EFFECTS OF AIR POLLUTION ON LIVER METABOLISM WITH RELEVANCE FOR CARDIOVASCULAR DISEASE – A MULTILEVEL ANALYSIS

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the School of Public Health (Epidemiology).

Chapel Hill
2007

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ABSTRACT

JENS LEVY: Effects of Air Pollution on Liver Metabolism with Relevance for Cardiovascular Disease – A Multilevel Analysis
(Under the direction of Dana Loomis)

The liver is a possible target organ for exposure to particulate air pollution, which has been associated with acute and chronic cardiovascular effects. The studies contained within this dissertation evaluate the effects of ambient measures of PM10, NO2 and SO2 in relation to individual cholesterol parameters, LDL and HDL, in manuscript one, and alanine aminotransferase (ALT) levels in manuscript two. I employed multilevel analysis on individuals nested in counties in a nationally representative sample survey data merged with ambient air pollution monitoring data. I explored the contribution of the mean county and deviation from the mean county pollution levels to evaluate the independent contribution of aggregate and individual exposures on individual outcome parameters.

In random intercepts models of LDL, a mean county average increase of 10µg/dL of PM10 and 10 ppb of NO2 was associated with an increase of 4.26 mg/dL (95% CI: -1.57, 10.06) and 3.61 mg/dL (95% CI: 0.98,6.30). To the extent that individual level variation exists, the individual level pollutant estimates support the positive effect of PM10 and NO2 at the county level. Some evidence exists that the individual level effects of PM10 and SO2 are higher at higher county mean levels of air pollution.

Log ALT levels are inversely related to PM10 exposure at both the county (-0.011; 95% CI: -0.040, 0.017) and individual level (-0.019 95% CI: -0.032, -0.005). The data suggest that log ALT is positively associated with county-level NO2 and though negative at mean

county levels, the individual-level effect is more positive at higher county levels of NO2. Though the direction of these results is not consistent with hepatotoxicity, these results suggest alterations in liver metabolism that are shared with current cigarette smoking and may signify pathological changes in the liver.

These data from around the country provide exposure contrasts that are evaluated against the alterations in the outcome measures at the appropriate level in the mixed models analysis. The cholesterol study provides evidence for a link between PM and atherosclerosis. The ALT study suggests a paradoxical relationship that may point to a meaningful alteration in metabolism with relevance to atherosclerosis.

DEDICATION

This work is dedicated to my wife Maria Cristina Flaminiano Garces who has supported me throughout many years, which included, at times, difficult separation and enduring patience.

ACKNOWLEDGEMENTS

I gratefully acknowledge my Committee Chair, Dr. Dana Loomis as well as the other members of my committee: Drs. Lucas Neas, JC Chen, Steven Marshall, and Joel Schwartz. I am also grateful for the help of Dr Annette Peters who originally proposed to look at cholesterol in relation to air pollution. I am further indebted to Dr Louise Ball who provided critical support through the environmental training grant.

Furthermore, this work benefited considerably from the help of my fellow students in particular Whitney Robinson and Emily Wenink who were vital in providing comments as well as social support.

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ABC1	ATP-binding cassette transporter 1
ACS	American Cancer Society
Ag	Silver
AIRS	Aerometric Information Retrieval System
ALT	Alanine aminotransferase
AM	Alveolar macrophage
APHEA2	Air Pollution and Health A European Appraoch 2
AST	Aspartate aminotransferase
ATP	ABC transporter proteins
BEAS	Bronchial epithelial cell line
CAD	Coronary artery disease
CAPS	Concentrated ambient particles
CB	Ultrafine carbon black
CETP	Cholesterol ester transfer protein
CFA	Coal fly ash
CRP	C-reactive protein
CU	Copper
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
EAg	Elemental silver
EPA	Environmental Protection Agency
ETS	Environmental tobacco smoke

FE	Iron
GM-CSF	Granulocyte macrophage colony stimulating factor
HDL	High-density lipoprotein
IL	Interleukin
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
MIP-2	Macrophage inflammatory protein-2
NAAQS	National Ambient Air Quality Standard
NADPH	Nicotinamide adenine dinucleotide phosphate
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF	Nuclear Factor
NHANES	National Health and Nutrition Examination Survey
Ni	Nickel
NO	Nitric Oxide
NO_2	Nitrogen Dioxide
Pb	Lead
PM	Particulate matter air pollution
PM_{10}	Particulate matter smaller than 10 μm in aerodynamic diameter
$PM_{2.5}$	Particulate matter smaller than 2.5 μm in aerodynamic diameter
PMN	Polymorphonuclear neutrophils
PON	Paroxonase
PSU	Primary sampling unit

RCT	Reverse cholesterol transport
RNA	Ribonucleic acid
OFA	Oil derived fly ash
ROFA	Residual Oil Fly Ash
ROS	Reactive oxygen species
sICAMs	Soluble intracellular adhesions molecules
SO_2	Sulphur Dioxide
TNF	Tumor necrosis factor
UFCB	Ultra-fine carbon black
UFP	Ultrafine particulates or PM smaller than 1 μm in aerodynamic diameter
UFPs	Ultra fine particles
VLDL	Very low-density lipoprotein
Vn	Vanadium
ZN	Zinc

CHAPTER I

INTRODUCTION

A vast amount of scientific evidence has accumulated that confirms the conclusion that particulate matter (PM) pollution and in particular particulate matter smaller than 2.5 μm in aerodynamic diameter (PM2.5), is associated with cardiovascular morbidity and mortality in addition to that from lung disease. The evidence has compelled the Environmental Protection Agency (EPA) to promulgate new regulations for PM2.5 with which the health effects have been most strongly linked. These standards are laid out in the Air Quality Criteria for Particulate Matter published by EPA in 1996.

However, the new standards have provoked significant controversy and regulatory action has not been implemented due to challenges from industry. Some inconsistencies and limitations in the epidemiologic data are notable. Ambivalence remains due to the fact that the associations have been observed at ambient levels not previously considered hazardous and the possibility that the observed findings could be an artifact of complex and sophisticated statistical models or confounding.[1] Also major gaps exist in the understanding of the biological mechanisms underlying the observed health effects. Among the questions that remain to be answered include: Are the significant exposures limited to the lung? Do the effects of PM exist only among some subset of the population who are vulnerable? What are the vulnerabilities? Are health effects limited to acute exposures or do acute effects of PM over time lead to significant health effects in the general population? Bringing communities

into compliance with the new standards is likely to be enormously expensive and have significant economic consequences. Additional research is necessary to inform the management of PM pollution.

Cardiovascular disease (CVD) and respiratory disease place tremendous burdens on the public health and the prevalence of exposures to PM pollution compel us to understand the scope of the problem in order to mitigate man-made and therefore modifiable causes of disease. As countries transition into industrialized economies, obesity and CVD become more prevalent. Furthermore, the air pollution in transitioning economies is some of the worst in the world.

Recently a substantial amount of mutually reinforcing evidence has emerged from both epidemiological and laboratory research, which points to the particular toxicities of certain components of fine particulate matter that may explain the patho-physiologic mechanism by which PM is causally related to adverse changes underlying CVD. These mechanisms by which PM exposures, presumably originating in the lung, include: 1) pulmonary and/or systemic inflammatory response involving endothelial dysfunction and pro-atherosclerotic changes involving coagulation and inflammation; 2) autonomic nervous system dysfunction in response to stimulation from the vagal nerves of the lung and/or systemic inflammation; and 3) toxicity to the myocardium. Individual connections along the line within each of these posited relationships can be evaluated in epidemiological studies and will advance a coherent chain of established cause-effect evidence by which PM pollution exposures cause significant CVD morbidity and mortality.

The investigations of CVD in relation to PM come at a time when there is an increasing appreciation of the role of the physical and social environment in cardiovascular risk. This

approach has more public health utility due to the emphasis on modifiable environmental exposures than essential differences that correspond to class and race. In the past, research has focused on lifestyle and genetic explanations for the causes of disease – perhaps reflecting a historical predilection to reflect on intrinsic characteristics of race and/or heredity and Mendelian genetics. This has come with a neglect of understanding the heterogeneous influence of the environment's impact and multiplicitous covariants of genes. Recent CVD research has examined the contribution of contextual relationships of individuals in relation to their social and physical environment of which air pollution is a significant part. Air pollution has both regional (macro) and small-scale (micro) variability in the environment relevant to ecological and individual level effects. Exposure measurements in environmental epidemiological studies that incorporate both sources of variability can elucidate important contextual effects or identify direct ecological effects on individual risk independent of those which are conferred by their individual analogue. Such studies can provide a more coherent understanding of the physical and social environment on CVD.

Questions about health effects of air pollution are relevant to the concern of racial and social disparities in health and social justice. Class and race is associated with increased exposures to environmental hazards i.e. Superfund sites, toxic emissions, and existing waste facilities.[2] Many environmental toxins produce hypertension and cardiac arrhythmias.[3] Studies indicate that disadvantaged communities (often non-white) experience higher than average exposures to air pollution[4] and higher burdens of deleterious effects from air pollution.[5] This may be because urban areas where a large percentage of racial minorities reside are prone to have higher levels of ambient air pollution due to heavy traffic and industry.[6] One study has found that in addition to environmental tobacco smoke (ETS) and

concomitant PM10 ambient air measures, proximity of the home to urban traffic emissions was a major determinant of personal PM 2.5 exposures.[7] Thus, environmental pollution and air pollution in particular may account for much of the socio-economic disparities in health in general and cardiovascular disease in particular.

In addition to cardiovascular effects noted above, PM has been linked with infant mortality in Mexico City[8] and in the U.S.[9] In Asia, PM & SO_2 (notably from coal stoves used for heating) was associated with low birth weight in children.[10,11] In the highly polluted area of Northern Bohemia, PM_{10} and $PM_{2.5}$ were associated with intrauterine growth retardation.[12] A critical question remains to be answered; how can ambient pollutants that may sometimes produce non-lethal effects on the target organ – the lung – have fatal consequences on a secondary organ system such as the cardiovascular system?

The pathophysiological mechanisms arising from exposure to the lung that is necessary to establish causation are long and involve understanding alterations at the level of the cell, tissue, organ, organ system and organism. Toxicological and controlled human exposure studies can provide critical links; however they do not necessarily represent the consequences to humans of real world exposures. Epidemiology alone cannot establish causation, but the iterative and mutually reinforcing investigation between epidemiology and toxicology is necessary to establish the health consequences of exposure to particulate air pollution.

In the accumulating literature that connects exposures to PM to cardiovascular disease one critical linkage has been overlooked. Alteration in liver metabolism has recently been recognized as a central component of cardiovascular disease and is involved with other characteristic metabolic alterations that together comprise what is known as metabolic syndrome. These include central adiposity, diabetes, glucose intolerance, low HDL, high

LDL, systemic inflammation and elevated blood pressure.[13] While virtually all physiological alterations involved in the initiation and progression of atherosclerosis have been examined in relation to PM – airway injury and inflammation,[14-16] oxidative stress,[17,18] endothelial dysfunction,[19] inflammation,[20,21] insulin resistence/diabetes,[22] autonomic nervous system dysfunction,[23] arrhythmias,[24] coagulation rheology,[25,26] myocardial infarction,[27] and atherosclerosis[28,29] – no studies have examined lipid levels in relation to PM. Furthermore, scant attention has been paid specifically to alterations in liver metabolism of which lipid metabolism is part.

In this dissertation, I employ multilevel techniques to investigate PM in relation to levels of LDL and HDL. I also examine PM in relation to alanine aminotransferase (ALT) that is a marker of hepatocytotoxicity. The multilevel analysis is employed in a data set that has the distinct advantage of including standardized data of the outcome measures and important covariates among individuals from around the United States. The geographical and temporal variation in this data set that is merged with ambient air pollution monitoring data provides valuable exposure contrasts to address the study questions. These data allowed me to examine air pollution that is both an ecological and individual exposure at the appropriate level, while accounting for the non-independence of individuals clustered in counties. Of particular value is that the exposures at the higher (ecological) level of aggregation (than that at which the outcomes are observed) are not treated as unrelated[30]. The unified expression of the exposures to air pollutants at the two levels allows for a statistical evaluation of the contextual dependencies of air pollution. Multilevel analysis therefore has some ability to address confounding issues that are typically overlooked in air pollution epidemiology,

which often relies on imprecise exposure measurement and lacks data on individual-level confounders.

The studies in this dissertation include a novel application of a methodology in addressing an important linkage that is missing in the literature of air pollution epidemiology. The results of these studies are of tremendous public health importance as exposure to particulate air pollution and the consequences for CVD are increasing as countries undergo industrial transition.

