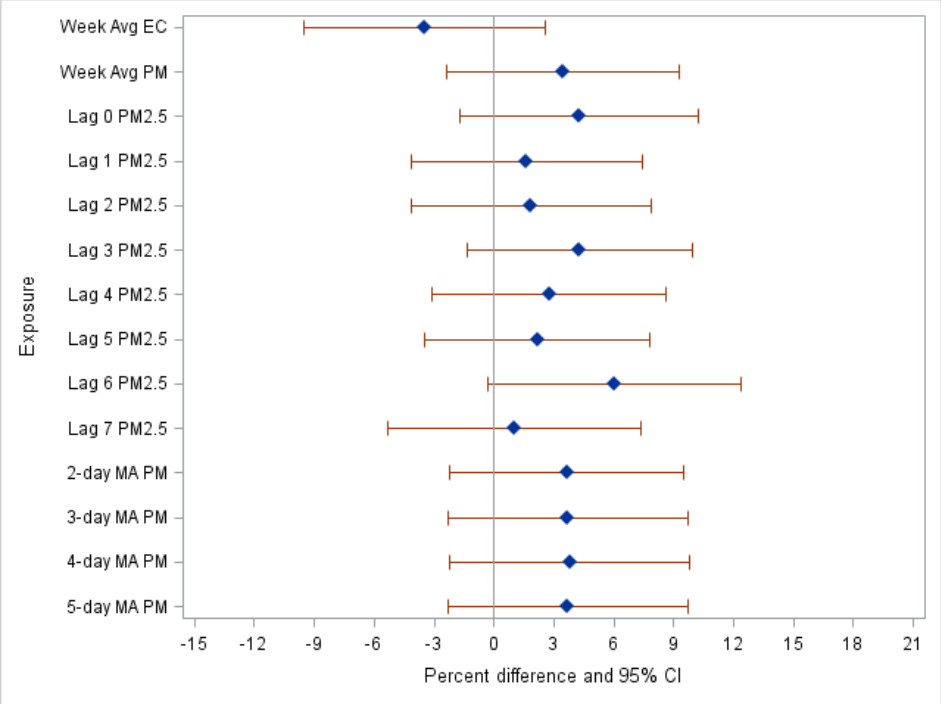


Figure 3. Associations between pulse wave velocity and an interquartile range increase in EC and $PM_{2.5}$ exposures in fully adjusted models

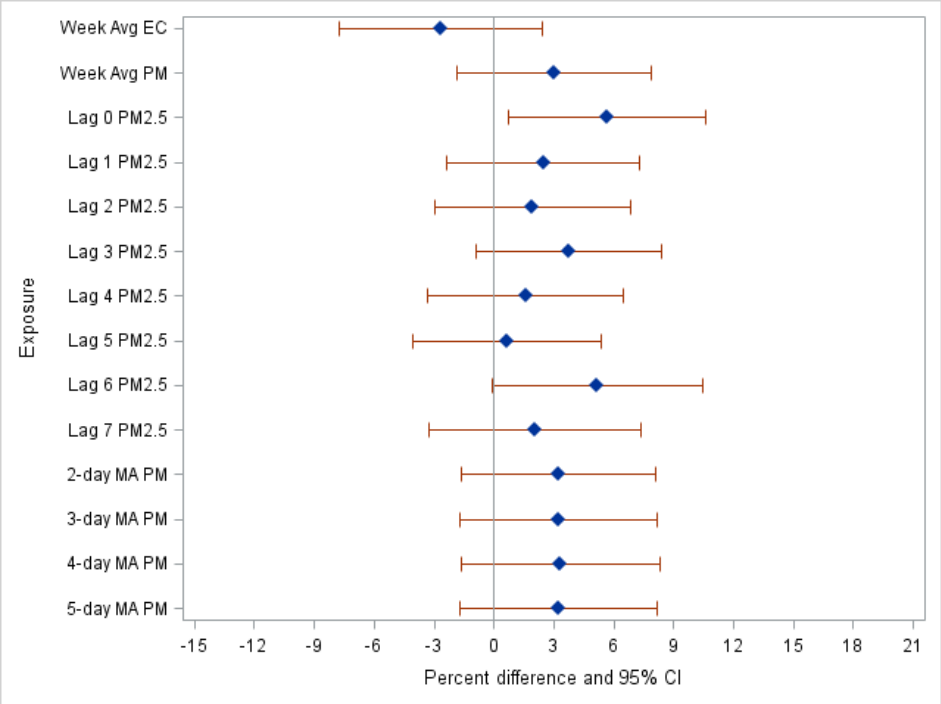


Figure 4. Associations between pulse wave velocity and an interquartile range increase in ADMS-Roads exposures in unadjusted models

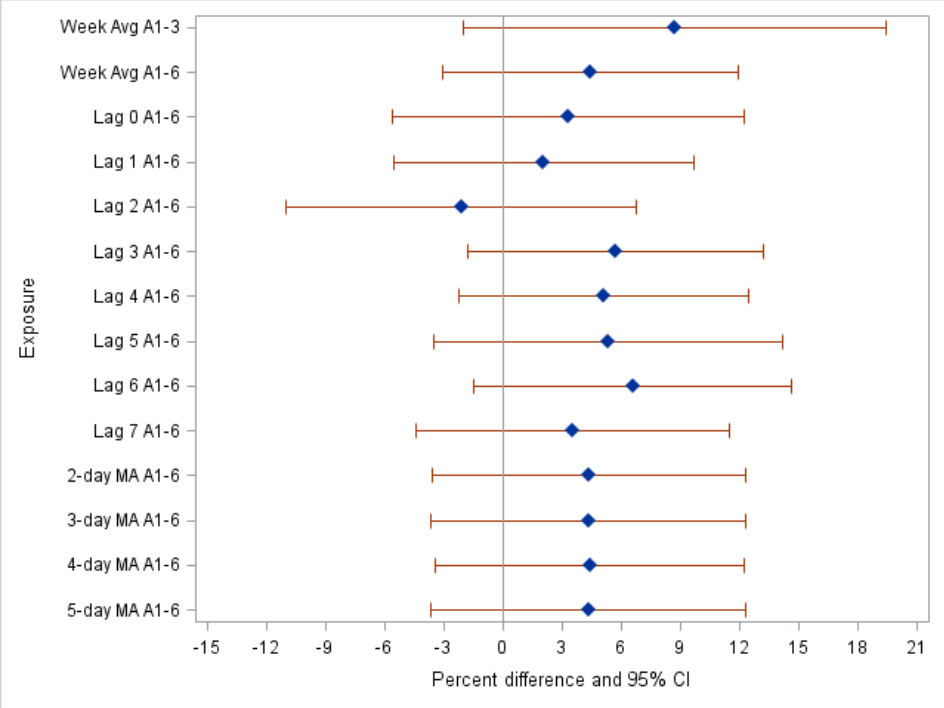


Figure 5. Associations between pulse wave velocity and an interquartile range increase in ADMS-Roads exposures in fully adjusted models

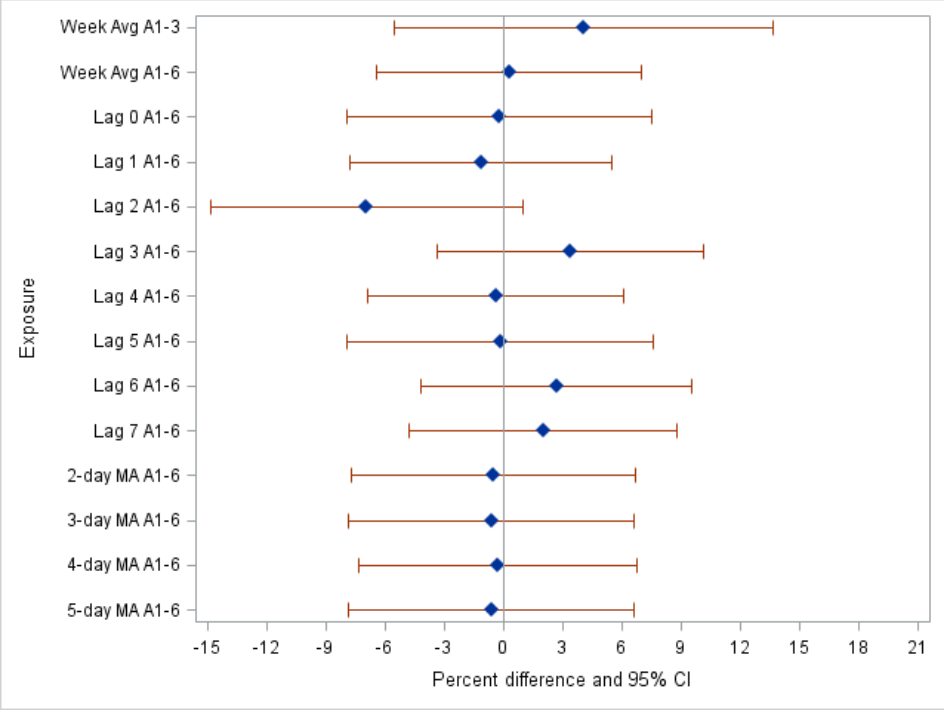
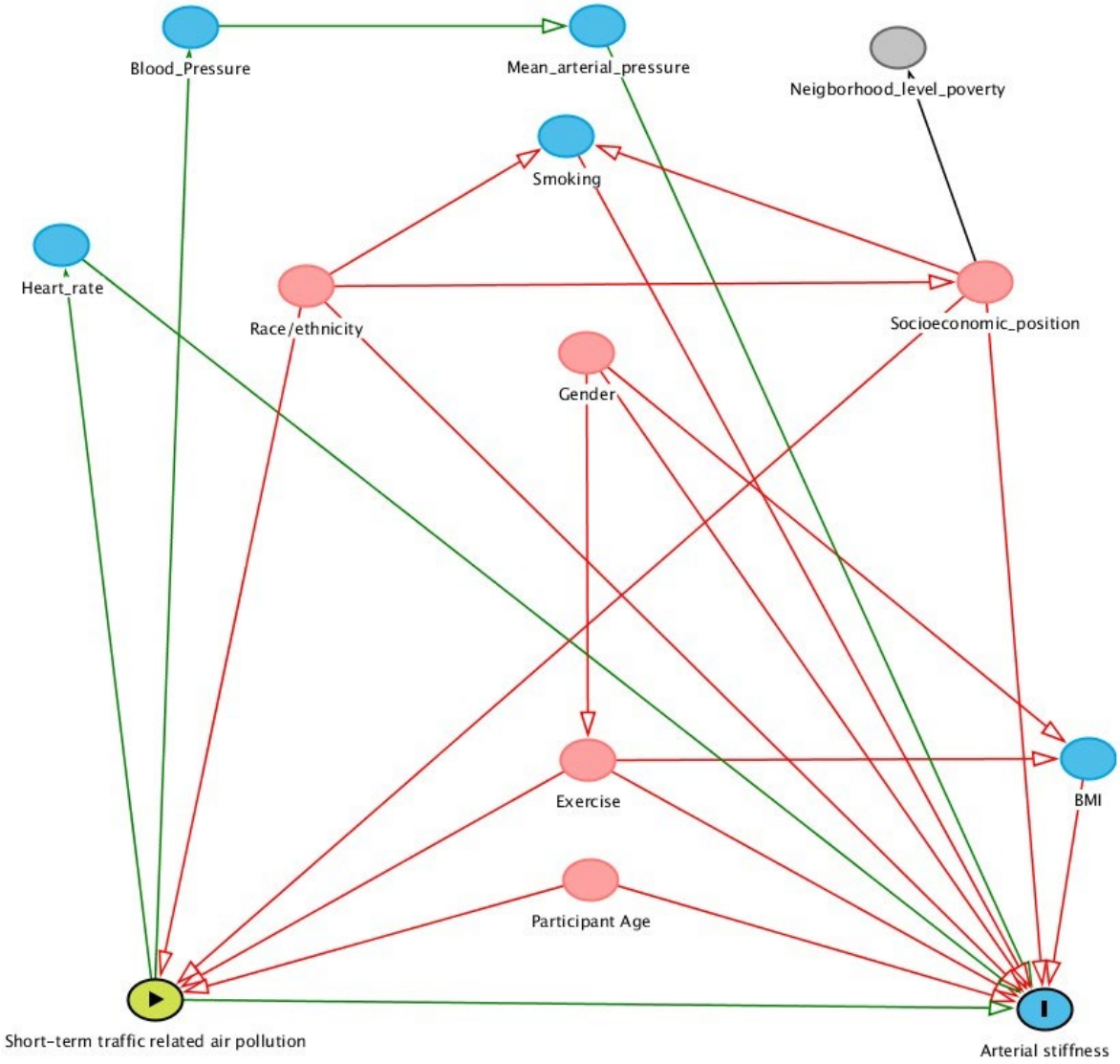


Figure 1. Directed acyclic graph for the relationship of short-term traffic-related air pollution exposures with measures of arterial stiffness



CHAPTER 4

Are Changes in Air Pollution Exposures Associated with Changes in Inflammation among Children and Youth with Type 1 Diabetes?

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4.1 Abstract

Background: Air pollution is associated with cardiovascular morbidity and mortality, and potential biological mechanisms underlying this relationship include systemic inflammation. Youth with type 1 diabetes (T1D) are at higher risk for cardiovascular diseases (CVD), however, few studies have examined the effects of air pollution exposures on inflammatory markers in this population.