CHAPTER II

BACKGROUND

A. Background - History

Cardiovascular effects from air pollution were first recognized as a serious public health problem after severe air pollution episodes in the Meuse Valley, Belgium in 1930, and in London in 1952, resulted in excess deaths from cardiovascular in addition to respiratory disease.[31] Beginning in the late 1980s new statistical methodologies were first applied to available data on daily mortality in relation to transient elevations in particle concentrations in North America, Latin America, Europe and Asia.[32-34,35-38] These studies found significant elevations of CVD mortality in association with elevations in PM that were not accounted for by season and temperature and furthermore, they occurred with particulate air pollution levels below the current National Ambient Air Quality Standard (NAAQS) and appeared to have no lower threshold.[39-41] Additional study indicated that mortality associations were with fine particulates ($PM_{2.5}$)[42,43] and sulfate. Furthermore, the evidence suggested that the results were not simply due to harvesting (loss of life brought forward by only a few days).[40,44] Other studies have begun to examine air pollution in relation to hospitalizations for cardiovascular related endpoints.[45,46]

In addition to the analysis of CVD morbidity and mortality in relation to short-term exposures to particulate air pollution, two prospective cohort studies reported that chronic exposure to PM increased the risk of premature mortality.[34,47] The loss of life due to ambient pollution has been estimated to be from one to three years.[48,49]

Based on the evidence, new standards for particulates smaller than 2.5 μm in diameter ($PM_{2.5}$) have been promulgated by the EPA. Since then new evidence has continued to come from time-series analysis, case-crossover analyses, and chamber studies.[31,50] The results have produced small but consistent estimates of relative risks of adverse health events from PM air pollution. While small these relative risks represent a major modifiable source of attributable risk and a major public health concern. However, resistance to the implementation and enforcement of the particle standards has arisen in part because the biological mechanisms are poorly understood. In vitro investigations as well as animal and human laboratory studies have been conducted in order to elucidate the underlying biological mechanisms to explain how non-lethal effects on the target organ (the lung) have fatal consequences on secondary organ systems such as the cardiovascular system.

B. Background-PM

In order to mitigate the effects of PM, it will be necessary to understand the components and/or characteristics of PM that are responsible for health effects. Unlike other criteria pollutants such as ozone, SO_2 or NO_2, particulate matter is not a defined entity but refers to a complex aerosol of solid and liquid, organic and inorganic materials. Particles can be formed by conversion of gases or directly emitted into the atmosphere from stationary or mobile combustion sources. Some particles are naturally occurring such as crustal material (including windblown soil); however, the particles that are of regulatory and/or health concerns are largely the result of the combustion of fossil fuels.[51]

With respect to the categorization of PM in relation to the study of adverse health consequences from PM, the first cut is made by size of the particle. Gravimetric assessments are made of particles of a certain diameter. Particles that are larger than 10 μm in aerodynamic diameter are not considered to have much impact on human health, while particles smaller than this size (PM_{10}) are considered respirable and as a consequence have intimate exposure with the lungs. A major new area of focus in the literature is the health effects of fine particles, those that are less than 2.5 μm in aerodynamic diameter ($PM_{2.5}$). Adverse effects of these smaller particles are very plausible given that, human exposures to substantial amounts of fine particles only began with the domestication of fire while pathogen resistance has arisen over millions of years of evolution. Prior to this, particles that were prevalent in the atmosphere to which we have adapted were primarily products of mechanical processes and are in excess of 2.5μm in aerodynamic diameter. Contemporary PM pollution is unique in that it reflects a remarkable redistribution in the environment of metals and combustion products with highly reactive chemical structures. Fine particles that exist today arise from processes associated with industrialization and have chemical structures and properties capable of stimulating or inhibiting signaling molecules that are mal-adaptive to the host.

Fine particles include finely divided carbonaceous material derived from the incomplete combustion of hydrocarbon fuels from point sources (notably from coal fires in earlier decades but now mainly from diesel engines) and mobile sources. Particles from petrol engines are a consistent contributor to fine PM. Diesel engines however, produce 100 times more particles than do gasoline engines at similar levels of performance.[52] Fine particles also include inorganic dusts (fly ash) dispersed from industrial processes, and secondary particles

such as sulfates and nitrates formed by reactions between gases in the atmosphere.[53] Additionally, fine particles proportionate to their smaller size and number will also have other compounds or elements adsorbed onto their surface. Diesel exhaust particles consist of a carbonaceous core onto which over 18,000 different high molecular weight compounds are adsorbed.[15] Notably, metallurgical operations emit vapors that tend to condense on fine particulates. Particles vary in size, geometry, chemical composition, and physical properties and are crudely characterized in epidemiological studies largely by particle size.

1. Particulate characteristics and toxicities

Beyond size, there are several characteristics of particles that relate to their pathologic potential and that have been evaluated in laboratory studies to evaluate their pathogenicity. Characteristics such as elemental composition, surface chemistry, soluble or bioavailable chemical constituents, biologic agents such as endotoxin, acidity, and metal content all relate to pathways that laboratory investigation have shown may mediate the associated health effects. The potential effects of particles on the respiratory system are diverse and complex. Effects related to chemistry and solubility among the classes of particulate air pollution that have been used in laboratory investigations are notably different. These include ambient urban air particles; highway derived dusts; dusts associated principally with power production i.e. coal derived fly ash (CFA); oil derived fly ash (ROFA) associated with oil burning power plants,[51] but increasingly emissions from diesel fuel combustion; and natural fugitive dust.

Recently, it has been suggested that much of the health effects of PM are mediated by ultrafine particles (particles less than 100 nm in size or UFPs).[54] Characteristics of urban air

pollution, UFPs are derived principally from anthropogenic sources of mobile and stationary combustion processes.[55] Levels are generally between 1 and 2 ug/m3.[56] However, during episodic increases, levels can reach as high as 20-50 ug/m3.

UFPs have unique toxicological properties compared with larger particles.[57] These particles associated with urban traffic are a small proportion of PM by mass but a large proportion by number of particles and surface area. Ultrafines are biologically more reactive than larger particles.[14] Evidence of their unique toxicity comes from experiments that show harmful effects in the lung from exposure to ultrafine levels of material that are not intrinsically toxic, i.e. carbon or titanium dioxide.[58,59] Ultrafines may be much smaller than cell structures. Furthermore, their large surface area allows absorption of substances from the environment onto their surface which increases the reactivity of the particle.

It is the anatomy and physiology of the respiratory surface in the lung that is the interface between a person and inhaled pollutants. In spite of the considerable defenses of the lung, changes in the lung from exposure to PM and in conjunction with co-pollutants have been demonstrated to precipitate systemic changes. The relevant characteristics of this interface relate primarily to the macrophages and epithelial cells of the respiratory tract, and airway surface liquid. The toxicity of PM may be modified by the mechanics of inspiratory and expiratory airflow; however, the principal consideration is with the epithelial cells and the integrity of the epithelial cell layer, mobile immune cells (macrophages) and the surfactant (and its lipid and protein components) in the bronchioles and alveoli that coats the air-liquid interface. The surfactant lowers air/liquid surface tension essential for maintenance of normal functioning and avoidance of alveolar and airway collapse but also has a significant role in protecting the host from antigens.[60] The tight junctions of adjoining epithelial cells provide a

significant barrier to solute movement between the liquid and intercellular space that communicates with the submucosa across the relatively porous basement membrane. The tight junctions maintain the positional integrity of the cells, thus allowing for the selective insertion of receptors and channels that confer polarity underlying transcellular transport and selective secretion of epithelial cell products to one or the other side of the epithelium.[60]

2. Deposition of inhaled particulate matter

Very small particles remain suspended in air for many weeks and a cloud of fine particulates may travel many miles and cross borders. Fine particulates ($PM_{2.5}$) readily penetrate buildings and are therefore also a significant component of indoor air.[61] It has been shown that indoor personal monitoring samples may contain as much chemical particulate matter as that found in fixed point outdoor sampling in the same general area.[62]

Particles that are in the coarse range ($>PM_{2.5}$) get deposited in the upper respiratory tract due to high velocities induced by turbulence and directional changes of the nasal, tracheal, and laryngeal passages. These particles are then removed by mucociliary clearance. Fine particles ($<PM_{2.5}$), particularly ultrafines ($<PM_{1.0}$), on the other hand demonstrate Brownian movement and are therefore likely to be deposited in the alveolar region of the lungs where removal of particles is largely by phagocytosis by alveolar macrophages.[63]

UFPs have a very high deposition efficiency (approaching 50% for 20 nm particles). In the terminal airways and proximal alveoli where the net flow of air is zero, the deposition efficiency increases from diffusion.[59] A unique toxicological property of inhaled ultrafine particles have been demonstrated by their propensity to penetrate the epithelium and reach interstial sites.[64] Such a mode of exposure would be particularly relevant for the liver since

the liver is the major organ of their uptake from the circulation.[55] Whether or not ultrafine particles can be translocated from the lungs into the systemic circulation is a source of controversy, but has tremendous significance since they have the potential to directly interact with extra-pulmonary organs like the endothelium, heart and liver. The evidence for this unique toxicity is mixed. A few studies have found evidence for translocation into the blood compartment after 1 hour, and could be measured in the circulation,[65] liver, [55,66] brain,[66,67] and heart and spleen.[66] In an exposure study of humans however, their was negligible evidence of translocation of 35 nm particles into circulation.[68]

3. Exposure measurement

Most air pollution studies use outdoor (ambient) monitors of urban background particulate matter as indicators for particle exposures. The validity of ambient monitor data to reflect personal exposure varies by pollutant.[69] One significant problem with exposure measurement is that most people spend most of their lives indoors and indoor sources of air pollutants are numerous. Thus exposures based on ambient pollutant monitoring may not reflect personal exposure. However, a study by Sarnat et al. indicate that ambient $PM_{2.5}$ concentrations are suitable surrogates for personal exposures and furthermore, ambient gaseous pollutant measures are also suitable surrogate for personal exposure to $PM_{2.5}$ but not for themselves.[70] The Sarnat study suggests that gaseous pollutants measured with ambient monitors are surrogates rather than confounders in epidemiological studies of $PM_{2.5.}$

Geographical differences of surrogacy -Local temperatures, precipitation, clouds, atmospheric water vapor, wind speed and wind direction influence atmospheric chemical processes.[71] Meteorological conditions that are related to short-term health outcomes can

confound PM estimates in studies of acute effects of air pollution. The chemical nature of particles can change significantly with location and time.[55] Thus the pathogenic potential for a given mass concentration in study conditions can vary significantly. However, their toxicological potential related to their size also exists independently of chemical composition. The actual constituents of PM pollution are highly variable and geographically and site specific and differ with respect to characteristics with relevance to their toxicity.

C. Background – CVD

Cardiovascular disease represents the largest burden of premature morbidity and mortality in industrialized societies, but is highly prevalent in low to middle income countries as well.[72] The burden of CVD is increasing along with the epidemic of obesity as economies become increasingly industrialized as is occurring in India and China. The etiology of atherosclerotic cardiovascular disease is multidimensional, involving endothelial dysfunction, lipid metabolism, rheology, homeostasis, glucose metabolism, inflammation and the autonomic nervous system. The underlying relationships have implications for susceptibilities and variance in epidemiological studies. Atherosclerosis develops over the course of many years. Individual risk factors include obesity, physical inactivity, diet, tobacco use, high LDL, low HDL, blood pressure, elevated blood glucose and male sex. In addition to individual risk factors characteristics, increasingly the context of individuals within the physical and social environment is being recognized as an important contributor to CVD.

Socioeconomic environment (disadvantaged neighborhoods) is associated with an increased incident coronary event hazard ratio[73] as well as insulin resistance.[74] While

neighborhood characteristics may correspond to important differences in chronic stress due to poverty or violence, or to sources of social support, availability and cost of healthful foods, they also correspond to environmental pollutants. Environmental exposures may account for the health disparities between and among race characteristics. Class and race is associated with increased exposures to environmental hazards i.e. Superfund sites, toxic emissions, and existing waste facilities.[2] Studies indicate that disadvantaged communities (often non-white) experience higher than average exposures to air pollution[4] and higher burdens of deleterious effects from air pollution.[5]

1. General CVD pathophysiology

The cascade of events involved in the initiation and progression of atherosclerosis occurs over the course of years. Theoretically, air pollution exposures could impact a number of pathogenic events that occur over the life-course. Atherosclerosis is a complex process characterized by the accumulation of lesions arising from foam cells formation in the space between the endothelium and smooth muscle of the arterial wall (the intima). The mechanisms by which these lesions arise involve the infiltration of low-density lipoprotein (LDL), monocytes and T-cells into the anti-oxidant poor environment of the arterial intima, the subsequent proliferation of the smooth muscle cells and increased production of extracellular matrix involving hemostatic proteins i.e. platelets and fibrinogen.

Three major elements are involved in the pathogenesis of atherosclerosis arising from oxidative changes involving the endothelium: 1) Modification of endothelial function; 2) Changes in vascular tone; 3) sequlae of hyperplasia of smooth muscle cells in the intima of

affected blood vessels.[75] Vascular events are associated with a rupture of the lipid rich plaques leading to platelet activation and fibrin deposition and occlusion of the lumen.