Objective: We examined associations of short-term exposures to traffic-related air pollutants (including $PM_{2.5}$ mass and elemental carbon [EC]) with changes in C-reactive protein (hs-CRP), fibrinogen and interleukin-6 (IL-6) among children and young adults with T1D.

Methods: We included participants with T1D from the Search for Diabetes in Youth (SEARCH) study who had repeat measurements of hs-CRP, fibrinogen and IL-6. Questionnaires and blood were taken at baseline and a follow-up visit. Pollutant exposures for 7 days prior to the blood draws were assigned to residential addresses for each participant. Using separate longitudinal mixed models adjusted for demographic and lifestyle variables, we examined whether changes in pollutant exposures predicted changes in inflammation.

Results: We observed consistent positive associations between acute $PM_{2.5}$ mass exposures and IL-6 (e.g., 0.25% change [95% CI: 0.15, 0.35] for an interquartile range (IQR) increase in weekly average $PM_{2.5}$ mass). Increases in $PM_{2.5}$ were not associated with significant changes in hs-CRP or fibrinogen. Relationships of EC with inflammatory biomarkers were generally null.

Conclusions: Our findings suggest that changes in acute exposures to $PM_{2.5}$ mass over time may influence changes in the release of a procoagulant cytokine early in the coagulation process rather than inflammatory markers further downstream. Additional studies are warranted to confirm these findings in other populations and to further explore the relationship between changes in inflammation and air pollution exposures over time.

4.2 Introduction

A growing body of epidemiologic evidence indicates that exposures to traffic-related air pollution are associated with cardiovascular disease (CVD) morbidity and mortality (Dockery et al. 1993; Pope and Dockery 2006; Brook et al. 2004; Brook et al. 2010). Potential underlying mechanisms for these effects include increased inflammation, oxidative stress, endothelial dysfunction and changes in vascular function (Brook et al. 2010; Gurgueira et al. 2002; Utell et al. 2002). Prior research suggests that ambient air pollution exposures may result in acute elevations as well as chronic changes in inflammatory biomarkers such as C-reactive protein (CRP), fibrinogen and interleukin-6 (IL-6) (Panasevich et al. 2009; Hoffmann et al. 2009; Zhang et al. 2013; Hajat et al. 2015; Viehmann et al. 2015; Hassanvand et al. 2017; Li et al. 2017). However, study results have been inconsistent and direct evidence of an association between air pollution exposures and measures of systemic inflammation remains limited.

The effects of air pollution on CVD appear stronger among individuals with diabetes (Dubowsky et al. 2006; Hart et al. 2015; Pinault et al. 2018), and it has been hypothesized that increased inflammation may be partially responsible for this enhanced sensitivity (Dubowsky et al. 2006). However, only a few studies have examined whether air pollution exposures are associated with inflammatory biomarkers among individuals with diabetes (Schneider et al. 2010; Khafaie et al. 2013; Rückerl et al. 2014; Wang et al. 2015; Puett et al. 2018), and results have been mixed depending upon the specific pollutants and time windows of exposure considered. Furthermore, the majority of existing work has focused on adult populations who primarily have type 2 diabetes. To date, very little research has examined the impact of air pollution on systemic inflammation in young

adults and children with type 1 diabetes (T1D), even though these individuals have a significantly increased long-term risk of CVD-related morbidity and mortality beginning from an early age (Maahs et al. 2010; Schnell et al. 2013). One prior cross-sectional analysis using data from the SEARCH for Diabetes in Youth (SEARCH) study reported positive associations of short-term elemental carbon (EC) exposures with IL-6 and hs-CRP, and $PM_{2.5}$ mass exposures with IL-6 levels among youth with T1D (Puett et al. 2018).

In the present study, we evaluated whether changes in short-term exposures to traffic-related air pollutants ($PM_{2.5}$ mass and EC) over time are associated with changes in hs-CRP, fibrinogen, and IL-6 over time among SEARCH participants with T1D in a repeat-measures analysis. We also examined effect modification by sociodemographic factors.

4.3 Methods

Study Population

The SEARCH study was initiated in 2000 to address knowledge gaps in the understanding of childhood diabetes with centers located at five original sites across the US, including Ohio (eight counties surrounding Cincinnati), Colorado (statewide), Washington (five counties surrounding Seattle), South Carolina (statewide) and California (single health plan membership from seven counties). Detailed study methods have been published elsewhere (Hamman et al. 2014). Briefly, SEARCH maintains an active registry of children and young adults diagnosed with diabetes prior to age 20 years to assess trends in prevalence (in 2001 and 2009) and annual incidence (ongoing since 2002) as well as trends by age, race/ethnicity, sex and diabetes type. SEARCH has also established a longitudinal cohort to explore risk factors for acute and chronic diabetes-related

complications and other issues such as quality of care and quality of life. Centers conduct active surveillance using networks of endocrinologists, as well as other health care providers, hospitals, community health centers, clinical and administrative data systems and electronic databases. Case determination and eligibility (based on age, residence, non-military, non-institutionalized, and health plan membership at the time of diagnosis) is confirmed using medical records or by the referring physician. The SEARCH protocol was reviewed and approved by Institutional Review Boards (IRBs) at each participating institution and is in compliance with the Health Insurance Portability and Accountability Act (HIPAA). Written informed consent was obtained from all participants aged ≥18 years or from parents/legal guardians if the participant was <18 years.

Participants included in the present analysis completed a baseline study visit (prevalent cases identified in 2001 and incident cases from 2002-2005) and returned for follow-up at 12, 24, and/or 60 months after the initial visit. In order to examine the greatest potential change in the exposure and outcome, for the current study, we included the follow-up visit furthest in time from the baseline visit which had sufficient plasma for the inflammatory marker analysis. Additional information collected at study visits included ascertainment of clinical and family history (income, education, etc.), treatment of diabetes, quality of life, anthropometric measures such as height and weight, laboratory measures including inflammatory biomarkers, as well as factors related to health behavior (e.g., diet, physical activity, sedentary behaviors, etc.).

Inflammatory Markers

Blood was collected at each study visit after an eight hour fast, and concentrations of hs-CRP, fibrinogen and IL-6 were assessed in resultant plasma samples. Fibrinogen and hs-CRP were measured immunochemically using Siemens reagent on a nephelomether autoanalyzer (BNII). The assay sensitivities were 0.004 mg/dL and 3.0 mg/dL, respectively. IL-6 was measured using a monoclonal antibody-based, high sensitive solid-phase ELISA method (R&D System, Inc.). The assay sensitivity was 0.039 pg/ml.

Air Pollution Exposures

Residential addresses at the time of each study visit were obtained for each participant and geocoded using ArcGIS 9.3 software (ESRI, Redlands, CA) and 2000/2006 Topologically Integrated Geographic Encoding and Referencing (TIGER) Road Network Files. A 30-meter (m) offset was used to account for residential distance to roadways. Geocoded addresses were then used to assign exposure estimates for $PM_{2.5}$ mass and EC.

In brief, a spatio-temporal modeling approach was used to estimate 24-hour average $PM_{2.5}$ mass and EC concentrations across the conterminous US from 1999 to 2011 (Yanosky et al 2014; Yanosky et al. 2018). Our spatio-temporal statistical models included $PM_{2.5}$ mass concentrations obtained from the US Environmental Agency's (EPA) Air Quality System (AQS), the Interagency Monitoring of Protected Visual Environments (IMPROVE) network and the Southern Aerosol Research and Characterization Study (SEARCH) network. In addition, EC measurements were obtained from the AQS and IMPROVE networks. Data on meteorological variables known to influence pollutant dispersion were obtained from the MERRA project (Rienecker et al. 2011), including wind

speed, wind direction, air temperature, total precipitation, and planetary boundary layer height. Traffic counts for road classes A1-A6 (A1: primary highway with limited access; A2: primary road without limited access; A3: secondary and connecting roads; A4: local, neighborhood and rural roads; A6: roads with special characteristics, including cul-de-sacs, access ramps and traffic circles) were obtained using Dynamap Traffic Counts v4.2 (Geographic Data Technology, Inc., Lebanon, NH) and spatially averaged. Additional inputs included data on county-level population density from the 2000 US Census (US census Bureau 2013) and elevation data from the USGS National Elevation Dataset (USGS 2013). The generic form of the spatio-temporal generalized additive mixed models (GAMMs) was (Yanosky et al. 2014):

$$y_{i,t} = \alpha + \alpha_t + \sum_q d_q(X_{i,q}) + \sum_p f_p(Z_{i,t,p}) + g_t(s_i) + g(s_i) + b_i + e_{i,t};$$

$$b_i \sim N(0, \sigma^2{}_b);\ e_{i,t} \sim N(0, \sigma^2{}_{e\,t})$$

where $y_{i,t}$ are monitor measurements for i=1…I sites and t=1…T 24-hr time periods, s_i is the projected spatial coordinate pair for the ith location. $X_{i,q}$ are GIS-based time-invariant spatial covariates for q=1…Q, $Z_{i,t,p}$ are spatio-temporal covariates for p=1…P, and α_t is an intercept representing the adjusted mean across all sites on a given day. $d_q$are penalized spline smooth functions of Q GIS-based time-invariant spatial covariates, f_p are penalized spline smooth functions of P spatio-temporal covariates, $g_t(s_i)$ accounts for residual spatial variability in the 24-hr average values, and $g(s_i)$ accounts for time-invariant spatial variability across the conterminous US. The random effect b_i represents unexplained site-specific variability.

We calculated the weekly average of seven days prior to the blood draws for $PM_{2.5}$ mass and EC. In addition, for $PM_{2.5}$ mass, daily exposures were calculated for each

individual lag day from lag 0 (the day of the blood draw) through lag 7 (seven days prior to the blood draw) and for moving averages of 2, 3, 4 and 5 days prior to the blood draws.

Covariates

We selected covariates a priori using a directed acyclic graph (DAG) that was developed based upon a review of the existing literature (Ergou et al. 2018; McEvoy et al. 2015; Lin et al. 2015; Carroll et al. 2009; Cevenini et al. 2010; Gruenewald et al. 2009; Green et al. 2016; Bind et al. 2012; Hajat et al. 2015a; Hajat et al. 2015b), including the following demographic and lifestyle variables: age, sex, race/ethnicity, neighborhood affluence, smoking status, physical activity and sedentary behavior (Figure 1). We also considered sex, race/ethnicity and smoking status as effect modifiers.

Data on all potential confounders were obtained at baseline and follow-up visits via standardized questionnaires. Information on smoking, physical activity and sedentary behavior was assessed for participants 10 years of age and older. Smoking status was ascertained by asking whether participants had any history of smoking tobacco (even one or two puffs); those who had previously smoked were subsequently asked to quantify the number of days in the past month that they smoked tobacco. Parents were asked to waive their rights to review answers to the smoking questions. Physical activity was assessed by asking participants to report the number of days in the past week that they spent exercising or engaging in activities that made them breathe hard or sweat for at least 20 minutes. Sedentary behavior was assessed using questions about the number of days in the past week spent watching television and playing video or computer games. Neighborhood affluence was determined by using residential addresses to obtain the percentage of the population

below poverty in the census tract of residence using data from the 2000 US Census (US Census Bureau 2013).

Data Analysis

Of the 3530 SEARCH participants with baseline data available for the study, 1057 participated in a follow-up visit. We excluded participants with missing outcome data (hs-CRP n=63, fibrinogen n=64, IL-6 n=264), as well as an additional 263 participants missing exposure estimates, 75 with geocode quality other than a street segment match or better, 9 who reported statin medication use, and 26 with missing or unknown information on covariates. Individuals taking statin medications were excluded because these drugs have known anti-inflammatory effects (Antonopoulos et al. 2012). After examination of the distributions of the inflammatory biomarkers and influential outliers were removed (hs-CRP n=1, fibrinogen n=2, IL-6 n=6), 616 participants were included in the analyses for hs-CRP, 618 in fibrinogen analyses and 441 in IL-6 analyses. Upon examination of exposure and outcome distributions, all were log transformed to approximate a normal distribution.

We examined the effect of time-varying air pollution levels on the change in inflammation from baseline to follow-up. Separate longitudinal mixed models were developed for each exposure and outcome with a person-level random intercept to account for the correlation among repeated measurements; study site (California, Colorado, Ohio, South Carolina and Washington) was included as a random effect. Base models were unadjusted for additional covariates. Fully adjusted models, as designated a priori through the use of a DAG, included age (continuous), sex, race/ethnicity (white, other race/ethnicity), percent below poverty in the census tract of residence (continuous),

smoking status (never/ever tried smoking), number of days smoked in the past month (continuous), time spent watching television or playing computer/video games in the past week (continuous) and days of vigorous exercise in the past week (0, 1-4, 5-7). Covariates in the models were time-varying, except for race/ethnicity and gender. From these models, we estimated the percent change in each outcome associated with an interquartile range (IQR) change in $PM_{2.5}$ mass or EC.

Effect modification by gender, race/ethnicity, and smoking status was assessed using interaction terms in fully adjusted models. We additionally conducted several sensitivity analyses. First, models were restricted to include only participants who did not change residences in order to assess the influence of exposure misclassification caused by participants moving between baseline and follow-up. We also included an additional covariate for seasonality (winter, spring, summer, fall) to account for potential confounding effects caused by seasonal trends in air pollution and/or inflammation. Fasting status (yes, no) was also included as a covariate in sensitivity analyses because it may influence inflammatory biomarker measurements. In addition, we ran models that excluded individuals <10 years of age since smoking and physical activity/inactivity covariates were not evaluated in this subpopulation. We also investigated the effects of an IQR increase in baseline levels of $PM_{2.5}$ mass and EC on changes in hs-CRP, fibrinogen and IL-6. SAS version 9.4 (SAS Institute Inc., Cary, NC) was used for all analyses. P-values <0.05 were considered statistically significant.

4.4 Results

Descriptive statistics of the SEARCH study participants at baseline are provided in Table 1. Average age was 10.6 years old (standard deviation [SD] 4.1). The study population was 47% female, 78% white and 22% other races/ethnicities. Approximately 8% of participants had tried smoking cigarettes. Among those that had tried smoking, the average number of days that they smoked out of the past 30 days was 2.1. The majority of participants were drawn from the Ohio site (34%), and the fewest were from California (12%). Approximately 6% of participants had not exercised in the past week, while 32% of participants exercised 1-4 days during the past week and 21% exercised 5-7 days in the past week. On average, participants watched television four days in the past week and played computer games three days in the past week. The mean percentage of residents living below poverty in the census tract of residence was 9%. Mean levels of inflammatory biomarkers were 0.09 mg/dl (SD 0.3), 341.9 mg/dl (SD 67.5) and 16.1 pg/ml (SD 19.3) for hs-CRP, fibrinogen and IL-6, respectively.

$PM_{2.5}$ mass and EC exposure levels during the study period are displayed in Table 2. At baseline, weekly average $PM_{2.5}$ mass levels were 11.1 $\mu g/m^3$ (SD 5.0) with an IQR of 6.2 and geometric mean (GM) of 2.4. At follow-up, weekly average $PM_{2.5}$ mass levels were 9.4 $\mu g/m^3$ (SD 4.0) with an IQR of 5.7 and GM of 2.2. Baseline concentration of weekly average EC was 771.1 ng/m^3 (SD 455.8) with an IQR of 511.6 and GM of 6.5. At follow-up, the concentration of weekly average EC was 604.9 ng/m^3 (SD 345.2) with an IQR of 386.6 and GM of 6.2. During the study period, the correlation between weekly average $PM_{2.5}$ mass and EC was 0.51.

hs-CRP

We observed positive but weak associations between time-varying $PM_{2.5}$ mass exposures and changes in hs-CRP levels in our unadjusted longitudinal mixed models for weekly average exposures, as well as individual lag days 0-7 and moving averages of 2-5 days (Table 3). For example, an IQR increase in weekly average $PM_{2.5}$ mass exposure was associated with a 0.12% increase in hs-CRP (95% CI: -0.02, 0.25). These associations were attenuated in our fully adjusted models for all time periods of exposure examined (Table 4) (e.g., an IQR increase in weekly average $PM_{2.5}$ mass was associated with a 0.8% increase in hs-CRP [95% CI: -0.05, 0.21]). In unadjusted models, an IQR increase in time-varying EC was weakly positively associated with hs-CRP (0.02% change [95% CI: -0.12, 0.16]) but the association reversed direction in fully adjusted models (-0.02% change [95% CI: -0.15, 0.12]).

Fibrinogen

Associations of time-varying $PM_{2.5}$ mass and EC with fibrinogen were weak and did not reach the level of statistical significance in any of our analyses (basic or fully adjusted models) for all time periods of exposure examined, including weekly average exposure as well as individual lag days 0-7 and moving averages of 2-5 days (Tables 3 and 4). For example, an IQR increase in weekly average $PM_{2.5}$ mass exposure was associated with a 0.002% change in fibrinogen (95% CI: -0.01, 0.02) in unadjusted analyses and a -0.001% change in fibrinogen (95% CI: -0.02, 0.01) in fully adjusted models. An IQR increase in weekly average EC exposure was associated with a -0.01% change in fibrinogen

(95% CI: -0.03, 0.01) in unadjusted models and a -0.02% change in fibrinogen (95% CI: -0.03, 0.003) in fully adjusted models.

IL-6

We observed consistent positive effects of time-varying $PM_{2.5}$ mass exposures on changes in IL-6 in our unadjusted and fully adjusted analyses for all time periods of exposure considered (e.g., weekly average, lag days 0-7 and moving averages of 2-5 days) (Tables 3 and 4). For example, an IQR increase in weekly average $PM_{2.5}$ mass exposure was associated with a 0.25% change in IL-6 in unadjusted models (95% CI: 0.15, 0.35) and fully adjusted models (95% CI: 0.15, 0.36). Associations of IL-6 with time-varying $PM_{2.5}$ mass were also elevated corresponding to exposures on individual lag days 0-7 as well as 2-5 day moving averages (e.g., an IQR increase in $PM_{2.5}$ mass on lag day 0 resulted in a 0.15% change in IL-6 in unadjusted analyses [95% CI: 0.06, 0.24] and fully-adjusted analyses [95% CI: 0.07, 0.24]). Associations of time-varying EC with changes in IL-6 were weak and did not reach statistical significance (e.g., -0.02% change [95% CI: -0.14, 0.10] for an IQR increase in weekly average exposure in fully adjusted models).

Sensitivity Analyses

Sensitivity analyses were conducted in separate fully adjusted models for each exposure (weekly average $PM_{2.5}$ and EC, as well as lag days 0-7 and moving averages of 2-5 days for $PM_{2.5}$) and outcome (hs-CRP, fibrinogen and IL-6) combination. Our results did not appreciably change with the addition of seasonality or fasting status as covariates (data not shown). We found no evidence of effect modification by sex, race/ethnicity or

smoking status. Additionally, the results did not considerably change in models restricted to include only non-moving participants or those age 10 years and older. We also considered the effects of an IQR increase in baseline levels of $PM_{2.5}$ mass and EC on changes in inflammation, and the majority of the observed associations were weak and did not reach statistical significance (Appendix 2, Tables A1 and A2).

4.5 Discussion

In this population of children and youth with T1D, elevations in IL-6 were associated with increased $PM_{2.5}$ mass exposures over the week prior to the biomarker assessment in a longitudinal, repeat-measures analysis over a period of 1 to 5 years. Associations of time-varying $PM_{2.5}$ mass with changes in hs-CRP and fibrinogen were weaker and less consistent. EC exposure was not associated with changes in hs-CRP, fibrinogen or IL-6. To the best of our knowledge, this was the first longitudinal study to examine associations of short-term exposures to traffic-related air pollutants, including $PM_{2.5}$ mass and EC, with changes in measures of inflammation among youth with T1D.

We observed consistent positive effects of time-varying exposures to $PM_{2.5}$ mass (corresponding to an IQR increase in weekly average exposure, as well as individual lag days 0-7 and 2-5 day moving averages) on changes in IL-6. A cross-sectional analysis using data from the same cohort also reported elevations in IL-6 associated with increased short-term exposures to $PM_{2.5}$ mass (Puett et al. 2018). Though direct comparisons with other studies are not possible due to the dearth of information among children and youth with T1D, we present here studies of adults, predominately with type 2 diabetes (T2D) for information purposes. T1D and T2D are both chronic diseases that affect glucose

regulation; however, T1D is an autoimmune disease with deficient insulin production whereas T2D is characterized by insulin resistance. In a repeat measures study of 22 adults with T2D (mean age 61 years), Schneider et al. (2010) reported significant increases in IL-6 in association with an increase of 10 $\mu g/m^3$ in $PM_{2.5}$ on lag day 2 (percent change of mean level: 20.2% [95% CI: 6.4, 34.1]). Also in line with our results, the authors found no significant associations of $PM_{2.5}$ exposures with hs-CRP or fibrinogen. However, in a longitudinal panel study of 187 adults (mean age 66.4 years) with T2D or impaired glucose tolerance, Rückerl and colleagues (2014) found no associations of IL-6 levels with $PM_{2.5}$ mass exposures on lag days 0-4 or the 5-day average.

Several other longitudinal studies of healthy adults have observed weak or null relationships between acute exposures to $PM_{2.5}$ mass and changes in IL-6. A recent analysis using repeat measures of inflammation from the Framingham Heart Study (n=3996; average age 53.6 years) found that increases of 5 $\mu g/m^3$ in 1-7 day moving averages of $PM_{2.5}$ mass were positively but not significantly associated with elevations in IL-6 (Li et al. 2017). However, the authors reported stronger positive effects among participants with diabetes. Thompson and colleagues (2010) conducted a repeat-measures analysis of 45 healthy adults (average age 26.2 years) and reported weak positive associations between $PM_{2.5}$ mass exposures on lag days 1-5 and changes in IL-6, but none reached statistical significance. Another longitudinal analysis using data from the Multi-Ethnic Study of Atherosclerosis (MESA) (n=2253; average age 62 years), reported null findings for changes in IL-6 associated with a 5 $\mu g/m^3$ increase in time-varying $PM_{2.5}$ mass concentrations on lag day 0, lag day 1, and moving averages of 2-5 days (e.g., for lag 0, 0% change [95% CI: -1, 1]) (Hajat et al. 2015). One possible explanation for

inconsistencies with our study results is that individuals with T1D may be more susceptible to the effects of $PM_{2.5}$ mass, a traffic-related pollutant, on the procoagulant cytokine IL-6. In addition, age could also play a role, with younger ages more vulnerable.

We did not find significant effects of time-varying $PM_{2.5}$ mass or EC on changes in fibrinogen or hs-CRP. A longitudinal analysis of data from the Normative Aging Study (average age 73.2 years), reported analogous findings for associations between $PM_{2.5}$ mass exposures (24-hour average, 3-day moving average and weekly average) and changes in fibrinogen and CRP (Bind et al. 2012). Similar exposures to black carbon (BC) were also not associated with changes in CRP; however, in contrast to our findings, the authors reported a 2.6% elevation in fibrinogen (95% CI: 0.9, 4.3%) for an IQR increase in BC at a 3-day moving average. Another repeat-measures study of 2,086 midlife women (average age 46.3 years) also reported no significant associations of 1-day exposures to $PM_{2.5}$ mass with changes in hs-CRP (0.1% increase [95% CI: -1.7, 1.9]) and fibrinogen (1.4% increase [95% CI -0.3, 3.1]) for a 10 $\mu g/m^3$ increase in time-varying $PM_{2.5}$ mass (Green et al. 2016). However, Li et al. (2017) found that 3-7 day moving averages of $PM_{2.5}$ mass were positively associated with changes in CRP. For example, a 5 $\mu g/m^3$ higher 5-day moving average $PM_{2.5}$ mass was associated with a 4.2% increase in CRP (95% CI: 0.8, 7.6). In that study, an increase of 0.5 $\mu g/m^3$ in 5-day moving average BC was also associated with 5.8% higher CRP levels (95% CI: 0.5, 11.4) while associations of $PM_{2.5}$ mass and BC exposure with fibrinogen were largely negative in the direction of effect. In addition, Hajat and colleagues (2015) reported weak positive associations between time-varying exposures to $PM_{2.5}$ mass on lag day 0 with changes in CRP (1% difference [95% CI: 0, 3]) and fibrinogen (1.16 mg/dl [95% CI: 0.28, 2.61]) corresponding to a 5 $\mu g/m^3$ increase in $PM_{2.5}$ mass.

Discrepancies with our results may be partially explained by differences in the characteristics of study participants (e.g. age differences) or locations.

Our most robust findings were for changes in short-term exposures to $PM_{2.5}$ mass over time with changes in IL-6 over time. Levels of IL-6 have been shown to predict future vascular events as well as correlate with endothelial dysfunction, arterial stiffness and sub-clinical atherosclerosis (Ridker 2016). IL-6 is a procoagultant cytokine that acts as a central mediator of the acute-phase inflammatory response (Gabay 2006). It directly influences the production of CRP, an acute-phase protein that is largely considered to be a downstream biomarker for atherothrombosis (Sesso et al. 2007; Ridker 2016). Fibrinogen is an end product of the coagulation cascade and a positive acute-phase protein that is also stimulated by IL-6 (Davalos and Akassoglou 2012). In relation to CRP and fibrinogen, IL-6 is further upstream in the inflammatory process (Ridker 2016). In previous work, longitudinal effects of $PM_{2.5}$ mass on changes in CRP and fibrinogen were stronger when longer moving averages of exposure were considered. For example, Green et al. (2016) reported a 21% change in hs-CRP (95% CI: 6.6, 37%) and a 5.2 mg/dl change in fibrinogen (95% CI: -4.5, 15) associated with a 10 $\mu g/m^3$ increase in time-varying annual average $PM_{2.5}$ mass concentrations. Due to inconsistencies in the epidemiologic evidence, additional longitudinal studies of air pollution and biomarkers of inflammation are indicated. Because the majority of existing work has focused on adult or elderly populations, future studies should include younger participants. In particular, youth with T1D have been overlooked in the existing literature.

Strengths of our study included the use of repeat blood measures from an established cohort with available data to account for a number of relevant confounding

factors in our analyses. In addition, ours is the first longitudinal study of air pollution and inflammation to focus on youth with T1D. Our study population was also racially and ethnically diverse. In addition, we utilized $PM_{2.5}$ mass and EC exposure estimates generated from a novel and extensively validated spatio-temporal statistical model that took into account various factors that could have influenced air pollution exposures, including daily meteorology, traffic intensity, and geographic variables such as land use and elevation.