Oxidative stress is central to the initiation, progression and destabilization of atherosclerotic plaques and involves cholesterol in its well established contribution to risk of CVD. Progression of atherosclerosis in men can be reduced in heavily smoking men by supplementing with vitamin C and E – antioxidants. Oxidative stress in endothelial cells, macrophages and smooth muscle cells results in the production of superoxide anions that in conjunction with high intimal levels of LDL may combine to generate high levels of oxidized LDL. This cell mediated LDL oxidation arises from an imbalance in the prooxidant systems (i.e. NADPH oxidase, lipoxygenase or myeloperoxidase) and the cellular content antioxidants such (i.e. reduced glutathione and superoxide dismutase.[76] Under pathologic conditions, macrophages further precipitate inflammation by secreting growth factors, cytokines and inflammatory mediators. Furthermore, the presence of oxygen radicals precipitates monocyte penetration from the blood stream into the intima (monocyte chemotaxis) beneath the endothelium and differentiation into macrophages, which phagocytizes the oxidized LDL. Macrophages become filled with oxidized LDL and become foam cells. Thus oxidative modification of LDL contributes to two main causes of atherogenesis. It precipitates macrophage lipoprotein derived accumulation of cholesterol and the induction of an inflammatory response.

The modification of LDL is a necessary step in atherogenesis. Incubation of macrophages with native LDL does not result in foam cell formation as macrophage uptake of native LDL is satiable and regulated by cholesterol content;[76] however, macrophages have scavenger receptors different from the LDL receptors that bind only modified forms of LDL, including

oxidized LDL and are not down-regulated by cellular content. Under continued oxidative stress, the LDL filled foam cells necrose, releasing their toxic contents into the intimal space, which in turn stimulates an inflammatory response in which neutrophils and additional macrophages are recruited to the lesion site.

This underlying pathological process involving oxidative stress and cholesterol is modified by mechanisms that are involved in the control of the vascular tone under normal physiologic conditions. Vascular tone has traditionally been thought of as being directed at the level of the smooth muscle cell only, which responds to sympathetic/parasympathetic nerve stimulation or circulating vasoactive hormones e.g. products of the rennin-angiotensin system. It has become increasingly clear however, that the endothelium plays a major role in the regulation of vascular tone through its affects on smooth muscle contractility.[75] The modification of vascular tone by humoral factors released from nerve terminals, cells in the kidneys or heart, or endothelial cells is referred to as neuroendocrine activation.

Continued accumulation of foam cells in the intima result in fatty streaks. Along with the subsequent migration of smooth muscle cells from the media to the intima and their proliferation, the recruitment of macrophages and T-lymphocytes leads to plaque formation with the attending elaboration of collagen and fibrin matrix. The oxidized LDL induced macrophage cholesterol accumulation and plaque formation are the hallmarks of early atherosclerosis. Plaques in turn disrupt the vasodialation / vasoconstriction balance in favor of contraction which in turn increases sheer stress that can cause plaque disruption. Thus we see that pathologic changes occur as a result of oxidized LDL and precipitate a number of events that result in disease.

Inflammation, lipids and the endothelium - The endothelium, which is the vessel wall-blood interface, is the largest autocrine, paracrine, and endocrine organ and is also a regulator of vascular tone, lipid breakdown, platelet activation, monocyte adhesion, thrombogenesis, inflammation and vessel growth.[77] In response to physical and chemical stimuli, the endothelium produces a number of active substances that are responsible for its many functions. One of the most notable is its production of Nitric Oxide (NO) which is the predominant vasodilator. Other significant proteins include Endothelin-1, Angiotensin II, and thromboxane which promote vasoconstriction and platelet aggregation, smooth muscle proliferation and collagen breakdown.

The strong association between cholesterol and atherosclerosis has consistently been found in epidemiological and experimental studies as well as clinical trials. A specific profile characterized by high low density lipoproteins (LDL) and low High density lipoproteins (HDL) is implicated. In the presence of hypercholesterolemia or hypertriglyceridemia, the endothelial function becomes impaired and this dysfunction may be the earliest anatomic evidence of atherosclerosis. Some of the key features associated with the presence of coronary disease are endothelial dysfunction, an increased vasoconstrictor response, enhanced interaction of circulating blood cells and the proliferation of smooth muscle cells.[75]

An example of the shared fate of blood lipids and the endothelium, many clinical trials have reported improved endothelial function within 1 hour of lipid apheresis or within 2 weeks of initiation of statin therapy in patients with atherosclerosis or CAD.[77] Even in healthy middle aged men, endothelial functioning improved as a consequence of reductions of serum LDL cholesterol levels.[78]

HDL- and reverse cholesterol transport (RCT) - HDL has been found to protect against LDL oxidation.[79] This protection may be due to protein components of HDL that bind transition metals. Ceruloplasmin and transferrin are metal binding acute phase proteins also associated with HDL.[80] HDL has other antioxidative properties. PAF-AH association with HDL hydrolyzes oxidized phospholipids and removes oxidized fatty acids.[81] Paroxonase (PON), another HDL associated protein, protects LDL from oxidative stress through its ability to hydrolyze phospholipids in oxidized LDL.[80] Also, HDL may also play a role in the reverse transport of potentially reactive hydroperoxide species for hepatic detoxification.

There are two different proposed mechanisms by which HDL is involved in removal of cholesterol from macrophage foam cells. The first is a lipoprotein mediated mechanism by which cholesterol is removed from plasma membranes of peripheral cells to lipid poor pre-β HDL particles. This requires lipoprotein A1 on HDL as well as an ATP-binding cassette transporter 1(ABC1) on the cell surface.[82] The second mechanism is a diffusion mechanism that is dependent on LCAT which converts free cholesterol in HDL into cholesterol ester and moves into the core of HDL particles and thus maintaining a free cholesterol gradient to allow continued diffusion of free cholesterol across the plasma membrane to HDL. HDL may then be endocytosed by liver parenchymal cells or HDL may exchange cholesterol ester for triglyceride from triglyceride rich lipoproteins that is facilitated by CETP.

LDL - High levels of LDL that attend high cholesterol levels may result in high intimal levels of LDL that are available to become oxidized LDL.[83] As noted above, oxidized LDL contributes to atherosclerosis by causing macrophage lipoprotein derived accumulation of cholesterol and the induction of an inflammatory response.[84] One consequence of this relates to endothelial dysfunction. Oxidized LDL has been shown to reduce NO synthesis and

release, and can also cause the destruction of NO. The limiting capacity of oxidative stress on NO compromises the balance between vasoconstriction and vasodilation. In vitro susceptibility to LDL oxidation and endothelium dependent vasomotion in humans has been demonstrated.[83]

2. PM - CVD pathways

The preceding review of the pathogenesis of atherosclerotic CVD provides necessary background for understanding the consequences one may expect from the putative mechanisms by which PM has been suggested to affect CVD. Much of the available epidemiological evidence has indicated that PM can elicit systemic haemostatic and inflammatory as well as autonomic alterations. However, the full range of the implications arising from these alterations is not known. The possible consequences of PM exposures can have multiple direct and indirect effects resulting in pro-atherogenic changes that are dynamic and involve oxidative stress, inflammation, lipids, endothelial dysfunction, changes in vasomotor activity, shear stress and plaque disruption and vascular remodeling. Putative changes arising from PM exposures are superimposed over existing states of disease from a lifetime of exposures. These may include those induced from previous cumulative exposures to PM and other environmental pollutants. However, evaluating changes in these outcomes in relation to pollutants can help establish a coherent understanding of the causal influence of PM over the cascade of events that is necessary to motivate regulatory action.

3. Effect modification: susceptible populations

The identification of categories of individuals who are susceptible to particulate air pollution has been explored in several epidemiological studies. In one study done in Montreal that utilized information from questionnaires obtained through the universal Quebec Health Insurance plan, increased daily mortality was related to particulate air pollution was consistently found among persons 65 years of age and over, persons with cancer, acute lower respiratory diseases, any form of cardiovascular disease, chronic coronary artery diseases and congestive heart failure; however, there was little evidence for the association among persons with acute or chronic upper respiratory diseases, airways diseases, hypertension, acute coronary artery diseases and cerebrovascular diseases.[85] Another study has found that diabetics were twice the risk of PM-associated cardiovascular admission to the hospital than non-diabetics and persons over 75 years of age were at increased risk. This suggests potential mechanisms that are influenced by diabetes.[86] Additional evidence for exploring interactions is apparent in a study of the Edinburgh artery study. Investigators tested the interaction between selected baseline risk factors of cardiovascular health. Although interaction was not found at conventional levels of significance, people with high concentrations of fibrinogen appeared to be more susceptible to adverse cardiovascular effects of particulate air pollution.[87]

Taken together with the pathophysiology of CVD involving inflammation, the evidence of effect modifiers by conditions related to the pathways by which PM is putatively related to CVD points to some important considerations in evaluating the health effects of PM. A coherent analysis will provide for the fact that the effect of PM is likely to differ across persons with conditions related to inflammation and CVD i.e. fibrinogen, insulin

resistance/diabetes, age, high blood pressure, adverse blood lipid profiles, existing heart disease (and medication use) and lung disease (i.e. asthma). Furthermore, these conditions are not mutually exclusive and may vary geographically in a way that may correlate with pollution mixtures. Additionally, smoking, race, and poverty may also represent groups with susceptibilities.

The Air Pollution and Health: A European Approach 2 (APHEA2) project indicated the possible effect modification by specific city characteristics. The effect of PM_{10} was higher in cities with high average NO_2 levels than those with low average NO_2 levels; higher in warm climate cities than in cold; and higher in cities with low standardized mortality rates than those with high standardized mortality rates.[88] On an individual level, the investigators found that estimated increase in the daily number of deaths for all ages was slightly higher in the elderly than among all ages.

D. Background-Biological Mechanisms

Although a large body of epidemiological evidence exists that provides coherent evidence that PM pollution is a significant contributor to premature death from cardiovascular disease, a significant gap exists between the evidence and the ability to abrogate risk from exposure to PM, due the lack in understanding of clear biological mechanism/s that would explain how exposures limited to the lung can effect the heart/ circulatory system. A host of mutually reinforcing epidemiological and toxicological studies has addressed the knowledge deficits in the mechanistic pathways. Evidence continues to come from human exposure studies, in-vivo, and in-vitro laboratory studies at the level of the cell, tissue, organ, and systemic communication that may play a role of intermediates

between exposure to the lung and physiological alterations along the spectrum, of the CVD pathway.

1. Pathways- pulmonary inflammation

The totality of the evidence supports the idea that exposure to PM results in a systemic inflammatory response arising from alterations in cell signaling pathways as a consequence of the interaction between the particle and the lung. The toxic components of PM may include acidity, transition metals, organics and biogenic materials in PM. The consequences of their toxicity may include alterations in signal transduction,[89] contamination by endotoxins,[90,91] and the generation of reactive oxygen species to form tissue damaging free radicals and its induction of an inflammatory process;[92,93] This results in an increase of proinflammatory cytokine expression of the cells in the lung, causes neutrophil, B-lymphocytes, eosinophils and monocyte influx in the airways and increased epithelial permeability as measured by total protein in the bronchoalviolar lavage.[16,94] Furthermore, these changes in the lung are accompanied by proatherogenic alterations in the blood even without changes in lung function.[95]

Individual linkages in the above pathway have been established and illustrate that toxicity of PM upon exposure to the lung is complex and may vary depending on strength and duration of exposure, the physicochemical properties of the particles as well as the circumstances such as the ph of the exposed tissue of the respiratory tract, copollutants and co-morbid conditions that may facilitate toxicity. Studies demonstrate the ability of PM to cause a range of in-vitro effects in macrophages and epithelial cells that include DNA damage, apoptosis (cell-death), ROS production, cytotoxicity, prostaglandin synthesis[96] and

cytokine expression. In the lung, the initial cytokine production occurs with phagocytosis of particles by macrophages and epithelial cells.[94,97-99]

Macrophages - Inhaled fine and ultrafine particles deposit in large numbers in the terminal airways and alveoli, beyond the ciliated portions of the airway where the macrophages play the most important role in removing particles. During phagocytic activity, macrophages release reactive oxygen species, and proinflammatory cytokines including TNF-α, IL-6, granulocyte macrophage colony stimulating factor (GM-CSF), IL-1β.[100] These cytokines are known to stimulate bone marrow to release leukocytes and platelets into circulation and stimulate the production of acute-phase proteins in the liver.

The evidence suggests that the toxicological properties of PM on macrophages differ. One study evaluated different PM constituents (Oil Fly Ash, diesel dust and ambient air particles collected from 4 urban centers - on a mass basis and not evaluated in its fractionations) with respect to its cytotoxicity and cytokine (IL-6 & TNF) production in human and rat AM. In-vitro production of LDH (indicative of cytotoxicity) was found after 2 hours –from exposure to Oil Fly Ash (OFA) but not from ambient air particles or diesel dust.[90] However, after 20 hours ambient air particles were also cytotoxic suggesting two possible pathways of cytotoxicity in macrophages. Sub-cytotoxic levels of OFA showed immediate production of ROS (measured by chemiluminesence) that did not correspond with cytokine responses, while a smaller level of ROS production was observed after exposure to ambient particles but not from diesel dust. Ambient air particles however, produced the strongest cytokine production of (IL-6 and TNF) at non-cytotoxic concentrations of particles. This cytokine induction was inhibited by poymoxin B but not iron chelators indicating the cytokine production in AM arose from endotoxins and not metals. Reactive oxygen species

precipitated by metals in PM may be responsible for cytotoxicity but not necessarily in the up-regulation of cytokines in alveolar macrophages.

With respect to the toxicity in macrophages, the evidence supports the role of PM – depending on particle composition – on exposure to AM to produce ROS and inflammatory mediators that may result in vascular permeability changes, airway constriction, tissue injury and inflammation.

Epithelial cells - Epithelial cells also phagocytose particles in the lung. In vitro studies demonstrate that cytokine expression in epithelial cells appears, primarily to result from oxidative stress involving transition metals found in diesel dust, OFA, ROFA and more variably in ambient pollutants. Metals found in particles include Iron (FE), copper (CU), Nickel (Ni), Zinc (ZN), lead (Pb), and Vn.[101] These metals readily generate ROS in Fenton like reactions. In vitro studies demonstrate that upon treatment with particles, epithelial cells increase production of GM-CSF, sICAM, IL-6, IL-8, TNF as well as the messenger RNAs coding these cytokines.[92,102-104] Oxidative stress from metals is demonstrated by the inhibition of cytokine production with treatment by metal chelators and free radical scavengers. The production of cytokines (IL-6, IL-8 and TNF-alpha) is also induced by the soluble components of PM. The production of cytokines is also mimicked by metals.[105]

As a consequence of an oxidative stress and the inflammatory response in the lungs, systemic alterations occur as a consequence of the cytokines that pass into general circulation. Oxidative stress precipitated by particulates in the lungs may be augmented by oxidants generated by recruited inflammatory leukocytes.[57] Furthermore, oxidative stress induced from exposure to PM may also increase the permeability of the lung epithelium.[106] Airspace epithelial permeability is known to be increased in cigarette smokers. The

mechanism by which cigarette smoke induces epithelial permeability is likely the result of an oxidant induced effect accompanied by a reduction in the antioxidant reduced glutathione.[107] Similarly, oxidative stress from PM could induce the opening of tight junctions shown to occur in smokers. The increased epithelial permeability may also enhance the ability of diffusible molecules produced in the lungs in response to particles to gain access to the circulation. Smokers may also represent a particularly susceptible population to PM.

Transition metals derived largely by fuel combustion and capable of producing Fenton like reactions exist along with ultrafine particulates. Much of the laboratory evidence points to the ability of metals in fine PM to precipitate the oxidative stress and cytotoxicity,[108] cytokine production and inflammation.[93,109,110] Intratracheal instillation of ROFA suspension to rats resulted in severe inflammation, pulmonary injury that included recruitment of neutrophils, eosinophils and monocytes into the airway.[111] The role of soluble transition metals in pulmonary injury is indicated by its replication by metal sulfate solution containing Fe, V, and Ni; furthermore the ROFA injury was abrogated by its depletion of these from the ROFA leachate. The production of acute lung injury and inflammation from intratracheal administered PM in rats is dependent on the dose of bioavailable transition metal and not on a mass basis.[109] ROFA samples with soluble metals produce a far greater neutrophil influx than other ROFA.[99] Similar neutrophil influxes to the lungs has been observed in rats exposed to soluble forms of Vanadium (V).[112] Furthermore, cytokine mRNA expression of macrophage inflammatory protein-2 (MIP-2) and KC (these being the principle neutrophil chemotactic factors in rats) were induced within 1 hour - and continued throughout 48 hours - of exposure.

The effects of metal containing particulates on macrophages show some heterogeneity. The oxidative burst in alveolar macrophages in one study are greatest with ROFA containing soluble V and less with ROFA containing Nickel (NI); however, protein leakage and indications of lung injury (LDH) was correlated with its soluble NI content.[113] This suggests the mechanisms of pulmonary injury differ between metals contained in PM. In another study the oxidant effect of concentrated air particulates (CAPS) collected at different times on hamster AMs showed significant variation.[108]

The inflammatory response of epithelial cells from particles containing transition metals involves the activation of NF-kappa B. Many cytokine genes are regulated in part by nuclear transcription factor kB. ROFA has been demonstrated in vitro to produce time and dose dependent increases in IL-6, IL-8 and TNF in epithelial cells that is dependent on activation of the NF-kB; this was inhibited by a metal chelator and free radical scavenging.[114] Provo particles caused cytokine induced (IL-6 , IL-8 and intercellular adhesion molecule ICAM-1), neutrophil chemotractant dependent inflammation of rat lungs and this cytokine secretion was preceded by nuclear factor-kappa B activation.[101] The copper found in the Provo extract replicated the activation of nuclear factor-kappa B and IL-8 in-vitro with cultured BEAS cells. Thus, activation of NF-kappa B may be a critical first step in the inflammatory response of epithelial cells to particles generated from oil combustion and containing transition metals.

Protein tyrosine phosphate homeostasis is a mechanism governing the synthesis of proinflammatory proteins in human epithelial cells. Oxidative stress from particle exposure elicits disruption of tyrosine phosphate homeostasis with implications for increases in anti-bacterial proteins (lysozyme and mucin).[115] Non-cytotoxic levels of ROFA is shown to

disrupt protein tyrosine phosphate homeostasis, as demonstrated by an increase in phosphotyrosine levels.[89] This effect was mimicked by vanadium containing solutions. Vanadium within ROFA may disrupt protein tyrosine phosphate homeostasis in BEAS cells that may increase the synthesis of proinflammatory proteins.

Whether generation of oxidative stress and inflammation from ultrafines can exist by mechanisms other than the ability to induce Fenton reactions through release of transition metals is unclear. However, in-vivo research supports the unique contribution of size related toxicity on inflammation, independent of metals. Ultrafine levels of titanium dioxide were toxic in exposed animals while the equivalent mass of fine particles was not.[116] The inflammogenicity of ultrafine carbon black (CB) relative to ultra-fine carbon black (UFCB) was evaluated in rats and evaluated from BAL fluid.[117] Ultrafine Carbon black has been shown to have greater inflammogenicity than non-ultrafine respirable Carbon black.[94,118] These studies suggest that it is the size of the particle that is toxic rather than it's chemical composition.

Relevant to the unique properties of ultrafine to induce toxicity, studies have shown that ultrafine CB and ultrafine latex particles induce alterations in calcium signaling in human monocytic cell lines and in macrophages lavaged from rats.[119] Intracellular calcium is involved in the control of transcription factors such as NF-KappaB.[57] The mechanism by which ultrafines may enhance calcium influx is unknown; however, in the presence of thapsigarin that releases endoplasmic reticulum calcium stores, ultrafines enhance the influx of extracellular calcium through plasma membrane calcium channels.[119] This suggests that in the presence of other proinflammatory mediators, ultrafines can have a substantial effect on

intra-cellular calcium–signaling pathways and possibly on expression of proinflammatory genes.

Investigations have shown that inhaled or intratracheally instilled ultrafine particles of titanium dioxide, iron oxide and India ink are found mainly in alveolar macrophages[116,120-123] Agglomerated particles may be phagocytosed by alveolar macrophages; however, even for agglomerated particles that are insoluble the elimination may be slow. Macrophages that phagocytose large numbers of particles have diminished clearance functions.[124] Furthermore, macrophages, which phagocytose a large number of ultrafine particles, are stimulated by the high particle load to release inflammatory mediators such as TNF.[59] Particles may overwhelm the macrophage defense and remain unphagocytosed. Unphagocytosed particles in the peripheral lung can result in sustained stimulation of epithelial cells. The consequence of this could be the increased production of IL-8 or MIP1α. Increased production of these chemokines has been found in rats exposed to carbon black,[125] a process that is important to the recruitment of neutrophils to the lungs.

Ultrafine particles have many characteristics which may impart toxicity to the lung with consequences in cytokine mediated systemic changes that are proatherogenic. However, their size may impart a unique toxicological property over and above that of the larger fractionations of particulates and relevant to the health consequences arising from target organs other than the lung. This will be discussed below.

2. Comparative toxicology

In vivo models provide evidence of the systematic consequences that include alterations in inflammatory and hemostatic factors, impaired vascular function and accelerated atherosclerosis as a consequence of particle exposures. The alterations in the blood include

elevations in fibrinogen,[16,126] blood viscosity (largely a function of fibrinogen),[25] CRP,[20,23,26] bone marrow and decreases in hemoglobin and red blood cell count.[26] Inconsistencies in some of these relationships exist. In a study that found elevations of CRP in relation to PM, no relationship with fibrinogen was found.[26] In a study of the Multi-Ethnic Study of Atherosclerosis, the results were incompatible with strong effects of PM on elevations of CRP.[21]

Oxidative stress - In-vivo experiments have demonstrated oxidative stress in the hearts and lungs of rats from exposure to concentrated $PM_{2.5}$.[18] Similarly, exposures to ambient $PM_{2.5}$ associated with being a bus driver in central Copenhagen compared to those in rural/suburban areas, was associated with increased concentrations of markers of lipid and protein oxidation in the blood.[17]

Bone marrow stimulation - In-vivo exposures to PM to rabbits have also been found to stimulate the bone marrow production of polymorphonuclear leukocytes (PMN) in the marrow and accelerated the production of band cells.[127] The magnitude of these changes was related to the amount of particles phagocytosed by the macrophages. An epidemiological study of military recruits exposed to a severe air pollution episode resulting from the forest fires in Southeast Asia, characterized by increases in PM and SO_2, had stimulation of bone marrow and release of immature PMNs into circulation[128] similar to that seen in the rabbit study. This evidence suggests that upon exposure to PM in the lung, macrophages are stimulated to produce pro-inflammatory mediators that result in the observed bone marrow toxicity. These cytokines stimulate bone marrow to release premature platelets as well as granular and toxic PMNs, which preferentially sequester in capillaries and can cause tissue damage.[100]

3. Atherosclerosis

An additional study that showed an increase in PMN and band cells in rabbits exposed to PM_{10} also showed an increase in the volume in atherosclerotic lesions in proportion to the number of alveolar macrophages that phagocytosed PM_{10}.[28] Furthermore, exposure to PM_{10} caused an increase in plaque cell turnover, the extracellular lipid pools and the total amount of lipids in the lesions. In a recent epidemiology study, exposure to PM based on GIS derived exposure gradients was associated with increases in intima-medial thickness among subjects involved in clinical trials in Los Angeles.[29]

4. Unique role of ultrafines in systemic distribution

While, it is generally believed that proatherogenic changes arise from cell signaling pathways as a consequence of inflammation in the surface of the lung, the ultrafine components of PM represent a unique toxicological potential independent of their metal content or their effects on the lung. Ultrafine particles or the soluble components of PM may penetrate the epithelium and cross over into the general circulation, resulting in direct toxicological effects of organs involved in CVD i.e. the endothelium of vasculature, the heart, liver, kidneys and platelets. The possibility exists that exposures may result in pulmonary retention and systemic distribution of PM with the possibility of other organs being the target of PM toxicity.

Some studies support the potential of ultrafine particles to penetrate the lungs and enter into systemic circulation. Takenaka and colleagues have shown that while agglomerated ultrafine elemental silver (EAg) particles larger than 100 nm are phagocytosed by alveolar macrophages, disaggregated particles were frequently found in the alveolar walls.[123] Furthermore, the investigators found that after a low concentration of ultrafine EAg were

detected in other organs such as the heart and the blood that shows that systemic distribution occurred. Thus one hypothesis by which particulates may be related to CVD is that ultrafine particles may enter the alveolar wall and gain access to the circulating blood through capillaries.

Another study has been performed which supports this hypothesis in humans. Nemmar et al. Showed that inhaled radiolabelled particles were detected in the blood after one minute and subsequently in the liver[129] - a result that was similarly found in hamsters.[130] This study has been criticized on account of the possibility that the radiolabels become unbounded from the particulates and bounded instead to plasma proteins. In contrast to Nemmar, Brown et al. found no significant accumulation of radiolabeled ultrafine particles in the liver.[131] Nonetheless, the potential of particles to enter the bloodstream is evident from the presence in the blood of proteins that are mainly if not exclusively produced in the respiratory tract i.e. Clara cell secretory protein CC16 & CC10, surfactant protein SP-A SP-B & SP-D, as well as mucin associated antigens KL-6, 17-B1 & 17-Q2.[132]

E. Background- Liver

The liver is the principle organ involved in the acute phase reaction. The involvement of the liver is therefore implied by the observations that PM stimulates systemic inflammation as demonstrated by associations of exposure to PM with CRP and fibrinogen. In addition, as the liver's central function is detoxification, the proposed mechanism by which ultrafine particles, metals or other soluble components of particles can be translocated from the lungs into the circulation also points to the involvement of the liver. Changes in the metabolizing capacity of the liver may be the first sign that a pathological process is beginning .[133]

1. Toxicity to liver from PM

The liver has been investigated as an obvious target organ in the study of the dosimetry following inhalation of PM. In a theoretical model, ultrafine particles administered directly into the blood circulation of rats by intravenous injection, accumulated in liver.[134] In another model, ultrafine carbon black particles in suspension were injected into mice to mimic particles translocated into the pulmonary veins in systemic circulation.[135] In-vivo fluorescence microscopy was used to measure the interactions of blood cells with the hepatic endothelium. UFPs were shown to increase the number of adherent platelets in the hepatic microvasculature of healthy mice. Accumulation of platelets is a procoagulatory/prtohrombotic effect. The change however did not occur with an inflammatory reaction nor did it induce hepatocellular tissue injury.