Our study also had a few limitations. Similar to the majority of previous studies, we were unable to account for the role of exposure to secondhand smoke in the home. We also did not have available data on the amount of time that study subjects spent away from the home. In addition, our results could have been affected by loss to follow-up since only 30% of participants had a repeat measure of inflammation available. Our study also focused specifically on youth with T1D and is not generalizable to other populations.

In conclusion, the results of our repeat-measures study indicate that increased short-term exposures to $PM_{2.5}$ mass are associated with higher levels of IL-6, a biomarker of inflammation. These effects were consistent across all time periods of exposure considered and were robust to adjustment for a number of demographic and lifestyle covariates as well as several sensitivity analyses. Our findings suggest that changes in acute exposures to $PM_{2.5}$ mass over time may influence changes in inflammation over time early in the inflammatory process.

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4.7 Tables and Figures

Table 1. Descriptive statistics of the SEARCH study population at baseline

Characteristic	N (%) or Mean (SD)
Age	10.6 (4.1)
Female	292 (47.3)
Race/Ethnicity	
White	483 (78.2)
Other races	135 (21.8)
Study Site	
South Carolina	92 (14.9)
Ohio	208 (33.7)
Colorado	119 (19.3)
California	76 (12.3)
Washington	123 (19.9)
Smoking Status	
Never smoker	320 (51.8)
Tried smoking	50 (8.1)
Missing/not asked (<10 years old)	248 (40.1)
Number of days smoked in the past 30 days	2.1 (2.0)
Days of vigorous exercise in past week	
0	38 (6.2)
1-4	200 (32.4)
5-7	131 (21.2)
Missing/not asked (<10 years old)	249 (40.3)
Days of TV watching in past week	4.1 (1.5)
Days of computer use in past week	3.0 (1.5)
Percent below poverty in tract of residence	9.0 (8.9)
Outcome variables	
CRP, mg/dl (n=616)	0.09 (0.3)
Fibrinogen, mg/dl	341.9 (67.5)
IL-6, pg/ml (n=441)	16.1 (19.3)

Table 2. Characteristics of $PM_{2.5}$ and elemental carbon during the study period

Pollutant	Mean	SD	IQR	GM
Weekly avg. $PM_{2.5}$ mass, $\mu g/m^3$				
Baseline	11.1	5.0	6.2	2.4
Follow-up	9.4	4.0	5.7	2.2
Weekly avg. EC, ng/m^3				
Baseline	771.1	455.8	511.6	6.5
Follow-up	604.9	345.2	386.6	6.2

Table 3. Associations of time-varying $PM_{2.5}$ and elemental carbon with inflammatory biomarkers in unadjusted models

	CRP N=616	Fibrinogen N=618	IL-6 N=441
	% Change (95% CI)	% Change (95% CI)	% Change (95% CI)
EC, weekly avg.	0.02 (-0.12, 0.16)	-0.01 (-0.03, 0.01)	-0.01 (-0.13, 0.11)
$PM_{2.5}$ averaging time			
Weekly avg.	0.12 (-0.02, 0.25)	0.002 (-0.01, 0.02)	0.25 (0.15, 0.35)*
Lag 0	0.01 (-0.10, 0.12)	-0.001 (-0.02, 0.02)	0.15 (0.06, 0.24)*
Lag 1	0.05 (-0.05, 0.16)	-0.003 (-0.02, 0.01)	0.14 (0.06, 0.22)*
Lag 2	0.07 (-0.04, 0.17)	-0.0002 (-0.01, 0.01)	0.09 (0.01, 0.17)*
Lag 3	0.08 (-0.03, 0.19)	-0.001 (-0.02, 0.01)	0.13 (0.05, 0.22)*
Lag 4	0.11 (-0.004, 0.22)	0.01 (-0.01, 0.02)	0.21 (0.12, 0.29)*
Lag 5	0.08 (-0.03, 0.19)	0.01 (-0.01, 0.02)	0.19 (0.11, 0.28)*
Lag 6	0.08 (-0.03, 0.19)	0.003 (-0.01, 0.02)	0.24 (0.16, 0.33)*
Lag 7	0.05 (-0.06, 0.17)	-0.003 (-0.02, 0.01)	0.18 (0.09, 0.27)*
2-day moving avg.	0.11 (-0.02, 0.24)	0.003 (-0.01, 0.02)	0.23 (0.13, 0.33)*
3-day moving avg.	0.11 (-0.02, 0.24)	0.003 (-0.01, 0.02)	0.22 (0.12, 0.32)*
4-day moving avg.	0.11 (-0.02, 0.24)	0.003 (-0.01, 0.02)	0.22 (0.12, 0.31)*
5-day moving avg.	0.11 (-0.02, 0.24)	0.003 (-0.01, 0.02)	0.22 (0.12, 0.32)*

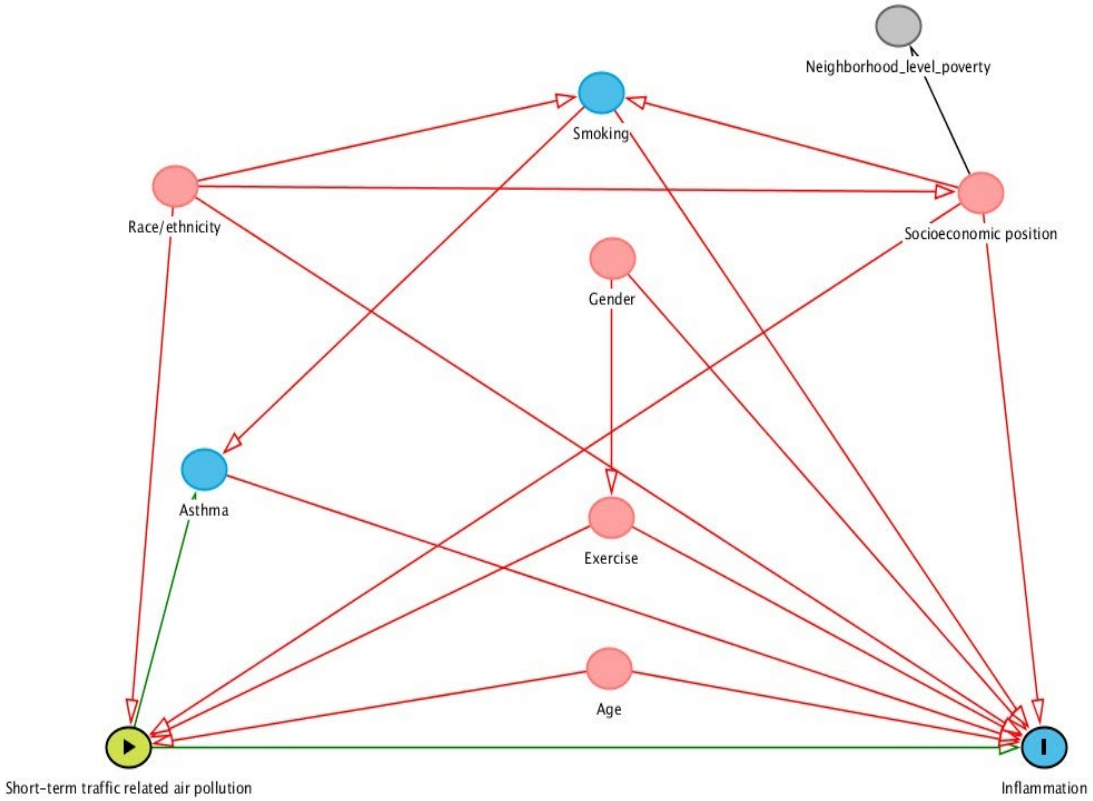
*$p<0.05$

Table 4. Associations of time-varying $PM_{2.5}$ and elemental carbon with inflammatory biomarkers in fully adjusted models

	CRP N=616	Fibrinogen N=618	IL-6 N=441
	% Change (95% CI)	% Change (95% CI)	% Change (95% CI)
EC, weekly avg.	-0.02 (-0.15, 0.12)	-0.02 (-0.03, 0.003)	-0.02 (-0.14, 0.10)
$PM_{2.5}$ averaging time			
Weekly avg.	0.08 (-0.05, 0.21)	-0.001 (-0.02, 0.01)	0.25 (0.15, 0.36)*
Lag 0	-0.01 (-0.11, 0.10)	-0.003 (-0.02, 0.01)	0.15 (0.07, 0.24)*
Lag 1	0.03 (-0.07, 0.14)	-0.01 (-0.02, 0.01)	0.15 (0.06, 0.23)*
Lag 2	0.04 (-0.06, 0.15)	-0.003 (-0.02, 0.01)	0.09 (0.01, 0.17)*
Lag 3	0.06 (-0.05, 0.17)	-0.003 (-0.02, 0.01)	0.13 (0.04, 0.22)*
Lag 4	0.09 (-0.02, 0.19)	0.01 (-0.01, 0.02)	0.21 (0.12, 0.29)*
Lag 5	0.06 (-0.05, 0.17)	0.004 (-0.01, 0.02)	0.19 (0.11, 0.28)*
Lag 6	0.06 (-0.05, 0.17)	0.001 (-0.01, 0.02)	0.25 (0.16, 0.33)*
Lag 7	0.03 (-0.08, 0.14)	-0.01 (-0.02, 0.01)	0.18 (0.09, 0.27)*
2-day moving avg.	0.08 (-0.05, 0.21)	-0.0001 (-0.02, 0.02)	0.23 (0.13, 0.33)*
3-day moving avg.	0.08 (-0.05, 0.21)	0.0001 (-0.02, 0.02)	0.22 (0.12, 0.32)*
4-day moving avg.	0.08 (-0.05, 0.21)	-0.0001 (-0.02, 0.02)	0.22 (0.12, 0.32)*
5-day moving avg.	0.08 (-0.05, 0.21)	0.0001 (-0.02, 0.02)	0.22 (0.12, 0.32)*

*$p<0.05$

Figure 1. Directed acyclic graph for the relationship between short-term exposure to traffic-related air pollution and inflammatory biomarkers



CHAPTER 5

The Relationship of Traffic-Related Air Pollution Exposures with Allostatic Load among Children and Youth with Type 1 Diabetes in the SEARCH Cohort

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5.1 Abstract

Background: Chronic exposures to traffic-related air pollutants may influence allostatic load (AL), particularly among sensitive subpopulations such as individuals with type 1 diabetes (T1D); however, these associations have not been explored in prior studies.

Objectives: We assessed the relationship of long-term exposures to particulate matter <2.5 μm in diameter ($PM_{2.5}$) and residential distance to major roadways with AL using data from the SEARCH for Diabetes in Youth Study (SEARCH). We also examined effect modification by sociodemographic factors.

Methods: Baseline questionnaires, anthropometric measures and blood were taken at an in-person clinic visit. A summary measure of AL was calculated using 10 biomarkers reflecting cardiovascular, metabolic and inflammatory risk. We estimated monthly and annual residential exposures to $PM_{2.5}$ mass and proximity to high traffic roadways for each participant prior to the clinic visit. Separate linear mixed models adjusted for demographic and lifestyle factors were conducted for each exposure.

Results: Among 2338 participants, no significant associations were observed for traffic-related air pollution exposures and AL variables in our main analysis (e.g., for an interquartile range [IQR] increase in annual average $PM_{2.5}$ exposure with AL, β=0.06, 95% CI: -0.07, 0.18). However, in effect modification analyses, stronger associations were observed among white compared to non-white participants for an IQR increase in monthly average $PM_{2.5}$ exposures with AL and the metabolic and inflammatory components, and for an IQR increase in annual average $PM_{2.5}$ exposures with inflammatory AL. Residing in close proximity to a heavily trafficked major road was associated with higher AL and

increased cardiovascular and metabolic AL among non-white compared to white participants, and higher cardiovascular AL among females compared to males.

Conclusions: Our results suggest that the effects of chronic exposures to traffic-related air pollution on AL and the individual AL subscales may differ by race/ethnicity and sex among youth with T1D.

5.2 Introduction

Allostatic load is a measure of cumulative biological risk that has been associated with adverse health outcomes such as cardiovascular disease (CVD) and is an independent predictor of all-cause morbidity and mortality (Logan and Barksdale 2008; Karlamangla, Singer and Seeman 2006). Prior research has linked certain physiologic parameters of AL such as increased blood pressure (BP) and elevations in inflammatory biomarkers including c-reactive protein (CRP), fibrinogen, and interleukin-6 (IL-6) with higher exposures to fine particulates and other traffic-related air pollutants (Hajat et al. 2015; Hoffmann et al. 2012). Although these studies suggest that AL may be influenced by environmental exposures such as ambient air pollution, currently there is a lack of data regarding these associations.

The concept of AL is intertwined with allostasis, or the ability of the body to successfully adapt to changing environments and stressful challenges (Logan and Barksdale 2008). Over time, frequent or chronic stimulation leads to wear and tear on the system as normal allostatic processes wear out or fail to engage. The resulting damage that accumulates is referred to as AL. In epidemiologic studies, AL is typically quantified using a multi-system framework that includes biomarkers of physiologic activity across a range of important regulatory systems (e.g., BP, body mass index [BMI], total cholesterol [TC]). Individual scores that represent the highest risk quartile (e.g., $\geq 75^{th}$ percentile) are allocated for each variable and summed based on the total number of parameters indicating high risk.

It has been hypothesized that individuals with diabetes experience chronic AL, which is thought to play a role in associated adverse health consequences, including CVD (Steptoe et al. 2014). There is also a sizeable body of evidence indicating that exposure to traffic-related air pollution is a risk factor for cardiovascular morbidity and mortality,

potentially through changes in systemic inflammatory responses, oxidative stress and/or vascular dysfunction (Brook et al. 2010; O'Neill et al. 2005). Furthermore, individuals with diabetes may be particularly vulnerable to the cardiovascular health effects associated with ambient air pollution. Increases in cardiovascular hospitalizations and mortality have been observed among individuals with diabetes during episodes of high ambient air pollution exposures (Zanobetti and Schwartz 2002; Goldberg et al. 2001). Although the underlying pathophysiology is not fully understood, existing evidence suggests that pro-inflammatory responses, chronic inflammation and/or changes in vascular function may play a role (Jacobs et al. 2010; Brook et al. 2010; O'Neill et al. 2005).

To date, there is a paucity of research investigating the relationship between exposures to traffic-related air pollutants and a summary index of AL. One prior study conducted in Taiwan examined the relationship of AL score with indoor $PM_{2.5}$ levels among office workers during a typical workday and reported no significant associations (Jung et al. 2014). To our knowledge, ours will be the first study to examine whether AL is associated with chronic exposures to outdoor concentrations of $PM_{2.5}$ and indicators of traffic-related air pollution. Additionally, we will focus on children and young adults with type 1 diabetes (T1D), a population with an increased long-term risk of CVD-related morbidity and mortality (Allemann et al. 2009). Although CVD events are generally not expected to occur during childhood, our findings will help uncover how early life factors may translate into increased risk for major health outcomes including CVD later in life.

Using data from the SEARCH for Diabetes in Youth Study (SEARCH), we examined whether AL was associated with chronic (monthly and annual) exposures to $PM_{2.5}$ and residential proximity to heavily trafficked major roads. We also assessed effect

modification of the associations by sociodemographic factors (e.g. race/ethnicity, gender) in order to investigate health disparities.

5.3 Methods

Study Design and Subjects

Participants included in this analysis were enrolled in the SEARCH for Diabetes in Youth Study (SEARCH), a multi-center observational study that maintains an active registry of US children and youth diagnosed with diabetes at age <20 years. The present analysis included participants from five SEARCH sites in the contiguous US: Ohio (eight counties surrounding Cincinnati, OH, including Butler, Clermont, Hamilton, and Warren counties in Ohio, as well as Boone, Campbell and Kenton counties in Kentucky, and Dearborn, Indiana), Colorado (statewide), Washington (five counties surrounding Seattle, including King, Kitsap, Pierce, Snohomish and Thurston), South Carolina (statewide), and California (health plan membership in seven counties of Southern California, including Los Angeles, Orange, Riverside, San Bernardino, Ventura, Imperial and Kern). Detailed study methods have been published elsewhere (Hamman et al. 2014). Briefly, SEARCH was initiated in 2000 with funding from the Centers for Disease Control and Prevention (CDC) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to address gaps in the understanding of diabetes in youth. SEARCH centers conduct active surveillance using networks of health care providers, hospitals, community health centers, clinical and administrative data systems, and electronic medical records. Case ascertainment is based on verification of physician diagnosed diabetes. Health care providers assign the clinical type for all registered cases. This analysis included participants

with type 1 diabetes (T1D) who completed a baseline in-person visit for anthropometric and laboratory measures, and collection of information on quality of care, quality of life, sociodemographic factors and health behaviors including diet and physical activity. The current study included prevalent cases identified in 2001 and incident cases from 2002-2005. The SEARCH protocol has been reviewed and approved by the Institutional Review Boards (IRBs) at each participating institution and is in compliance with the Health Insurance Portability and Accountability Act (HIPAA). Written informed consent was obtained from all participants age >18 years or from parents/legal guardians if <18 years.

Exposure Assessment

Residential addresses at the time of each clinic visit were geocoded to an X, Y coordinate system using ArcGIS 9.3 software (ESRI Inc., Redlands, CA) and Topologically Integrated Geographic Encoding and Referencing (TIGER) Road Network Files for 2000 and 2006 using a 30 m offset. Daily average concentrations of $PM_{2.5}$ mass were estimated across the conterminous US from 1999-2011 using spatio-temporal generalized additive mixed models (Yanosky et al. 2014; Yanosky et al. 2018). Average daily (24-hour) exposure estimates for $PM_{2.5}$ were assigned to each participant based on geocoded residential addresses, and monthly and annual averages were calculated corresponding to the month and the year prior to the clinic visit, respectively.

The spatio-temporal model included air pollution monitor measurements from the US Environmental Protection Agency's Air Quality System (AQS) database, the Interagency Monitoring of Protected Visual Environments (IMPROVE) network, and the Southern Aerosol Research and Characterization Study (SEARCH) network, and also

incorporated meteorological covariates that are known to influence pollutant dispersion such as wind speed, wind direction, air temperature, surface roughness, total precipitation and sensible heat flux, which were obtained from the MERRA Project (Goddard Earth Sciences Data and Information Services Center 2010) for the same time period as the monitored pollution concentrations. We obtained daily traffic counts from Geographic Data Technology, Inc. (Lebanon, NH) Dynamap Traffic Counts v4.2, which were spatially joined to the ESRI StreetMap Pro 2007 network of road segments to obtain the US Census Feature Class Codes rode class (A1 [primary roads or highways with limited access], A2 [primary roads or highways without limited access], A3 [secondary and connecting roads including state and county highways], A4 [local, neighborhood, and rural roads], and A6 [roads with special characteristics, including access ramps and exits]) and spatially averaged using ArcGIS. We additionally included county-level population data from the 2000 US Census (US Census Bureau 2013) and elevation data from the USGS National Elevation Dataset (USGS 2013). During development, the model was subject to extensive validation techniques. Predictive accuracy was moderate for 24-hr averages (CV R^2=0.532) and higher for longer averaging times (CV R^2=0.707 and 0.795 for monthly and annual averages, respectively).

Residential distance to the nearest road and concentrations of annual average daily traffic were calculated for road classes A1-A4. Roads with annual average daily traffic counts ≥10,000 vehicles/day were considered heavily trafficked. Our primary analysis focused on examining associations between long-term traffic-related air pollution exposures, including monthly and annual average $PM_{2.5}$ mass as well as residential proximity to a major road (classes A1-A3, collectively) with heavy traffic, and AL. We

categorized the distance to major roadways as <100 meters (m), 100-<200 m, 200-<400 m, and ≥400 m because existing research indicates that exposures to traffic-related air pollutants are highest within 400 m of a major road and concentrations decrease with distance (Zhou and Levy 2007; WHO 2013).

Assessment of Allostatic Load

The AL model was operationalized to include cardiovascular, metabolic and inflammatory biomarkers guided by prior research (Jung et al. 2014; Rainisch and Upchurch 2013; Robertson et al. 2015; Bird et al. 2010) and based on SEARCH measures. In total, 10 physiological parameters were used to create a summary score of AL. Cardiovascular biomarkers included diastolic blood pressure (DBP) and systolic blood pressure (SBP). Inflammatory biomarkers included C-reactive protein (CRP) and fibrinogen. Biomarkers of metabolic risk included body mass index (BMI), glycated hemoglobin (HbA1c), high density lipoprotein (HDL), total cholesterol (TC), triglycerides and waist circumference (WC). For each of the indicators, cut-points identifying high risk were determined based on the sample distribution using the 75th percentile (with the exception of HDL, where the 25th percentile was utilized as the cut-point); youth who exhibited high risk levels of biomarkers received a score of 1 for that parameter. A composite AL index was then created by summing the total number of parameters identified as high risk. In this analysis, the range of AL scores is 0 to 10, with higher values signifying greater systemic dysregulation.

Blood pressure was measured three times; the second and third blood pressure determinations were used to calculate the mean systolic and diastolic blood pressure.

Height, weight and WC were measured in duplicate according to NHANES protocol in light clothing without shoes. Height and weight were measured to the nearest 0.5 cm and 0.1 kg using a stadiometer and digital scale, respectively. BMI was then calculated as weight in kilograms divided by the square of height in meters. WC was measured at the nearest 0.1 cm at the uppermost lateral border of the right ilium. The following measures were analyzed using a single aliquot of stored plasma from blood drawn at the baseline study visit after an 8 hour fast. HbA1c was measured using a dedicated ion-exchange high-performance liquid chromatography instrument (Tosoh Bioscience, San Francisco, CA). Measurements of TC, HDL-C and triglycerides were performed enzymatically on a Hitachi 917 autoanalyzer. CRP and fibrinogen were measured immunochemically using Siemens reagents on a nephelomether autoanalyzer (BNII).

Covariates

We constructed a directed acyclic graph (DAG) to assess potential sources of bias and confounding in our analyses (Figure 1). Based on the DAG and a review of the relevant literature, we considered the following variables as covariates: age, sex, race/ethnicity, physical activity, smoking status and socioeconomic status (SES) (Dowd, Simanek and Aiello 2009; Brody et al. 2014; Yang and Kozloski 2011; Mair, Cutchin and Peek 2011; Gay et al 2015; Yang and Kozloski 2011; Tomfohr, Pung and Dimsdale 2016).

Key demographic data were obtained at case ascertainment and verified at initial contact with the patient using a structured questionnaire that queried date of birth, date of diagnosis, gender, and race/ethnicity. Parental education level and family income were ascertained during the in-clinic visit in a parent/guardian interview. Neighborhood

affluence was assessed by using residential addresses to obtain the percentage of the population below poverty in the census tract of residence from the 2000 US Census (US Census 2000). Physical activity level and smoking status were assessed for patients age 10 or older by interviewer-administered questionnaire with questions based on the CDC-sponsored Youth Risk Behavior Surveillance System. Questions ascertained usual participation in both sedentary and non-sedentary activities, including the average amount of time per week spent watching television and playing video/computer games, and the average number of days per week spent engaging in physical activity that made them sweat or breathe hard for at least 20 minutes. Cigarette smoking was assessed by querying whether participants had ever smoked tobacco, and among those that had, how many days they smoked tobacco in the past 30 days. Parents/legal guardians were asked to waive their right to review their children's responses to the smoking questions.

Statistical Analysis

Our study population initially consisted of 3530 individuals. We excluded participants with missing information on biomarkers included in the AL score (n=436), exposure estimates (n=279), percent below poverty in the census tract of residence (n=29), clinic site (n=4), and gender (n=1). We additionally excluded individuals residing >1000 m from major roads (n=289) as well as those living within 1000 m of a major road if the annual average daily traffic count was <10,000 vehicles/day (n=96). The final study population for our analysis included 2338 participants.

Separate linear mixed models with study site included as a random effect were used to estimate associations between long-term $PM_{2.5}$ concentrations or residential distance to

major roadways with heavy traffic and AL score. The cardiovascular, inflammatory and metabolic dimensions of AL were also considered individually. Basic models were unadjusted. Demographic models included age (continuous), race/ethnicity (white, non-white), sex and percent below poverty in the census tract of residence (continuous). Fully adjusted models additionally included physical activity level (continuous), sedentary behaviors (continuous), history of smoking tobacco (never/ever tried smoking), and the number of days that the subject smoked tobacco in the past 30 days (continuous). For monthly and annual exposures to $PM_{2.5}$ (continuous), we calculated β estimates corresponding to an interquartile range (IQR) increase in pollutant exposure. For residential proximity to heavily trafficked major roadways (categorical), we evaluated associations for distances of <100 m, 100-<200 m, and 200-<400 m compared to living ≥400 m away. Effect modification by race/ethnicity, sex, smoking status and age (<10 years old, ≥10 years old) was assessed in fully adjusted models using interaction terms and stratification. We additionally conducted sensitivity analyses including participants residing >1000 m from major roads and those living in proximity to major roads where the traffic count did not exceed 10,000 vehicles/day, as well as evaluating the relationship of AL with distance to each of the major road classes individually. Seasonality was also considered as a covariate in sensitivity analyses. All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). Statistical significance was determined by p-values <0.05 corresponding to two-sided tests.

5.4 Results

We defined the AL score as the number of biomarkers that fell above the 75th percentile of the sample distribution for each measure (or fell below the 25th percentile for HDL). Cardiovascular, inflammatory and metabolic AL scores included only the biological indicators included in each category, respectively. The cut-off point for each component of AL and the number of participants considered to be high risk for each variable are displayed in Table 1.

Characteristics of the study population are presented in Table 2. Among 2338 participants, approximately 78% were white and 49% were female. The mean AL score was 2.6 (standard deviation [SD] 2.2). Average age was 12.5 years (SD 4.3), and the majority of subjects had never tried smoking (56%). Estimated monthly and annual average $PM_{2.5}$ concentrations were 13.0 (SD 5.8) and 13.4 (SD 5.2) $\mu g/m^3$, respectively. There was a strong correlation between monthly and yearly $PM_{2.5}$ exposure estimates (r= 0.86). Approximately 20% of participants resided within 100 m of a major road, 18% lived within 100-<200 m, 35% lived within 200-<400 m, and 28% resided ≥400 m away. Additional parameters are shown in Table 2.