Investigations of actual translocation of particles into the lung compartment have been hampered by methodological difficulties. Inhaled ultrafine particles were found to accumulate in the liver of hamsters[136] rats [137] and humans.[129,136] However, these studies are questioned due the methodological difficulty that the label may come off. In one study that was not vulnerable to this labeling concern, translocation to the liver via the blood compartment was demonstrated. However, an alternate route of exposure from the ingestion due to the animals cleaning themselves, may have occurred as well.[55]

In spite of the evidence for particulate translocation and the evidence of systemic inflammation, remarkably few epidemiological studies have focused on the role of the liver in mediating the CVD health effects of PM. I found one epidemiological study of the effect of PM air pollution on liver toxicity represented by ALT levels. In this a positive relationship was observed between urban traffic and liver function tests. Increased levels of ALT were

found in municipal police employees doing traffic duty compared to those doing office work.[138] This occupational study is likely to reflect unusually high exposure.

2. The liver as mediator of inflammation, cytokine production, CVD

The role of the liver in cardiovascular disease has recently been appreciated. Hepatic inflammation in nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), characterized by elevations in ALT is associated with low-grade inflammation is highly prevalent in obese individuals and is recognized as part of the metabolic syndrome.[139] The metabolic syndrome is a host of conditions that includes systemic inflammation indicated by high levels of CRP, glucose intolerance, insulin sensitivity, diabetes, obesity, high blood pressure, high LDL, low HDL and now NAFLD.

Rather than being a simple marker of occult liver disease, ALT levels in the absence of a more specific measure of NAFLD, reflect the involvement of the liver and visceral fat in CVD. Both visceral fat and subcutaneous fat are major sources of soluble factors that modulate energy homeostasis and tissue remodeling. These fat derived factors include hormones (leptin,k adiponectin and resisten), cytokines, (tumor necrosis factor TNF-α, IL-6, transforming growth factor-β TGF-β, and tissue factor, neurohormonal mediators (angiotensinogen), and clotting cascade regulators. These adipose-derived factors (adipokines) interact to regulate one another's production and biological activity in fat as well as the liver. The resulting network of interdigitating signals coordinates complex responses to changes in energy substrate supply and demand, directing appropriate tissue catabolism and anabolism, to optimize survival.[140]

Obesity modifies the liver to create a proinflammatory hepatic milieu. Altered adipokine production by expanded visceral adipose depots may initiate this process. For example,

excessive levels of TNF-α relative to its antagonist adiponectin, favors increased biological activity of TNF which further inhibit adiponectin. Reduced adiponectin activity promotes hepatocyte steatosis by enhancing fatty acid uptake, inhibiting fatty acid oxidation and reducing lipid export... Faced with excessive TNF- and fatty acids but little adiponectin, hepatocytes store lipids.

In turn the retention of fatty acids activates NF-kB within hepatocytes, increasing various mediators such as IL-6, TNF- , and IL-8, creating a proinflammatory milieu in the liver.

Where it had been assumed that the production of IL-6 that is responsible for the increased production of CRP in the liver has been derived in other tissues, it is now recognized that it comes from fatty liver. This is particularly significant since increased sustained IL-6 from the liver causes systemic insulin resistance. Also, local increases in TNF- and IL-8 promote hepatocyte oxidant stress and eventual apoptosis.

Obese livers are selectively depleted of CD4+ NKT cells because of increased rate of NKT apoptosis. NKT cells are the predominant source of interleukin-4 and interleukin-13, important anti-inflammatory TH-2 cytokines. Thus hepatic depletion of CD4+ NKT cells with obesity is accompanied by TH-1 polarization of other cytokine-producing liver mononuclear cells. This leads to excessive production of proinflammatory cytokines such as TNF-α and interferon-γ.

Thus with visceral fat, the liver becomes a proinflammatory milieu that results in sustained increases of IL-6 that causes systemic insulin resistance, hepatocyte oxidant stress and apoptosis and infiltration of inflammatory cells to the liver. Increased hepatocyte death and inflammatory cells accumulate to cause NASH. Increases in ALT therefore correspond to metabolic alterations that occur in the liver and which contribute to the progression of

CVD. Elevated ALT is associated with younger age, male sex, Mexican American ethnicity, impaired glucose metabolism and insulin resistance, obesity, central adiposity, high leptin, triglycerides, and C-peptide. Central adiposity, insulin, and leptin are the most highly associated factors.[141]

In the interest of exploring how ALT as a sign of increased inflammation in the liver, it is notable that NAFLD corresponds with alterations in the response of the liver to oxidative stimuli. Studies of liver disease have shown that in normal liver tissue, hepatocyte are robust to damage from foreign agents; however alterations in liver tissue associated with metabolic disorders make it vulnerable to inflammation and cell death from oxidative stress. Studies of NAFLD suggest a two-hit hypothesis to explain adaptations of the liver that are associated with CVD.

Studies of alcoholic and non-alcoholic liver disease indicate that TNF-alpha is a key factor in orchestrating the response to physiologic and pathologic stimuli in the liver, and may be modified by nuclear factor kB (an antiapoptotic transcription factor). Tumor Necrosis Factor-alpha (TNF-α) is the principle factor in mediation of hepatic inflammation, apoptosis and necrosis of liver cells, and paradoxically also mediates regeneration of liver tissue after injury.[142] Normal hepatocytes are resistant to TNF-α induced apoptosis. In normal liver tissue, exposure to reactive oxygen species and bacterial endotoxins does not induce gene transcription for TNF-α as it does in other tissue. TNF induced activation of nuclear factor-kB is likely to be involved in this protecting hepatocytes. Nuclear factor kB may neutralize the cell death initiated by TNF-alpha in normal liver tissue. Inhibition of TNF induced activation transcription factor nuclear factor-kB promotes cell death of hepatocytes exposed to TNF-alpha.

The effects on liver inflammation have been hypothesized to involve pathogenic stimulus on an existing metabolic disturbances that involve an increase in TNF. Thus the two-hits is one that increases the exposure of hepatocytes to TNF-alpha and another that interferes with a hepatocytes normal ability to protect itself from TNF-alpha induced cell death. The effect of TNF-alpha on hepatocytes in vivo is strongly influenced by other cytokines in liver tissue which are upregulated in the fatty liver.

Obese patients have increased production and activity of uncoupling protein-2. The synthesis of uncoupling protein-2 in a fatty liver may help inhibit hepatocyte apoptosis, increasing hepatocyte survival. However, because cells with increased uncoupling-protein activity have partially depolarized mitochondria, they may also be more vulnerable to loss of the mitochondrial inner membrane potential, with consequent depletion of ATP and necrosis if exposed to secondary insults such as endotoxin and TNF-alpha. The uncoupling of protein 2 may be once component of a general adaptive response that preserves the viability of hepatocytes in fatty livers but also increases the vulnerability of these cells to subsequent insults.

As a consequence of obesity related alterations in the liver, metabolic adaptations improve hepatocyte survival while also making it vulnerable to future insults. These changes that are associated with inflammation in the fatty liver can have idiosyncratic consequences from pathogenic stimuli. The result of these metabolic alterations suggest the possibility that exposure to air pollution may alter the balance in the rate of hepatic apoptosis and proliferation of hepatocytes in favor of proliferation in normal liver tissue. Previous studies have shown decreases in ALT for both smoking and coffee consumption.[143] In an animal study, exposure to combustion exhaust gases containing a high percentage of SO_2 caused

decreases in ALT activity in guinea pig livers.[144] Exposure to wood smoke was noted to cause a decrease in ALT activity in rodents.[145]

F. Epidemiology and particulate matter

1. Effects of acute exposure

Collectively, the evidence air pollution epidemiology demonstrates that particulate matter air pollution can accelerate the development of atherosclerosis and worsen its sequelae.[31] The methodology involved in the accumulated evidence has heavily relied on temporal day-to-day variations of outdoor particulate concentration in relation to day-to-day variation in mortality, emergency room visits, hospitalizations, exacerbations of ischemia or arrhythmia controlling for other time related factors. These time-series studies and more recently case-crossover designs[27,146] measure only short-term exposure. The inference of these studies is largely limited to acute effects of PM air pollution. It is uncertain to what extent the excess deaths demonstrated in these studies represents a significant increase in mortality that would be reflected in changes in age-specific death rates in the general population, or merely the deaths of already ill persons being brought forward by only a few days (a harvesting effect). Furthermore, time-series studies are typically conducted one city at a time.

A valuable methodological tool has more recently been applied to the setting of risk of mortality from short-term fluctuations of PM. Multi-level modeling has been used to measure the effects under the heterogeneous circumstances between locations and to evaluate the contributions of other co-pollutants simultaneously. A study of effects of PM_{10} on day to day mortality between 1987 and 1994 was done in an analysis of 20 cities encompassing 50 million people.[40] In this study, a two-stage Bayesian approach was used to combine the effect estimates of the cities together and includes city level variables in the analysis. The

authors found that the rate of death from all causes increased .51% and .68% for cardiovascular and respiratory deaths for each increase in 10 μg/cubic meter of PM while controlling for other criteria pollutants. In another study that combined data, Stieb and colleagues performed a meta-analysis of daily time-series studies of air pollution and mortality around the world, combining 109 studies from single and multipollutant models. In the multipollutant model excess all-cause mortality from PM_{10} and SO_2 remained significantly different (referring to the frequentist interpretation) from zero.[147]

2. Chronic exposures

Measuring the effects of chronic exposure to PM air pollution is more difficult. The exposure contrasts that exist within populations from temporal variation in acute air pollution exposures diminish as exposure lags increase in the study of chronic effects. As a consequence, epidemiological studies have relied upon geographical variation in average PM air pollution. Inference from these studies is threatened by the necessity of utilizing exposure contrasts arising between populations. However, where they exist, cohort studies with adequate information to control for differences that may exist between geographically defined populations can account for the differences that may exist between populations. Cohort studies provide evidence of PM pollution exposure and heath relationships with relevance to the public health impact from exposures to PM air pollution, which occurs over time. Such studies have examined mortality as the outcome for which reliable data exists.

The few longitudinal cohort studies which have been done suggest that PM is associated with significant loss of person-time measurable in changes in age-specific death rates. The Six-Cities study,[148] the American Cancer Society (ACS) cohort,[34] and the Seventh day Adventist study are well known longitudinal studies. These data were used to examine the

differences in excess mortality from lung and cardiovascular disease at ambient PM community levels commonly found in the U.S. Two of the three analyses have found geographic differences in cardiovascular as well as respiratory mortality were correlated with ambient community levels of PM air pollution and sulfates. The Seventh Day Adventists in California found that long-term inhalable particles (measured PM_{10}) were related to all cause mortality (from natural causes), nonmalignant respiratory mortality, and lung cancer mortality in males. They did not, however, find a relationship with cardiovascular disease.[149] A subsequent analysis of the ACS cohort doubled the follow-up time of the original analysis and included new $PM_{2.5}$ data and also included dietary variables that account for total fat consumption, vegetables and citrus and high-fiber grains. The analysis showed that fine particulate and sulfur oxide-related pollution were associated with all cause, lung cancer and cardiopulmonary deaths. Coarse particle fraction and total suspended particles were not associated with mortality.[150] Another study found that living in proximity to a major road was associated with a two-fold risk of cardiopulmonary mortality.[151]

Between acute effects on both morbidity and mortality, and chronic effects on mortality, are associations between chronic effects and specific cardiovascular morbidity for which there is considerably less evidence. One notable exception is a study that provided the first epidemiological evidence of the specific association between PM air pollution and atherosclerosis.[29] In this study, annual mean PM exposures at the residence of individuals were associated with carotid intima-medial thickness that is a direct measure of atherosclerosis.

3. Methodological challenges

The single largest challenge in air pollution epidemiology is exposure measurement, particularly for the study of chronic effects of air pollution for which it is impracticable to use personal monitoring equipment. Reliance on ambient monitor data to estimate exposure is likely to continue. Though it has been demonstrated to be a reliable measurement of personal exposure indoors,[70] the most obvious problem is that people cross in and out of micro-environments every day. This problem is compounded by several other methodological problems. The first is that the actual offending exposures are unknown, although laboratory and human exposure studies implicate specific constituents and characteristics that are variously associated with pollutants, in particular $PM_{2.5}$, measured with ambient monitors. The relative contribution of ambient $PM_{2.5}$ and its gaseous copollutants are correlated. Thus, the independent effects of $PM_{2.5}$ that have been reported in the literature may be confounded by copollutants. However, ambient measures of gaseous copollutants have been shown to be poorly correlated with personal exposures to their respective pollutants; however, they have been found to be correlated with personal exposure to $PM_{2.5}$. Therefore, rather than being confounders, gaseous pollutants can be considered to be surrogates of $PM_{2.5}$.[70,152] Nonetheless, as surrogates, the gaseous pollutants are likely to correspond to different components of $PM_{2.5}$ which may confer unique pathogenic properties. Furthermore, the value of ambient monitoring data of gaseous pollutants as surrogates for $PM_{2.5}$ exposure may vary over space and time. In addition, the ability of ambient measures of gaseous as well as particle matter pollution to discriminate between individual exposures among people living within the same area is limited. Triangulating exposures to a particular

residence using ambient monitors may depend on the number of monitors and their relative positions.