In univariate models, an IQR increase in annual average $PM_{2.5}$ exposure was positively associated with AL (β= 0.30, 95% Confidence Interval [CI]: 0.11, 0.49), as well as the metabolic and inflammatory dimensions (β= 0.16, 95% CI: 0.04, 0.28; β= 0.11, 95% CI: 0.04, 0.18). However, after adjustment for covariates, the majority of these associations were attenuated below the level of statistical significance (e.g., for annual average $PM_{2.5}$ mass and AL score; β= 0.06, 95% CI: -0.07, 0.18). In our main analyses (Table 3), an IQR increase in monthly average $PM_{2.5}$ exposure was not associated with AL or any of the AL

subscales in fully adjusted models. An IQR increase in annual average $PM_{2.5}$ mass remained marginally associated with the inflammatory AL score in fully adjusted models (β= 0.05, 95% CI: -0.01, 0.11).

In our unadjusted distance to road analyses (Table 3), participants who lived <100 m from heavily trafficked major roadways had a significantly higher AL score than those who lived ≥400 m away (β= 0.32, 95% CI: 0.05, 0.58); the metabolic dimension was also elevated (β= 0.18, 95% CI: 0.001, 0.36). In addition, the inflammatory AL score was higher among those who lived <100 and 100-<200 m from a major road compared to those residing ≥400 away (β= 0.11, 95% CI: 0.03, 0.20; β= 0.10, 95% CI: 0.01, 0.20). These associations were attenuated below the level of statistical significance in fully adjusted models. No other consistent associations were observed between residential proximity to major roadways with high traffic and AL or the individual AL subscales.

In models adjusted for demographic and lifestyle covariates, we observed evidence of effect modification of the associations by race/ethnicity (Table 4). The total AL score was significantly higher among white participants (β= 0.04, 95% CI: -0.06, 0.15) and lower among non-white participants (β= -0.18, 95% CI: -0.36, -0.01) corresponding to an IQR increase in monthly average $PM_{2.5}$ mass (p interaction= 0.04). The metabolic and inflammatory AL components were also elevated among white participants (β= 0.06, 95% CI: -0.01, 0.14; β= 0.02, 95% CI: -0.02, 0.07) and lower among non-white participants (β= -0.11, 95% CI: -0.23, 0.01; β= -0.07, 95% CI: -0.14, 0.001) for an IQR increase in monthly average $PM_{2.5}$ mass (p interaction= 0.02 and 0.048, respectively). For an IQR increase in annual average $PM_{2.5}$ mass, the inflammatory AL score was higher among white participants (β= 0.10, 95% CI: 0.03, 0.18) compared to non-white participants (β= -0.06,

95% CI: -0.16, 0.03) (p interaction= 0.02). In the distance to road analysis, total AL was elevated among non-white participants (β= 0.73, 95% CI: 0.19, 1.3) and reduced among white participants (β= -0.09, 95% CI: -0.35, 0.17) residing 100-<200 m from roadways compared to participants living ≥400 m away (p interaction= 0.01). Cardiovascular AL was also higher among non-white subjects and lower among white subjects residing <100 m (β= 0.20, 95% CI: 0.02, 0.37; β= -0.05, 95% CI: -0.14, 0.04) and 200-<400 m (β= 0.21, 95% CI: 0.05, 0.37; β= -0.05, 95% CI: -0.12, 0.03) from high traffic roadways in comparison to participants residing ≥400 m away (p interaction= 0.02 and 0.01, respectively). Living 100-<200 m from major roadways with heavy traffic was also associated with a higher metabolic AL score among non-white participants (β= 0.35, 95% CI: -0.02, 0.72) and a lower metabolic AL score among white participants (β= -0.10, 95% CI: -0.28, 0.07) compared to living ≥400 m away (p interaction= 0.03). No other consistent differences were observed by race/ethnicity.

We also found some evidence of effect medication by sex (Table 5). Comparing participants residing <100 m from a heavily trafficked major road to those ≥400 m away, the cardiovascular AL score was higher among females (β= 0.10, 95% CI: -0.01, 0.21) and lower among males (β= -0.10, 95% CI: -0.22, 0.01) (p interaction = 0.02). The cardiovascular AL score was also elevated among females living 100-<200 m away from heavily trafficked major roadways (β= 0.09, 95% CI: -0.03, 0.20) and lower among males (β= -0.10, 95% CI: -0.22, 0.01) compared to participants residing ≥400 m away (p interaction = 0.01). No other consistent differences were observed by sex.

The observed associations did not differ by age or smoking status. In sensitivity analyses, the addition of participants residing >1000 m from major roadways and subjects

who lived near major roads that were not heavily trafficked did not substantially affect our results. The addition of seasonality as a covariate in our fully adjusted models did not appreciably alter the observed associations. We also evaluated associations between proximity to major roadways with AL and the AL subscales by individual road class (e.g., A1, A2, and A3) in separate models, and no significant associations were observed.

5.5 Discussion

In this study of children and young adults with T1D, we did not observe overall associations between long-term exposures to traffic-related air pollution exposures and AL or the individual AL subscales in our main analyses. However, several of the reported associations were modified by race/ethnicity or sex. An IQR increase in monthly average $PM_{2.5}$ exposure was associated with higher total AL as well as increased cardiovascular and inflammatory AL scores among white compared to non-white participants; higher annual average exposure to $PM_{2.5}$ was also linked with increased inflammatory AL in white participants. Among those residing in close proximity to major roadways with heavy traffic, total AL and the cardiovascular and metabolic dimensions of AL were elevated among non-white compared to white participants. We also observed sex differences in the cardiovascular AL score among female participants residing <100 m and 100-<200 m from a major high traffic roadway compared to males. To our knowledge, these relationships have not been examined in the existing literature.

We identified one prior study that investigated the relationship of indoor $PM_{2.5}$ exposure with AL among 115 office workers in Taiwan (Jung et al. 2014). $PM_{2.5}$ concentrations were measured during an 8-hour workday using aerosol monitors.

Biomarkers included in the AL score were BMI, body fat percentage, SBP, DBP, heart rate, IL-6, tumor necrosis factor alpha, cortisol, epinephrine, norepinephrine, and creatinine. Indoor $PM_{2.5}$ exposure was not significantly associated with total AL (β= -0.014, SE= 0.006) or the cardiovascular/respiratory (β= -0.002, SE= 0.003), anthropometric (β= -0.002, SE= 0.003), neuroendocrine (β= -0.006, SE= 0.003), immune (β= -0.006=5, SE= 0.003) and metabolic (β= -0.002, SE= 0.008) components. Direct comparisons with our findings are difficult due to differences in exposure duration and assessment of AL; however, our main results for monthly average $PM_{2.5}$ exposures were relatively similar in magnitude.

In the present study, we reported effect modification of the associations between traffic-related air pollutant exposures and AL and its subscales by race/ethnicity. Although these relationships have not been examined in prior studies, racial/ethnic differences in AL have been widely reported in the existing literature. Many studies have indicated that racial/ethnic minorities have higher overall AL load scores compared to their white counterparts, and that racial/ethnic differences in AL exist among younger age groups including adolescents (Duru et al. 2012; Santos-Lozada and Daw 2018; Rainisch and Upchurch 2013). We report mean AL scores of 3.0 and 2.5 for non-white and white participants, respectively. In comparison, Rainisch and Upchurch (2013) reported average AL scores of 2.81, 2.41 and 2.38 for non-Hispanic black, Mexican American and non-Hispanic white adolescents aged 12-19 years.

In our study, chronic exposures to $PM_{2.5}$ were associated with higher AL and increased inflammatory and metabolic AL scores in white participants, whereas residence in close proximity to major roadways with heavy traffic was associated with higher AL

and increased cardiovascular and metabolic AL scores in non-white participants. Prior studies have had limited power to explore racial/ethnic differences in the associations between exposure to ambient air pollutants and cardiovascular or metabolic outcomes. A recent analysis which investigated racial differences in exposures to $PM_{2.5}$ and black carbon (BC) and their association with CVD risk factors reported that black participants had a higher rate of incident CVD events and all-cause mortality than whites that was only partially explained by higher exposure to $PM_{2.5}$ (Erqou et al. 2018). It has been hypothesized that the effects of psychological stress and environmental pollutants are often concurrent and may produce synergistic effects leading to worse outcomes among racial/ethnic minorities and economically disadvantaged populations (Wright 2011). The complex interrelationships between socioeconomic status, race, and exposure to environmental pollutants requires further study.

We also identified sex differences in the association between residential proximity to heavily trafficked major roadways and the cardiovascular dimension of AL, with stronger associations reported among females compared to males. Several previous studies have indicated that long-term exposure to ambient air pollutants increases the risk of CVD in women, although findings have been somewhat mixed regarding gender differences in susceptibility (Pope and Dockery 2006; Miller et al. 2004; Gehring et al. 2006; Chen et al 2005). Our results indicating that sex may modify the effects of traffic-related air pollution on cardiovascular AL adds support to the existing body of literature.

Several biomarkers that are typically included as components in the multi-system AL index have been associated with ambient air pollution exposures in previous studies. Both short and long-term exposures to $PM_{2.5}$ have been shown to increase BP

measurements, particularly among high cardiovascular risk individuals and persons with diabetes (Giorgine et al. 2016; Hoffmann et al. 2012). Similarly, inflammatory biomarkers such as CRP have also been associated with exposure to $PM_{2.5}$ (Li et al. 2017; Hajat et al. 2015). However, prior studies in the existing air pollution literature have not considered AL as an outcome measure. Although total AL score was not significantly associated with our exposure metrics after adjustment for demographic and lifestyle covariates in the main analysis, the null results may be partially explained by factors related to the length of our study or characteristics of the study population. For example, alterations in the physiologic parameters included in the AL score might be more strongly associated with cumulative exposure. In addition, our study population was comprised of children and young adults who typically have lower AL scores than older adults or the elderly (Crimmins et al. 2003). Future work should incorporate additional age groups and consider different time periods of exposure across the lifespan.

Strengths of our study include the large sample size and racial/ethnic diversity. In addition, the availability of data from the established SEARCH study allowed us to incorporate multiple parameters of physiologic functioning in our assessment of AL, many of which were included in the original operational definition (Seeman et al. 1997). Furthermore, we utilized a novel spatiotemporal model with high resolution to estimate monthly and annual average $PM_{2.5}$ concentrations at the residential address of each participant, and we additionally included residential distance to major roadways as an exposure metric.

Our study also has limitations. We estimated exposures at participants' residential addresses and do not have available data on the amount of time that subjects spent away

from the home. We were also unable to include information on exposure to second-hand smoke in the household. In addition, the biomarker measurements included in the AL score represent biological activity at a single point in time and may not characterize typical physiologic activity for an individual or reflect variability in the measures. We were also unable to incorporate biomarkers of immune or neuroendocrine function, such as cortisol or epinephrine, in the AL score due to lack of data availability.

In conclusion, ours is the first study to investigate the effects of long-term exposures to traffic-related air pollution on AL. While we did not find associations between monthly or annual average $PM_{2.5}$ exposures or proximity to heavily trafficked major roadways and AL scores in our main analysis, our results indicate effect modification by race/ethnicity and sex. Additional research is needed to confirm or refute these findings. In particular, the racial/ethnic and sex differences should be further explored.

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3.7 Tables and Figures

Table 1. Allostatic load variables among participants from SEARCH for Diabetes in Youth

Variable Name	High Risk Threshold	High Risk n, %
Cardiovascular Risk Factors		
DBP (mmHg)	≥72.0	608 (26.0)
SBP (mmHg)	≥111.3	621 (26.6)
Inflammatory Risk Factors		
CRP (mg/dL)	≥0.133	588 (25.2)
Fibrinogen (mg/dL)	≥386.0	591 (25.3)
Metabolic Risk Factors		
BMI (kg/m^2)	≥23.9	589 (25.2)
HbA1c (%)	≥8.9	643 (27.5)
HDL (mg/dL)	≤46.0	639 (27.3)
TC (mg/dL)	≥185.0	602 (25.8)
Triglycerides (mg/dL)	≥92.0	588 (25.2)
WC (cm)	≥83.6	585 (25.0)

Table 2. Summary statistics among SEARCH for Diabetes in Youth participants

Variable	N (%) or Mean (SD)
Age (years)	12.5 (4.3)
Female	1146 (49.0)
Race/Ethnicity	
Non-white	525 (22.5)
White	1813 (77.5)
Smoking	
Ever tried cigarettes, even one or two puffs	403 (17.2)
Never tried cigarettes	1302 (55.7)
Missing	633 (27.1)
Days of vigorous exercise in past week	
0	241 (10.3)
1-4	932 (39.9)
5-7	531 (22.7)
Missing	634 (27.1)
Clinic	
South Carolina	199 (8.5)
Ohio	503 (21.5)
Colorado	713 (30.5)
California	302 (12.9)
Washington	621 (26.6)
Percent below poverty in census tract of residence	8.2 (0.08)
Allostatic Load Score	2.6 (2.2)
Cardiovascular	0.5 (0.7)
Metabolic	1.6 (1.5)
Inflammatory	0.5 (0.7)
Monthly average $PM_{2.5}$ ($\mu g/m^3$)	13.0 (5.8)
Median (IQR)	10.9 (6.7)
Annual average $PM_{2.5}$ ($\mu g/m^3$)	13.4 (5.2)
Median (IQR)	11.1 (8.0)
Distance to nearest major road (m)	
<100	465 (19.9)
100-<200	413 (17.7)
200-<400	806 (34.5)
≥400	654 (28.0)

Table 3. Associations of monthly and annual average $PM_{2.5}$ concentrations and residential distance to heavily trafficked major roadways with allostatic load among youth with T1D

	N	Total AL		AL subscales					
				Cardiovascular		Metabolic		Inflammatory	
		B	95% CI	β	95% CI	β	95% CI	β	95% CI
Unadjusted Results									
Monthly Average $PM_{2.5}$, µg/m^3	2338	-0.004	-0.15, 0.14	-0.02	-0.07, 0.03	0.01	-0.08, 0.11	0.02	-0.03, 0.06
Annual Average $PM_{2.5}$, µg/m^3	2338	0.30	0.11, 0.49*	0.04	-0.03, 0.12	0.16	0.04, 0.28*	0.11	0.04, 0.18*
Distance category, m									
<100	465	0.32	0.05, 0.58*	0.02	-0.06, 0.11	0.18	0.001, 0.36*	0.11	0.03, 0.20*
100-<200	413	0.06	-0.22, 0.33	-0.04	-0.13, 0.05	-0.01	-0.20, 0.17	0.10	0.01, 0.20*
200-<400	806	0.15	-0.08, 0.38	0.01	-0.06, 0.09	0.07	-0.08, 0.23	0.07	-0.01, 0.14
Fully Adjusted Results									
Monthly Average $PM_{2.5}$, µg/m^3	2338	-0.02	-0.11, 0.07	-0.02	-0.06, 0.02	0.01	-0.05, 0.08	-0.003	-0.04, 0.03
Annual Average $PM_{2.5}$, µg/m^3	2338	0.06	-0.07, 0.18	0.002	-0.07, 0.07	0.04	-0.04, 0.13	0.05	-0.01, 0.11
Distance category, m									
<100	465	0.10	-0.13, 0.32	-0.006	-0.09, 0.07	0.04	-0.12, 0.19	0.05	-0.03, 0.14
100-<200	413	0.06	-0.17, 0.30	-0.01	-0.10, 0.07	-0.01	-0.17, 0.15	0.07	-0.02, 0.16
200-<400	806	-0.02	-0.22, 0.17	-0.01	-0.08, 0.06	-0.03	-0.17, 0.10	0.01	-0.06, 0.09

* = Statistical significance at $p<0.05$

Table 4. Associations of monthly and annual average $PM_{2.5}$ concentrations and residential distance to major roadways with allostatic load in fully adjusted models among youth with T1D, stratified by race/ethnicity

				AL Subscales					
	N	Total AL		Cardiovascular		Metabolic		Inflammatory	
		β	95% CI	β	95% CI	β	95% CI	β	95% CI
Non-White Participants									
Monthly Average $PM_{2.5}$, $\mu g/m^3$	525	-0.18	-0.36, -0.01*	-0.01	-0.07, 0.05	-0.11	-0.23, 0.01	-0.07	-0.14, 0.001
Annual Average $PM_{2.5}$, $\mu g/m^3$	525	-0.14	-0.38, 0.09	0.01	-0.07, 0.08	-0.09	-0.25, 0.07	-0.06	-0.16, 0.03
Distance category, m									
<100	128	0.43	-0.09, 0.95	0.20	0.02, 0.37*	0.19	-0.17, 0.55	0.04	-0.16, 0.24
100-<200	108	0.73	0.19, 1.3*	0.16	-0.02, 0.34	0.35	-0.02, 0.72	0.23	0.02, 0.44*
200-<400	189	0.39	-0.10, 0.87	0.21	0.05, 0.37*	0.17	-0.16, 0.50	0.01	-0.18, 0.20
White Participants									
Monthly Average $PM_{2.5}$, $\mu g/m^3$	1813	0.04	-0.06, 0.15	-0.02	-0.07, 0.03	0.06	-0.01, 0.14	0.02	-0.02, 0.07
Annual Average $PM_{2.5}$, $\mu g/m^3$	1813	0.15	-0.01, 0.32	-0.01	-0.09, 0.07	0.10	-0.002, 0.20	0.10	0.03, 0.18*
Distance category, m									
<100	337	0.05	-0.20, 0.31	-0.05	-0.14, 0.04	0.02	-0.15, 0.20	0.07	-0.03, 0.17
100-<200	305	-0.09	-0.35, 0.17	-0.04	-0.13, 0.06	-0.10	-0.28, 0.07	0.04	-0.06, 0.14
200-<400	617	-0.10	-0.31, 0.12	-0.05	-0.12, 0.03	-0.07	-0.22, 0.07	0.02	-0.06, 0.10

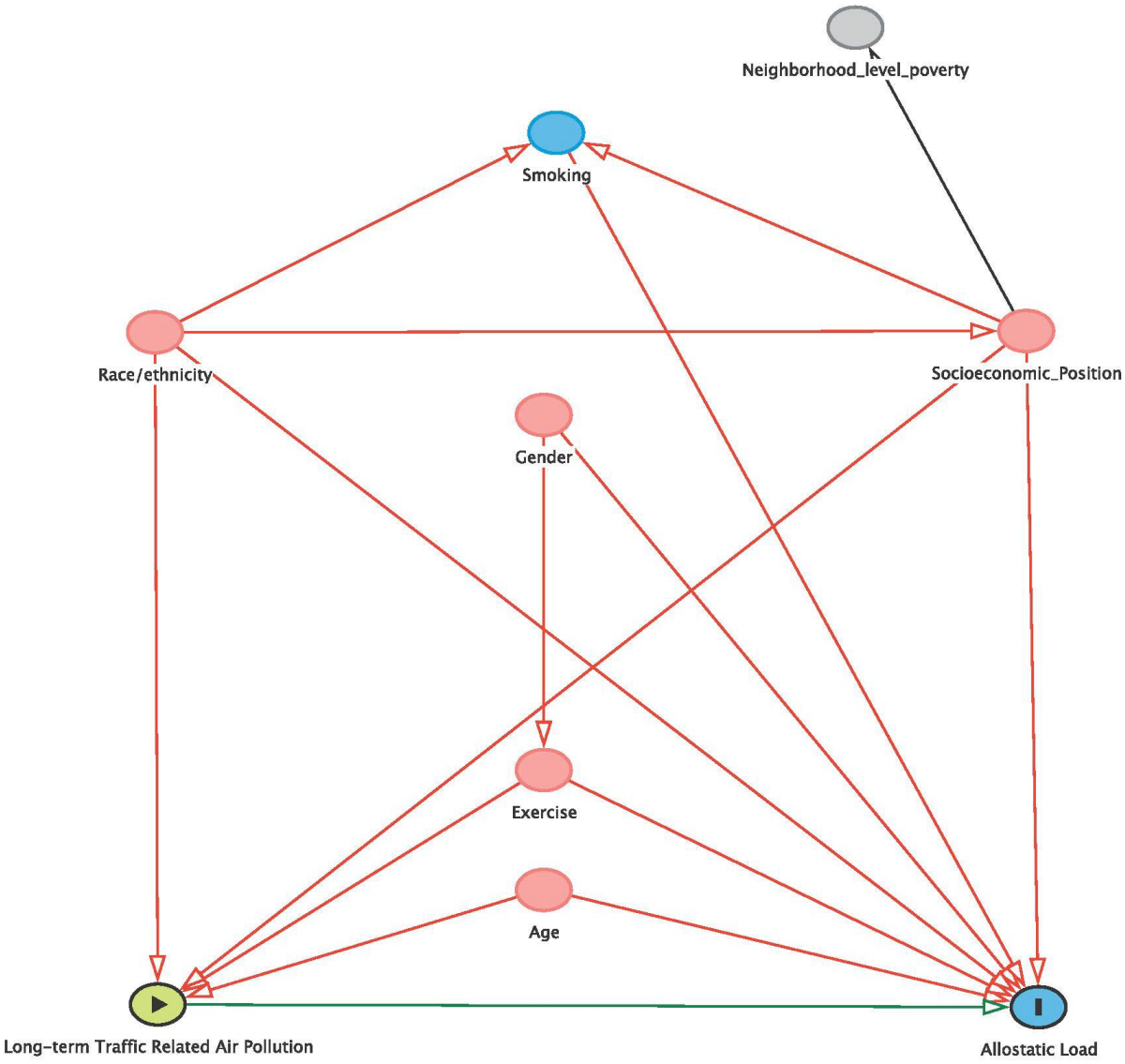
* = Statistical significance at $p<0.05$

Table 5. Associations of monthly and annual average $PM_{2.5}$ concentrations and residential distance to high traffic roadways with allostatic load among youth with T1D, stratified by sex

				AL Subscales					
	N	Total AL		Cardiovascular		Metabolic		Inflammatory	
		β	95% CI	β	95% CI	β	95% CI	β	95% CI
Female Participants									
Monthly Average $PM_{2.5}$, $\mu g/m^3$	1146	0.05	-0.08, 0.18	0.005	-0.05, 0.06	0.03	-0.05, 0.12	0.02	-0.03, 0.08
Annual Average $PM_{2.5}$, $\mu g/m^3$	1146	0.18	-0.02, 0.38	0.05	-0.04, 0.14	0.08	-0.05, 0.20	0.11	0.02, 0.20*
Distance category, m									
<100	235	0.30	-0.03, 0.63	0.10	-0.01, 0.21	0.12	-0.11, 0.34	0.07	-0.06, 0.20
>100-200	201	0.12	-0.22, 0.46	0.09	-0.03, 0.20	-0.03	-0.26, 0.20	0.05	-0.08, 0.19
>200-400	399	0.01	-0.28, 0.30	0.02	-0.08, 0.11	-0.01	-0.20, 0.19	-0.002	-0.12, 0.11
Male Participants									
Monthly Average $PM_{2.5}$, $\mu g/m^3$	1192	-0.11	-0.23, 0.02	-0.04	-0.10, 0.02	-0.02	-0.11, 0.07	-0.04	-0.09, 0.01
Annual Average $PM_{2.5}$, $\mu g/m^3$	1192	-0.05	-0.23, 0.12	-0.04	-0.12, 0.05	0.002	-0.12, 0.12	-0.01	-0.07, 0.05
Distance category, m									
<100	230	-0.11	-0.43, 0.20	-0.10	-0.21, 0.01	-0.05	-0.27, 0.16	0.03	-0.08, 0.15
>100-200	212	-0.01	-0.33, 0.31	-0.10	-0.22, 0.01	-0.02	-0.24, 0.20	0.10	-0.02, 0.22
>200-400	407	-0.06	-0.33, 0.20	-0.03	-0.12, 0.07	-0.07	-0.25, 0.12	0.02	-0.08, 0.12

* = Statistical significance at $p<0.05$

Figure 1. Directed Acyclic Graph (DAG) for the relationship of long-term traffic-related air pollution exposures with allostatic load



CHAPTER 6

CONCLUSIONS

This dissertation focuses on the pre-clinical cardiovascular impacts of traffic-related air pollution in a population of children and young adults with T1D. In the previous three chapters, we investigated: 1) associations of short-term exposures to $PM_{2.5}$, ADMS-Roads traffic-related PM and EC with PWV, 2) relationships between short-term exposures to $PM_{2.5}$ and EC with repeat measures of CRP, fibrinogen and IL-6, and 3) associations of long-term exposures to $PM_{2.5}$ and residential distance to the nearest major high traffic roadway with AL and its individual subscales (e.g. cardiovascular, inflammatory and metabolic AL). Our findings suggest that acute and chronic exposures to ambient air pollutants impact measures of arterial stiffness, biomarkers of inflammation and AL among youth with T1D. More specifically, an IQR increase in $PM_{2.5}$ mass exposure on the day of the PWV assessment was associated with increased PWV in the first manuscript (Chapter 3). In the second manuscript (Chapter 4), an IQR increase in fine particulates was associated with elevated levels of IL-6 corresponding to weekly average $PM_{2.5}$ exposure, as well as exposures on individual lag days 0-7 and moving averages of 2-5 days. In our final analysis (Chapter 5), no significant associations were observed for monthly and annual exposures to $PM_{2.5}$ mass or residential proximity to high traffic roadways and AL variables in the main analysis; however, we identified effect modification by race/ethnicity and sex. Stronger associations were observed among white compared to non-white participants for an IQR increase in monthly average $PM_{2.5}$ mass with total AL as well as the metabolic and inflammatory components, and for an IQR increase in annual average

$PM_{2.5}$ mass with inflammatory AL. Residing in close proximity to a heavily trafficked major road was associated with higher AL and increased cardiovascular and metabolic AL among non-white compared to white participants, as well as higher cardiovascular AL among females compared to males. Overall, these results add to the growing body of epidemiologic evidence suggesting that air pollution is associated with CVD-related risk factors. In the concluding chapter, we discuss the strengths and limitations of this work, in addition to public health implications. We also suggest directions for future research.