The following illustrates the difficulties that follow from the above. Due to the high diffusion capacity of fine particulates, most people in one community have high exposure to PM associated with fine particles associated with a power plant. In contrast another area has greater variation but low average measures of $PM_{2.5}$ derived from urban traffic. While they each have fine particles, for those in one community with the same exposure on a mass basis as someone in the other community, their biologically effective dose with respect to eliciting alveolar macrophage vs. epithelial cytotoxicity perhaps is different. In addition, those who live in proximity to highways may have very different exposures characterized by intensity and duration.

Given the same measured exposure to $PM_{2.5}$, the exposure characteristics in one community may confer a different risk than that in another community. The risks however may be relevant to two different mechanistic pathways. The consequences of each pathway however, may have relevance to either short-term or long-term risk. These risks are also modified by earlier exposures that are correlated with current risk. In addition, the physical and social environment that is related to CVD risk may be correlated with the different community air pollution exposure characteristics.

This may result in either confounding or modification of the effects of associations with ambient air pollution monitor exposures. The confounding may occur within or between the levels of the community. These problems are compounded by errors in measurement of the respective pollutants. The misspecification of the exposure or the measurement errors can be

associated in either an independent of dependent manner with the characteristics of the community or individuals within a community.

While many of these problems are intractable, others may be either addressed through study methodology or tolerated as random error. However, one may also recognize the dependencies of scale in these problems. Although $PM_{2.5}$ generally behaves as a regional pollutant in the eastern US, there can be considerable small-scale variability due to point source emissions (a smelter) or features such as street canyons in large cities.[31] The different sources of variation over time and space can be exploited to tease apart the associations that are of value in statistical inference.

Mixed models are useful in separating out variation from nested hierarchies. It is ideal to include repeated measures as at least one component of the hierarchy. However, exposure variation in time and space can be partitioned in mixed models in order to elucidate separate dependencies. Although each dependency may have sources of systematic or random error, mixed models can remove the influence of their joint effects.

G. Background-Synopsis

Epidemiological studies of the health effects of particulate matter health effects usually rely on ambient monitoring for exposure measurement. The resulting exposure measurement error is the single largest methodological difficulty in air pollution studies. Specifically, the difficulty is that the particular components and/or characteristics of PM that may be responsible for the observed health effects are various and poorly measured, particularly by measurements based on mass concentrations in relation to aerodynamic diameter. Laboratory investigations point to particles associated with the combustion of fossil fuels from urban traffic and associated with fine and ultrafine particles. Ambient monitoring data of PM, NO_2

and SO_2 are each valuable as surrogate measures for the latent exposures in air pollution. However, each has unique qualities that differ in their relationship with fine and ultrafine PM.

A vast literature of epidemiological studies has demonstrated that elevations of ambient PM air pollution are associated with increased morbidity and mortality related to cardiovascular disease, in particular that related to ischemic heart disease and arrhythmic cardiac disease.[72] Our understanding of the initiation and progression of CVD involves the interaction of the endothelium of the vasculature with lipids, macrophages, lymphocytes, coagulation, and the smooth muscle of the vasculature in response to stimuli.[153] In addition to this, contemporary research implicates the liver as a major factor involved with other risk factors (LDL, HDL, obesity, diabetes, glucose intolerance, and blood pressure). Alterations associated with increased CVD risk include increased ALT levels in the blood that correspond with CRP that in turn is a sign of inflammation. Epidemiological studies of intermediate endpoints on the pathway can help elucidate the specific causal mechanisms by which PM precipitates disease.

Investigations of the particular pathophysiological mechanisms underlying the epidemiological and laboratory observations are consistent in demonstrating that PM precipitates a systemic inflammatory response as well as autonomic nervous system dysfunction.[154,155] Cell signaling involved in systemic responses to inflammatory stimuli is largely mediated by the liver. In addition, the liver is central to the host of pro-atherogenic alterations that are associated with metabolic syndrome. In obese people, visceral fat occurs with alterations in liver metabolism. Systemic inflammation that is precipitated by PM air

pollution will therefore be either mediated by the liver or have effects on the liver with possible implications for liver metabolism.

Though ambiguous, evidence exists that ultrafine particles have the potential to enter into systemic accumulation and interact directly with circulation platelets, the endothelium, liver, brain and heart directly. The principle function of the liver is to metabolize xenobiotics. Therefore, the liver is a target organ of any gases, particles or soluble components that may enter into circulation. The effects on the liver from either direct exposure from ultrafines or inflammatory stimuli from indirect exposure to the lung are likely to be dependent on other factors that govern the synthesis of transcription factors in the liver cells.

While the liver has been studied in relation to dosimetry, little attention has been paid to the significance of the liver in mediating the cardiovascular health effects of PM. In addition to the source of acute phase reactants, the liver metabolizes cholesterol that is pivotal in the initiation and progression of atherosclerosis. ALT is a likely candidate to express toxicity arising from intimate contact between translocated particles and the liver. However, little is known about the contribution to variations in ALT in the normal range. Recently, studies have indicated that ALT may reflect inflammation in the liver that is associated with metabolic syndrome.

In view of the considerations of exposure and the likely manifestations of CVD related changes arising from PM exposure, epidemiological studies must face the following methodological concerns: 1) Localities have their own unique pollution mixtures due to local pollution sources, meteorology, and background levels from natural sources or long range transport and therefore exposures within a region are likely to be quantitatively and qualitatively similar ; 2) The pathogenic components and characteristics of PM may vary

across time/season; 3) people in a geographical location are likely to have had similar environmental exposures over time that impact existing levels of the measures related to disease; thus, short-term perturbations of these measures in relation to PM are superimposed over long-term effects from repeated exposures; 4) Geographic differences that correspond to differences in average PM exposures may also correspond with other cultural, socio-economic and social differences with consequences for CVD mortality.; 5)Different components and characteristics of PM may exert effects on multiple mechanistic pathways in CVD. 6) PM is crudely characterized in epidemiological studies with respect to its pathological potential ; and 7) PM exists in a heterogeneous mixture with other pollutants, some that may exert similar effects i.e. on stimulation of inflammation in the lung or afferent nerves in the lung with consequences for CVD.

CHAPTER III

RATIONALE AND SPECIFIC AIMS

A. Rationale

The overall goal of the research contained in this dissertation is to evaluate the alterations in liver metabolism as a consequence of exposure to particulate matter (PM) air pollution. PM air pollution is related to cardiovascular disease (CVD). Contemporary research has shown that PM exposure associated with fine particles exerts oxidative and inflammatory effects in the lung and systemic inflammation consistent with physiological alterations associated with the initiation and progression of CVD. Furthermore, evidence exists that ultrafine particles associated with the combustion of fossil fuels are capable of translocation from the lung into general circulation thereby exerting direct toxicity on extrapulmonary organs and activation of platelets. Regardless of whether the effects are direct or indirect, they are likely to involve the liver in the course of detoxification or as the mediator of the acute phase response.

The studies in this dissertation evaluate two possible consequences of the involvement of the liver with relevance to understanding the consequences represented by nearly ubiquitous man made particle air pollution. The liver is the principal organ in the orchestration of the acute phase reaction of the body to inflammatory stimuli. In addition to its involvement in inflammation, the liver governs lipid metabolism. Though much of this research remains to be worked out, the physiology of each is not unrelated.

While many existing studies have examined the relationship between PM and other CVD related endpoints, none have evaluated specifically the alterations in lipid metabolism that are integral to the physiology of ischemic heart disease. Also if gases, ultrafine particles or soluble components of PM do enter into systemic circulation, it is reasonable to expect that they may have direct toxic effects on hepatocytes. With the exception of the dosimetry of ultrafine particles upon inhalation, only few laboratory studies have examined PM effects on the liver. Alanine aminotransferase (ALT) is the most obvious parameter to examine to study hepatotoxicity from PM. ALT is the most specific marker of hepatocyte cell destruction.[156] To my knowledge, only one epidemiological study has examined ALT in relation to cytotoxicity or oxidative or inflammatory stimuli in the liver.[138]

Particulate air pollution is a heterogeneous mix of solids, condensed gases, and liquids suspended in air that continually vary in their size and composition over space and time. The nature of air pollution is such that it has both ecological and individual level significance for exposure. Statistical power to identify associations is largely driven by exposure contrasts; however, the nature of PM exposures is such that the pathogenic potential associated with a per-unit increase of exposure dose is variable. Furthermore, it does not vary uniformly in relation to particle size, constituents and chemistry. Furthermore, these properties of particulate air pollution that are relevant to toxicity are measured with surrogates, usually ambient air monitor data. While the use of these data have been shown to be valid as measures of personal exposures, the correspondence between personal exposures to the underlying pollution characteristics and ambient monitor data is also likely to vary across circumstances of space and time.

The NHANES data includes sampled participants clustered geographically in counties from around the United States with standardized data. These data that have been merged with ambient monitor data from around the country can provide information on physiologic parameters on people under a host of environments. The characteristics and constituents of particulate air pollution that are relevant to their toxicological potential vary over space and time. Because persons in the same county will have qualitatively and quantitatively similar exposures, the between county variation provides a valuable exposure gradient. Within counties, small area variation can provide a valuable exposure gradient within the same exposure milieu and among other common contextual characteristics. As a consequence of variability that exists between counties (at the aggregate level) and within county levels (the individual level), two sources of variability are available to address the study questions, each with different value in making statistical inference. However, variability in the within county level exposures may vary about an underlying effect. This variability may imply important etiologically relevant differences in either biological effects or measurement error.

Multi-level analysis is useful to separate the sources of variance in relation to the exposure for the purpose of simultaneously exploring the relationship across the two levels of exposure contrasts. It also allows the lower level exposure effect estimates to vary around a fixed effect and also over higher exposure levels.

B. Study questions/specific aims

Aim 1: To evaluate the relationship between short-term particulate air pollution exposure measures and levels of ALT measured in the blood.

Hypothesis 1: Living in counties with high PM_{10}, NO_2 and SO_2 pollution is associated with higher levels of ALT.

Hypothesis 2: Individual exposures related to small area and short-term temporal variation in exposures to PM_{10}, NO_2 and SO_2 in the previous week, relative to others in a county, is related to higher levels of ALT

Aim 2: To evaluate the relationship between chronic exposures (in the previous year) to particulate air pollution and levels of LDL and HDL.

Hypothesis 1: Living in counties with high PM_{10}, NO_2 and SO_2 pollution is associated with higher LDL cholesterol and secondarily lower HDL cholesterol levels.

Hypothesis 2: Individual exposures related to small area geographical variation in PM_{10}, NO_2, and SO_2 in the previous year, relative to others in a county, is related to high LDL cholesterol and secondarily to low HDL cholesterol.

CHAPTER IV
METHODS

A. Overview of methods

I conducted a secondary data analysis using the National Health and Nutrition Examination Survey (NHANES) III data merged with data from the Aerometric Information Retrieval System (AIRS) data. I evaluated the hypothesis that PM may precipitate liver mediated pro-atherogenic changes manifesting in alterations in LDL and HDL cholesterol levels (chapter 5), and alanine aminotransferase (ALT) levels (chapter 6). These data have been used before in order to evaluate the association of acute exposures to criteria pollution with hematological variables.[157] Typically NHANES data are analyzed using survey regression methods that generate individual level estimates with variance estimates that are adjusted for sampling design. NHANES subjects are individuals nested in counties that are the primary sampling units (PSUs). The pollution levels sampled from around the country provide a variety of exposure levels that provides a spectrum of exposures and variation from geographical or temporal variation within the counties provide an additional source of variation. In the current study, I applied multilevel analysis to the hierarchies of the nested data structure in NHANES to allow for the simultaneous examination of group level and individual level exposures that is characteristic of air pollution. These models simultaneously account for the correlated errors that exist within groups arising from the sampling structure of the data.

B. Study population- NHANES III data

The National Health and Nutrition Examination Survey (NHANES) is a survey conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention. This survey has been designed to collect information about the health and diet of people in the United States. The Third National Health and Nutrition Examination Survey (NHANES III), 1988-94, was conducted on a nationwide probability sample of approximately 40,000 persons from over 81 counties with 89 survey sites done over two phases – first phase (between 1988 to 1991) & second phase (1991 to 1994). As exposure data is only available for the first wave, the analysis will only be performed on these data.