6.1 Strengths

This work has many notable strengths. For all three studies, we utilized existing data from a large, on-going, multi-center study (SEARCH), which was both time and cost efficient. The use of detailed SEARCH data also allowed us to account for a number of relevant confounding factors in our analyses. In addition, our study population encompassed a large age range, including children and young adults diagnosed with T1D between the ages of 0-19 years, and was representative of the US population of youth <20 years of age (Hamman et al. 2014). This signifies a substantial departure from the bulk of existing air pollution-related research in persons with diabetes, which has largely been conducted among adults and the elderly – the majority of whom have T2D. Very little existing research has focused on the impact of air pollution in children with diabetes – the majority of whom have T1D. Therefore, we addressed a major gap in the epidemiologic evidence by focusing on a high-CVD risk population that has received little attention to date. Furthermore, our study population was racially and ethnically diverse, affording us the ability to investigate potential disparities in health by race/ethnicity – which the

majority of previous studies have been unable to assess. We also utilized novel spatio-temporal statistical models that underwent an extensive validation process to estimate concentrations of air pollutants at the residential address of each participant for each specific time period of exposure considered.

Additionally, in the first manuscript, we utilized PWV measurements to non-invasively assess arterial stiffness, which is currently considered the gold standard method. Also, few existing studies have assessed associations of PWV with short-term exposures to traffic-related air pollution, and none have specifically focused on children and young adults or populations with T1D.

A major strength of our second manuscript is the ability to incorporate longitudinal data on measures of inflammation and air pollution exposures because the majority of prior work has been cross-sectional. We also advanced the understanding of the impact of air pollution exposures on inflammatory processes, a step towards the longer-term goal of elucidating the biological mechanisms underlying the relationship between ambient air pollution and CVD.

Our third manuscript is the first study to investigate the effects of outdoor air pollution on AL and its individual subscales, thereby setting the stage for future work. This will be particularly important because we identified differences in the associations by race/ethnicity and gender, which require further exploration. Moreover, another strength of this analysis is that our assessment of AL incorporated many of the biomarkers that were included in the original operational definition of AL.

6.2 Limitations

There are also limitations to this research. A major limitation of our exposure models is that we were only able to estimate concentrations of air pollutants at the residential address of each participant, as we did not have available information on the time-activity patterns of study participants. This may have introduced exposure misclassification, which we expect to be non-differential. Although environmental tobacco smoke is known to influence exposures to PM (Mueller et al. 2011), we were unable to assess exposure to secondhand smoke in the household of residence. We were also unable to account for the role of traffic noise, which has been linked with factors related to CVD morbidity and mortality in prior studies (Münzel et al. 2018; Stansfeld 2015). In addition, this research is specifically focused on children and young adults with T1D and has limited generalizability to other populations. Furthermore, certain covariates included in our statistical models, such as physical activity and smoking behaviors, were only assessed in SEARCH participants >9 years of age, which may have introduced some bias. Another consideration is that our study population was comprised of the 60% of participants who completed an in-clinic SEARCH visit, which raises the possibility of selection bias.

In the first manuscript, we were limited by a small study population (approximately 350 individuals) because PWV has only been assessed in a subset of SEARCH participants with T1D to date. Although we detected positive relationships between $PM_{2.5}$ variables and PWV, the majority of these associations did not reach the level of statistical significance. Therefore, low power to detect significant associations may have been an important limiting factor.

In our second manuscript, we were also limited by a relatively small study population (n=618 for fibrinogen, n=616 for CRP, n=441 for IL-6) and therefore limited power to detect significant associations due to a low number of SEARCH participants with repeat measures of inflammatory biomarkers. Although we identified consistent associations between time-varying $PM_{2.5}$ measures and changes in IL-6 over the week prior to the biomarker assessment, resulting changes in inflammation were relatively small in magnitude (e.g., 0.25% increase in IL-6 for an IQR increase in weekly average $PM_{2.5}$ mass). In addition, because the analysis was longitudinal, the possibility remains that some participants may have been lost to follow up in-between study visits.

In the final manuscript, we were unable to incorporate immune and/or neuroendocrine biomarkers – such as cortisol and norepinephrine – into our assessment of AL due to a lack of data availability. In addition, all of the biomarker measurements included in the AL score represent snapshots of a single point in time and do not reflect inherent variability in the measures; they also may not characterize the typical range of physiologic activities for an individual.

6.3 Public Health Implications

Overall, this research adds supports to the existing body of evidence which suggests that ambient air pollution is associated with risk factors for CVD. Our research focused on identifying these risks at an earlier age than the majority of previous studies that have primarily focused on adults or the elderly, which could potentially result in a lifetime risk reduction.

In addition, our results have the potential to advance the development of ambient air quality guidelines, which could help reduce the substantial impacts of air pollution-related CVD risks. This is especially important because exposure to ambient air pollution is pervasive, and CVD is one of the leading causes of morbidity and mortality not only in the US, but also globally. The World Health Organization (WHO) estimates that worldwide exposure to ambient air pollution accounts for approximately 25% of all deaths from ischemic heart disease (WHO 2019). Therefore, even small reductions in CVD risk could have a major positive impact on public health. Furthermore, youth with T1D have a high lifetime risk of developing CVD and may be considered a susceptible subpopulation with regard to the effects of air pollution on CVD. Air quality regulations are aimed, in part, at protecting the health of susceptible subpopulations.

This research also has important clinical implications. Advancing the understanding of air pollution-related impacts on youth with T1D has the potential to have a marked impact on treatment approaches and behavioral recommendations. For example, our results underscore the importance of healthy lifestyle guidelines for youth and children with T1D living in more polluted areas.

6.4 Directions for Future Work

Additional studies investigating the links between air pollution and cardiovascular risk factors are needed. In particular, future work should incorporate more refined methods of exposure assessment such as measuring personal exposures to air pollutants, if possible. Other suggested refinements include the addition of information on indoor contributors to exposure such as secondhand smoke. It will also be important to factor in time-activity

patterns or time spent away from the home. In addition, future work should include larger and more diverse study populations to more fully assess potential differences by factors such as age, gender and race/ethnicity. Future studies should also consider the potential effects of gene-environment interactions and epigenetic changes following air pollution exposures.

In our first study, we reported positive associations of short-term exposures to $PM_{2.5}$ with PWV, a measure of arterial stiffness. Our results indicate that more research is required in this area, particularly since there are very few existing studies to date. Future work should incorporate additional biomarkers of arterial stiffness, such as AIx and BrachD. We also suggest that future studies include repeat measurements of arterial stiffness biomarkers to more fully characterize relationships with air pollution exposures. Because youth with T1D exhibit increased arterial stiffness from a young age (Gourgari et al. 2017; Urbina et al. 2010), additional studies should focus on this subpopulation. Larger studies with better power to detect significant associations are also needed.

Our second study found consistent positive associations between acute exposures to $PM_{2.5}$ mass and changes in levels of IL-6. Additional studies with repeat measures of systemic inflammation are indicated due to inconsistencies in the existing epidemiologic evidence. The biological mechanisms underlying these relationships also require further study. More work should focus on children and young adults with T1D due to the overall lack of existing data. Larger studies with better power to detect significant associations are also needed.

While our third study did not detect associations between chronic exposures to outdoor air pollution and AL variables in the main analysis, more research is needed to

characterize the relationships of air pollution exposures with AL and the AL subscales. Our study was the first to assess these relationships and may provide guidance for future work. We suggest that future studies incorporate cumulative lifetime exposures and also explore contributions of individual air pollutants such as ultrafine particles, NO_2 and SO_2. It will additionally be important to assess relationships of AL with air pollutants in different age groups and study populations. We identified effect modification by gender and race/ethnicity, and these differences also require further investigation. Another possible direction for future research is to explore the use of more extreme distribution-based cut-points for AL biomarkers, such as the use of the 90^{th} percentile rather than the 75^{th} percentile, to explore whether effects are stronger among individuals who are at highest risk. Longitudinal studies are also needed to characterize whether changes in air pollution exposures are associated with changes in AL.

APPENDICES

Appendix 1

DATE:	July 31, 2017
TO:	Robin Puett
FROM:	University of Maryland College Park (UMCP) IRB
PROJECT TITLE:	[313113-13] Air Pollution, Subclinical CVD and inflammatory Markers in the SEARCH Cohort
REFERENCE #:	11-0570
SUBMISSION TYPE:	Amendment/Modification
ACTION:	DETERMINATION OF EXEMPT STATUS
DECISION DATE:	July 31, 2017
REVIEW CATEGORY:	Exemption category # 4

Thank you for your submission of Amendment/Modification materials for this project. The University of Maryland College Park (UMCP) IRB has determined this project is EXEMPT FROM IRB REVIEW according to federal regulations.

We will retain a copy of this correspondence within our records.

If you have any questions, please contact the IRB Office at 301-405-4212 or irb@umd.edu. Please include your project title and reference number in all correspondence with this committee.

Appendix 2

Table A1. Associations of baseline $PM_{2.5}$ and elemental carbon with inflammatory biomarkers in unadjusted models

	CRP N=616	Fibrinogen N=618	IL-6 N=441
	% Change (95% CI)	% Change (95% CI)	% Change (95% CI)
EC, weekly avg.	0.04 (-0.12, 0.21)	-0.003 (-0.03, 0.02)	-0.06 (-0.17, 0.06)
$PM_{2.5}$ averaging time			
Weekly avg.	0.15 (-0.003, 0.30)	0.003 (-0.02, 0.02)	-0.02 (-0.13, 0.09)
Lag 0	-0.01 (-0.14, 0.11)	-0.002 (-0.02, 0.02)	-0.04 (-0.13, 0.05)
Lag 1	0.07 (-0.05, 0.20)	-0.003 (-0.02, 0.01)	-0.04 (-0.12, 0.05)
Lag 2	0.07 (-0.06, 0.19)	-0.002 (-0.02, 0.01)	-0.09 (-0.17, -0.002)*
Lag 3	0.08 (-0.05, 0.20)	-0.004 (-0.02, 0.01)	-0.05 (-0.14, 0.04)
Lag 4	0.14 (0.02, 0.26)*	0.01 (-0.01, 0.03)	0.03 (-0.06, 0.11)
Lag 5	0.11 (-0.02, 0.24)	0.01 (-0.01, 0.02)	0.002 (-0.09, 0.09)
Lag 6	0.13 (0.005, 0.25)*	0.01 (-0.01, 0.02)	0.06 (-0.03, 0.15)
Lag 7	0.09 (-0.04, 0.21)	0.003 (-0.02, 0.02)	0.02 (-0.07, 0.11)
2-day moving avg.	0.13 (-0.02, 0.29)	0.002 (-0.02, 0.02)	-0.04 (-0.15, 0.07)
3-day moving avg.	0.13 (-0.02, 0.28)	0.002 (-0.02, 0.02)	-0.04 (-0.15, 0.07)
4-day moving avg.	0.13 (-0.02, 0.28)	0.002 (-0.02, 0.02)	-0.04 (-0.15, 0.07)
5-day moving avg.	0.13 (-0.02, 0.28)	0.002 (-0.02, 0.02)	-0.04 (-0.15, 0.07)

*p<0.05

Table A2. Associations of baseline $PM_{2.5}$ and elemental carbon with inflammatory biomarkers in fully adjusted models

	CRP N=616	Fibrinogen N=618	IL-6 N=441
	% Change (95% CI)	% Change (95% CI)	% Change (95% CI)
EC, weekly avg.	-0.03 (-0.19, 0.14)	-0.01 (-0.04, 0.01)	-0.06 (-0.18, 0.05)
$PM_{2.5}$ averaging time			
Weekly avg.	0.10 (-0.05, 0.25)	-0.01 (-0.03, 0.01)	-0.01 (-0.13, 0.10)
Lag 0	-0.04 (-0.16, 0.08)	-0.01 (-0.02, 0.01)	-0.04 (-0.13, 0.05)
Lag 1	0.04 (-0.08, 0.16)	-0.01 (-0.02, 0.01)	-0.04 (-0.12, 0.05)
Lag 2	0.03 (-0.09, 0.15)	-0.01 (-0.02, 0.01)	-0.09 (-0.17, -0.004)*
Lag 3	0.04 (-0.08, 0.16)	-0.01 (-0.03, 0.01)	-0.05 (-0.14, 0.04)
Lag 4	0.10 (-0.02, 0.23)	0.004 (-0.01, 0.02)	0.03 (-0.06, 0.12)
Lag 5	0.08 (-0.05, 0.20)	0.001 (-0.02, 0.02)	0.01 (-0.08, 0.10)
Lag 6	0.09 (-0.03, 0.21)	0.001 (-0.02, 0.02)	0.06 (-0.03, 0.16)
Lag 7	0.05 (-0.07, 0.17)	-0.01 (-0.02, 0.01)	0.02 (-0.08, 0.11)
2-day moving avg.	0.08 (-0.07, 0.23)	-0.01 (-0.03, 0.01)	-0.03 (-0.15, 0.08)
3-day moving avg.	0.08 (-0.07, 0.23)	-0.01 (-0.03, 0.01)	-0.04 (-0.15, 0.07)
4-day moving avg.	0.08 (-0.07, 0.23)	-0.01 (-0.03, 0.01)	-0.04 (-0.15, 0.07)
5-day moving avg.	0.08 (-0.05, 0.23)	-0.01 (-0.03, 0.01)	-0.04 (-0.15, 0.07)

*p<0.05

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