The survey was designed to obtain nationally representative information on the health and nutritional status of the civilian non-institutionalized population of the United States through interviews and direct physical examinations. The study over-sampled minority populations, the very young (23% in NHANES vs. 9% in the population) and the elderly populations. African Americans (12,000) and Mexican Americans (12,000) each represent 30% of the sample where they represent 12% and 5% of the national population. Persons 60 years of age and older (12,000) represent 20% of the sample vs. 16% in the national population. (Source: http://www.cdc.gov/nchs/products/catalogs/subject/nhanes3.htm)

The first stage of the sampling began by selecting 81 counties as primary sampling units using selection probability proportionate to county population. Thirteen large counties were chosen with certainty. Due to their large size, these 13 counties were divided into 21 survey locations. The remaining U.S. counties were grouped into 34 strata and two were selected from each. The resulting sampling structure has 81 PSUs selected and 89 locations. These strata were divided into geographic aggregates of blocks and area segments corresponding to

units for which Census estimates are available. Within these area segments, selection of households and group quarters were listed from which a subsample was designated for screening. The result enabled production of national, approximately equal, probability samples of households in the United States, but with higher rates among those geographic strata with high Mexican-American populations. Within each stratum, screening rates were used to produce the desired number of persons with the rarest age-sex domain in the race and ethnic group defining the geographic stratum. The differences in the probability of selection resulting from over-sampling are incorporated into the weights.

The data collection included an initial screening of households, followed by an interview at the household during which the interviewer administered a questionnaire. The questionnaire was designed to find out about educational levels, ethnicity, occupational information, health insurance coverage, family income and characteristics about the household itself. The home visit was also used to schedule examinations. More than 73% of the sample persons made an appointment, appeared at the MEC and completed the examination. Interviewers re-contacted those who broke their appointment or refused. About 14% of these people agreed to take part, raising the examination rate by 4% to 77 percent.

The data analyzed here includes that which was collected on people in 44 locations during the first phase between October 1988 and October 1991.

C. Exposure data- Aerometric Information Retrieval System

Air pollution data was obtained from the Aerometric Information Retrieval System (AIRS) of the U.S. Protection Agency. AIRS contains information on all of the routine pollution monitoring in the United States. Pollutant exposures were assigned by means of geocoding. Each participant in NHANES III was assigned a longitude and latitude

corresponding to the population centroid of the census block group in which they lived. Block groups are collections of adjoining blocks, selected to be uniform in socio-economic status, with populations (in 1990) of about 1,000 persons. The longitude and latitude of each monitor in the United States was obtained by AIRS. Persons were assigned exposure values equal to the average of measurements from all monitors in their county of residence and adjoining counties, with the average weighted in proportion to the inverse of the square of the distance between their residence and the monitor. I created an exposure variable derived from this measurement value subtracted from the mean of all values (the grand mean). I refer to this as the unpartitioned exposure measure to distinguish it from the partitioned exposure measure described below. Pollution monitor data is missing for some participants in counties where there were no monitors and some counties had pollution data for some pollutants but not others. Therefore, analyses for individual pollutants do not include all of the same subjects.

In the current analysis, the use of the weighted average of pollutant measurements provides geographic variability in exposure within a county to be reflected in the exposure measurement of chronic exposure to PM_{10}, SO_2 and NO_2. The true geographical variation is driven by local point source and mobile source pollution as well as geographical and meteorological differences in the diffusion of air pollution.

The variation of air pollution between counties can contribute to average health related outcomes among residents in the county, which is a different level of inference from associations derived from variation within a county. In the absence of significant variation within a county, average county level exposures are the best measurement for individuals residing in the county. In relation to an outcome, analysis of air pollution in these data ought

to reflect the level of analysis from which the variation in exposure is derived. To this end, I parameterized the pollution exposure variables to be used in mixed models in order to arrive at separate estimates reflecting the county level (ecological) effect and the within county effect that has a more individual level of inference.

Because ambient monitors measure different characteristics of pollutants that may relate to the underlying latent pollutant characteristics in different ways, no one pollutant is particularly representative of the pertinent exposure. From the literature, particulate air pollution that is associated with fossil fuel combustion is the most specific characterization of the underlying exposure. PM_{10}, NO_2 and SO_2 are each indirect measures of this pollution, although each may reflect unique latent characteristics associated with fine PM. We used each in order to explore whether effects were associated with pollution from gasoline engines in urban traffic (NO_2) or pollution from combustion from diesel (SO_2).

Exposure Variables:

Pollutants obtained from AIRS data, geocoded to participants residence address with inverse variance weighting for multiple monitors.

Main exposures:

PM_{10} $\mu g/m^3$

SO_2 ppb

NO_2 ppb

Mean of prior year measures of pollutant (Manuscript #1 -Lipids)

Mean of prior week measures of pollutant (Manuscript #2 -ALT)

Partitioning of air pollution exposure measures - For each pollutant, I created a variable that is the county mean of subjects prior year (week for ALT) concentration exposures to the

air pollutant and subtracted the grand mean of the pollutant over all counties over the same period. The result is a county level average air pollution exposure measure expressed as a deviation from the grand mean. To the extent that true variation in air pollution exposure is derived from between county variation, inference is limited to the population (ecologic or county) level, such that living in a polluted area is associated with increase/decreased average lipid (or ALT) levels. For each individual, I also created a variable that is the individual's mean pollutant concentration at residence during the previous 12 months (1 week for ALT) minus the county average; this estimates an individual's air pollution exposure expressed as a deviation from the county mean. Inference on this parameter has a specific individual-level interpretation, allowing for measurement error, independent of its ecological analogue. It also tends to capture more local, rather than regional sources of pollution.

The equations below illustrate the creation of the county level and individual level partitioned pollution exposure measurement for the i^{th} individual in the j^{th} county:

PM_j = j^{th} county mean PM- grand mean PM

PM_{ij} = i^{th} individual in county j PM – j^{th} county mean PM

The resulting parameters employed in a mixed model that partitions the variation at the county and within county level as described below allow the other parameters to be interpreted as the effects at the mean county and mean individual exposure (relative to their county) to air pollution. I refer to this parameterization of the air pollution variable as the partitioned air pollution levels, in reference to the partitioning of the exposure measures to correspond with the analogous between-county and within-county variation

D. Statistical methodology

In the NHANES data, individuals are clustered within PSUs. In the analysis, the design

effect will be accounted for by including a random intercept corresponding to the PSU. The mixed models account for the correlation of errors within PSUs as a consequence of unmeasured risk factors that cluster within PSU, such that two people in the same PSU are more alike than two people in different PSUs. This source of variation is not of etiologic interest but is a nuisance that is accounted for in the analysis. However, clustering may also occur on account of cumulative exposures before the measures were collected for the study. This source of variation may cause correlated errors that are not typically of etiologic interest. The unique geographical/meteorological/co-pollution mixtures in each PSU may however correlate with unique pathogenic properties of PM of which the PSUs are a sample of all possible combinations. Therefore, the PSUs have unique group properties such that the outcomes for individuals within the group may be correlated. Where there are shared common exposures to air pollution within a county, then the county level exposure is a surrogate of the individual level exposure. Within PSUs, the variations of exposures to PM are of direct etiologic interest. Multi-level models will allow for the simultaneous examination of the effects of group level predictors along with individual level predictors, and allowing for the non-independence of observations within the groups.

The mixed model to be presented below is a generalized description to specifically identify the model as a linear model with an additional random component over the 1st level error. This additional error level denoted by the Z matrix allows county level variables to vary about the fixed components.

1. Mixed model formulae

The model for the General Linear Mixed Model is:

$$y = X\beta + Z\mu + e$$

where:

y= an n x 1 vector of n observations of the continuous outcome variable , y.

X= n x k matrix of predictor variables with 1s in the first column and p predictor variables measured for n observations (k=p+1)

β=k x 1 vector for fixed effect parameters, to be estimated by the model.

Z is an n x r design matrix

μ is an r x 1 vector of unknown random effects parameters assumed to follow a multivariate normal distribution.

e is an n x n matrix of residual variation, assumed to follow a multivariate normal distribution.

The overall variance-covariance of y is V.

$$V = ZGZ' + R$$

Where:

ZGZ' is the random effects component and R is the generalized residual variation

V is a block diagonal structure with n x n blocks within m x m block structure (n being the number of measures within a subject and m being the number of subjects).

As an example, the multilevel analysis equations for a given continuous outcome (LDL and HDL in manuscript 1 or ALT in manuscript #2) at the lower (1st) level with other 1st level covariates and that incorporates second level pollution parameters (PM) is shown here:

1st level

$$Y_{ij} = \beta_{0j} + \beta_{1j} (PM_{ij} - PM_j) + \beta_2(Age) + \beta_3(V_p) + r_{ij}$$

2^{nd} level

$\beta_{0j} = \gamma_{00} + \gamma_{01}$ (MEAN PM_j) + μ_{0j}

$\beta_{1j} = \gamma_{10} + \gamma_{11}$ (MEAN PM_j) + μ_{1j}

With substitution the equation reduces to the following:

$$Y_{ij} = [\gamma_{00} + \gamma_{01} (\text{MEAN } PM_j) + \gamma_{10}(PM_{ij} - PM_j) + \gamma_{11} (\text{MEAN } PM_j) (PM_{ij} - PM_j)$$

$$+ \beta_2(\text{Age}) + \beta_3(V_k)] + [\mu_{0j} + \mu_{1j}(PM_{ij}-PM_j) + r_{ij}]$$

Where:

Y_{ij} is the cholesterol value of the ith person in the jth county.

PM_j is the mean county level PM_{10} measures, expressed as a deviation from the grand mean PM_{10}.

PM_{ij} is the individual level PM_{10} measure, expressed as a deviation from the county mean PM_{10}.

μ_{0j} is a vector of deviations about the fixed effect for the model intercept γ_{01}.

μ_{1j} is a vector of deviation about the fixed effect for the model slope of γ_{10}.

r_{ij} is a vector of errors associated with the individual-level

For modeling purposes the variables in the first bracket will correspond to the fixed effects and the variables in the second bracket will correspond to the random effects. As a result of this I can fit a model with the variables included as shown in the first bracket and including the variables corresponding to the mean levels of PM for each PSU as random effects. One such benefit is that I can evaluate the covariance between the random effect for the slope of short term PM exposures across the levels of the intercept values that account for the mean levels of air pollution. If the covariance (τ_{01}) is significant, this would indicate that the effect of short term PM is conditional on the average levels of PM while controlling for other 1st level variables. Control variables will include variables that are risk factors for the outcomes but that are not believed to be descendents of the effect of short term PM effects. As an example, I will not include systolic blood pressure in a model that predicts LDL levels as their relative levels are both affected by inflammation. However, I will try to include variables that correspond to etiologically related measures that are not affected by short term PM exposures, such as age, BMI, and diabetes (insulin resistance).

2. Model assumptions

The exposure measurement values were derived from merging geocodes of the centroid of the census block group in which the NHANES participants resided to the air pollution monitor data based on a weighted average as described above in the description of the exposure data. These data are meant to represent individual level information. Inference from statistical models using these individual level data is based on the assumption that individual measures from a person under a particular exposure level can stand in for that which would be observed under another such that the errors are exchangeable. This assumption is valid if the times at which the outcomes are measured in people are

independent or random with respect to the likelihood of exposure to the pollution measures. This assumption is reasonable given that the NHANES methodology is dictated by considerations that are not likely to have much to do with pollution levels. Another assumption is that the errors at the individual level are not correlated with the errors at the macro (county) level.

3. Statistical Analysis

In each of the two analyses, I employed multilevel analysis (Proc Mixed, SAS, Cary, NC) to model the individual level lipid (HDL and LDL) or ALT values as the dependent variable, among the participants in NHANES nested within counties. I performed two mixed model types. The first was a simple random intercepts model that allowed the intercept, representing the county average adjusted mean lipid levels, to vary by county. For each pollutant, I fit a model that included the partitioned pollution parameters. In each model, I included variables that based on subject matter knowledge, are known risk factors for an adverse lipid profile or were involved in the sampling methodology (sex, age, and race-ethnicity).

In the random intercepts model including the partitioned pollution parameters , the interpretation of the resulting parameter estimates is the effect per unit change at average county prior 1-year (1 week for ALT) pollutant levels and average prior 1-year (1 week for ALT) within county pollution levels. The pollutant parameter effects themselves are independent of one another if the model is adequately specified and the county level errors are independent of the within county errors.

In the simple random intercepts model with the partitioned pollution parameters, the within county errors may not be exchangeable and may vary systematically with the average county means, or vary randomly. The within county errors would not be homogeneous in the

case where there is a dose response effect, such that at higher county mean pollution levels the within county effect was higher (or lower). Another reason the within county errors were not homogeneous would be in the case where the exposure measure based on ambient monitors in one county was more accurate than in other countries. As the number of monitors or the spread of the population of the residences within the counties are not known in these data, these differences in measurement error may vary randomly. However, counties with greater variability in air pollution levels are likely to reflect greater discrimination in air pollution exposures. Where such greater variation occurs with higher mean county pollution levels, the within county errors would vary systematically with mean air pollution levels. For this reason I also fit a model that allowed the within county coefficients to vary systematically in relation to the county mean pollution level (the cross-level effect), and where the within county variation was sufficient to do so, the county level coefficients were allowed to vary randomly about a fixed effect in a random coefficient model.

For each pollutant-response relationship, I evaluated a random coefficients model, that allowed the within county pollutant effects to vary randomly, and that included a fixed effect for the interaction of the average within county pollutant effect across the range of mean county levels. In performing the random coefficients model, I used the robust variance estimator and unstructured covariance in order to let the data determine the covariance between the random intercepts and slopes. The unstructured covariance was used for the theoretical reason that it is plausible that the within county slope could be related to the underlying population characteristics related to the adjusted mean county level of the outcome variable represented by the random intercept. This would result in the random slopes covarying with the random intercepts reflected by the τ_{01}. One consequence of this is

that in the case where there is not a lot of within county variation, one may get very data dependent solutions to the covariance between the random slopes and intercepts. Therefore, the random coefficients model is presented for qualitative evaluation and statistical inference is limited to the fixed effects models.

Upon running the random coefficient models, I evaluated the likelihood ratio test subtracting the deviance statistic of the random coefficients model from the deviance statistic of the random intercept model, in order to evaluate if the slopes differed significantly. Having specified an unstructured covariance in the model, the likelihood ratio test had two-degrees of freedom. If there was no evidence that the within county pollutant slope changed systematically with the county means, as defined as a p-value greater than 0.15, I dropped the cross-level interaction term from the model and evaluated the likelihood ratio test of the random coefficients model again without it.

E. Cholesterol analysis (MS1)

The unique features of the analysis of LDL and HDL cholesterol in relation to exposure to PM_{10}, NO_2 and SO_2 are described here. The principle difference in relation to the methods is that LDL and HDL, are not particularly labile characteristics and are considered to be determined by influences over time culminating in their status as a risk factor for future CVD events and are tied to other risk factors characterized as metabolic syndrome. We therefore consider cumulative exposure over time as the relevant time frame to evaluate the effects of PM exposures on the lipid profile. The two principle determinants of the lipid profile are LDL and HDL, where high levels of LDL and low levels of HDL confer greater risk.

Outcome Variables:

HDL-Cholesterol (Hitachi 704Analyzer/Boehringer-Mannheim Diagnostics) in mg/dl

LDL –Cholesterol calculated from measured total cholesterol and fasting triglycerides using the equation developed by Friedewald, Levy and Fredrickson in mg/dl

Total Cholesterol - high density cholesterol - triglyceride/5. in mg/dl.[158]

Total Cholesterol (Hitachi 704Analyzer/Boehringer-Mannheim Diagnostics) in mg/dl

Triglycerides. (Available only on 4yrs of age and older). (Hitachi 704 Analyzer/Boehringer-Mannheim Diagnostics) in mg/dl

Exposure variables:

I create a partitioned pollution exposure parameter for each of the prior 1 year average pollutant measurements. As an example, the county and within county parameter for PM (measured by PM_{10}, NO_2, and SO_2) was calculated as follows:

PM_j = j^{th} county mean of prior 1-year PM- grand mean of prior 1-year PM

PM_{ij} =i^{th} individual in county j prior 1-year PM – j^{th} county mean prior 1-year PM

The resulting county level parameter represents the deviation of the county mean of the prior 1 year exposure to PM_{10}, from the mean prior 1 year exposure across all counties. The resulting within county level parameters represents the individual's deviation from the county mean of the prior 1-year PM_{10} measurements.

1. Statistical Analysis

Mixed models as described above were fit with LDL and HDL as the dependent outcomes. Each model was fit alternately with each partitioned pollution parameter in the random intercept model. The results of these models constitute the principle statistical results with the resulting effect estimates for county and individual level being the basis of inference regarding the effects of PM.

2. Sensitivity Analysis

Due to the assumption of the mixed models that differences in the county effects are not due to differences in the distribution within the counties of characteristics causally associated with the outcome, I alternately fit models that included alternate specifications of the most deterministic characteristics of the outcome based on substantive knowledge of the lipid outcomes. For cholesterol, the base model included a linear and quadratic term for both age and BMI as well as an interaction term for sex with the linear and quadratic form of age. In the other models I employed alternate specifications related to age, sex and BMI that included:1) a model without the interaction between sex and the age variables;2) a model with an interaction between sex and the BMI variables (instead of age); 3) A piecewise linear parameterization of age with the cutpoint corresponding to 60, reflecting the overrepresentation of people of this age group in the NHANES data; 4) The piecewise linear parameterization of age with an interaction between sex and the piecewise age parameters; 5) The most naïve specification of age and BMI, a single continuous variable for each, without any interactions.

It is also assumed that no important variables are left out of the model that would make the errors at the individual level and the county level not exchangeable. I therefore ran

models that adjusted for additional possible confounders including: 1) a variable (1=yes, 0=no) for whether the participant had lived at the same address for more than a year 2) The sum total of the number of times exercised in the last month; 3) education; 4) Household size 5 or more (index) vs. 4 or less (referent); 5) Use of wood stove in the past 12 months; 6) Use of fireplace in the past 12 months; 7) Use of gas stove in the past 12 months; 8) Number of times eating seafood as an indicator of omega-3 fatty acid intake; 9) Coffee drinking that is associated with cholesterol levels; 10) Use of hypertension drugs.

To facilitate comparison, the resulting parameter estimates for county and within county level pollutant estimates derived from each of the alternate model specifications were plotted with their 95% confidence interval.

3. Effect measure modification

In studies of acute effects of air pollution, people with diabetes and older people have been found to be at elevated risk for adverse effects of particle matter air pollution. I therefore evaluated if there was evidence that the joint effect from each air pollutant and alternately diabetes and age, was different from their independent effects. To do this, I included interaction terms in each pollutant-lipid model. As a means to evaluate if there was statistical evidence that diabetes, or age did in fact modify the risk from air pollution, I used a cutoff of p less than 0.20, understanding that these tests are underpowered. The interaction terms included the within-county air pollutant parameter.

F. Alanine aminotransferase (MS2)

The unique features of the analysis of ALT in relation to exposure to PM_{10}, NO2 and SO_2 are described here. The principle difference in relation to the methods as compared to the

cholesterol analysis is that ALT as a measure of xenobiotic detoxification does fluctuate over the short term in response to stimuli. However, it too has determinants from influences over time that culminate in its reflecting the altered inflammatory state of the liver associated with NAFLD and tied to the other risk factors characterizing metabolic syndrome. As it was our intention to investigate the role of air pollution on the liver as the principle organ that would be involved in detoxification from stimuli arising from exposure to PM, we consider prior 1-week exposures to pollutants available in this data set as the relevant time frame of exposure. However, to examine short-term fluctuation it was necessary to pay particular attention to adjusting for the waist to hip ratio reflecting visceral fat as the principle determinant of underlying ALT levels. Elevations in ALT in relation to PM would represent cytotoxicity in the liver.

Outcome Variable:

Alanine Aminotransferase (Hitachi 737 Anelyzer/Boehringer-Mannheim Diagnostics) in U/L.

The distribution of ALT was positively skewed as were the residuals in models of ALT. The dependent variable of the mixed models was therefore log transformed ALT to normalize the errors of the model. Furthermore, large values of ALT exist that are due to causes, such as hepatitis, and alcohol intake that would overwhelm any subtle changes due to air pollution. I therefore, restricted the analysis to exclude patients with explained elevated aminotransferases as defined in a previous study of aminotransferases in NHANES III subjects.[159] Elevated aminotransferases among men were defined as either ALT greater than 40 or AST greater than 37; among women it was defined as either AST or ALT greater than 31. Participants with elevated aminotransferases were excluded if they also had hepatitis B

surface antigen, transferring saturation greater than 50%, or daily alcohol consumption of greater than 1 drink (10 gms) for women, or two drinks for men.

Exposure variables:

I create a partitioned pollution exposure parameter for each of the prior 1 week average pollutant measurements. As an example, the county and within county parameter for PM was calculated as follows:

PM_j = j^{th} county mean PM- grand mean PM

PM_{ij} = i^{th} individual in county j PM – j^{th} county mean PM

The resulting county level parameter represents the deviation of the county mean of the prior 1 week exposure to PM_{10}, from the mean prior 1 week exposure across all counties. The resulting within county level parameters represents the individual's deviation from the county mean of the prior 1-week PM_{10} measurements.

1. Statistical Analysis

I employed multilevel analysis (Proc Mixed, SAS, Cary, NC) to model the individual level log ALT values as the dependent variable, among the participants in NHANES nested within counties. I performed two mixed model types. The first was a simple random intercepts model that allowed the intercept, representing the county average adjusted mean lipid levels, to vary by county. For each pollutant, I fit a model that included the partitioned pollution parameters. In each model, I included variables that based on subject matter knowledge, are known risk factors for an adverse lipid profile. These included, age, sex, BMI, waist to hip ratio, saturated fat intake, alcohol consumption, race-ethnicity (black,

Mexican-American, and others, relative to the referent category whites), alcohol consumption, poverty income ratio (low and medium relative to the referent high).

Smoking and second hand smoke share exposures to the lung and may share pathomechanistic pathways by which acute exposures may effect alterations in liver metabolism. Due to underreporting of smoking and the desire to measure the dose response of persons exposed to environmental tobacco smoke, I used serum cotinine measurements to reflect active and passive smoking based on a validation study of reported smoke exposure in NHANES III.[160] Participants with cotinine levels greater than 15 ng/ml were designated as active smokers. Cotinine levels less than 15 ng/ml were considered passive smokers.

In the random intercepts model including the partitioned pollution parameters, the interpretation of the resulting parameter estimates is the effect per unit change at average county prior 1-year pollutant levels and average prior 1-year within county pollution levels. The pollutant parameter effects themselves are independent of one another if the model is adequately specified and the county level errors are independent of the within county errors. However, given that the within county pollutants may not all be equivalent across counties, I evaluated a random coefficient model, that allowed the within county pollutant effects to vary randomly, and that included a fixed effect for the interaction of the average within county pollutant effect across the range of mean county levels.

In performing the random coefficients model, I used the robust variance estimator and unstructured covariance in order to let the data determine the covariance between the random intercepts and slopes. Upon running the random coefficient models, I evaluated the likelihood ratio test subtracting the deviance statistic of the random coefficients model from the deviance statistic of the random intercept model, in order to evaluate if the slopes differed

significantly. Having specified an unstructured covariance in the model, the test was a two degree of freedom test. If there was no evidence that the within county pollutant slope changed systematically with the county means, as defined as a p-value greater than 0.20, I dropped the cross-level interaction term from the model and evaluated the likelihood ratio test of the random coefficients model again without it.

2. Sensitivity Analysis

Due to the assumption of the mixed models that differences in the county effects are not due to differences in the distribution within the counties of characteristics causally associated with the outcome, I fit models that included alternate specifications of the most deterministic characteristics of the outcome based on substantive knowledge of the lipid outcomes. For ALT, adiposity is the greatest single determinant of ALT in the absence of excessive alcohol or hepatitis the base model included a linear term for both age and BMI as well as an interaction term for sex with BMI. In the other models I employed alternate specifications related to age, sex and BMI that included 1) a county level variable for average age with an individual level variable for age; 2) county level variable for average age with an individual level variable for age expressed as a deviation from mean county level age; 3) The same as 2 above but with a cross-level interaction for age; 4) individual level age and a quadratic term for age; 5) linear and quadratic terms for age with interaction terms for both with sex 6) age with a county level term for BMI; 7) age with a linear and quadratic term for BMI; 8) age with a linear and quadratic term for BMI with interactions with sex 9) age with linear and quadratic terms for alcohol and interactions with sex.

The mixed models also assume that no important variables are left out of the model that would make the errors at the individual level and the county level not exchangeable. I

therefore ran models that adjusted for additional possible confounders including: 1) a variable (1=yes, 0=no) for whether the participant had lived at the same address for more than a year 2) The sum total of the number of times exercised in the last month; 3) education; 4) Household size 5 or more (index) vs. 4 or less (referent); 5) Use of wood stove in the past 12 months; 6) Use of fireplace in the past 12 months; 7) Use of gas stove in the past 12 months; 8) Number of times eating seafood as an indicator of omega-3 fatty acid intake; 9) Coffee drinking that is associated with cholesterol levels; 10) Use of hypertension drugs.

To facilitate comparison, the resulting parameter estimates for county and within county level pollutant estimates derived from each of the alternate model specifications were plotted with their 95% confidence interval.

3. Effect measure modification

The pathophysiology underlying stimuli to the liver which has been suggested by NAFLD research indicates that hepatocytes are vulnerable to insults after prior injury. To evaluate whether the stimulus of exposure to the different air pollutants is similar across levels of factors that are associated with ALT levels, I included a term for the product of the within-county pollutant variable and alternately male sex, age, BMI, metabolic syndrome, vitamin C and waist to hip ratio.

CHAPTER V

RESULTS

A. Manuscript 1: Chronic exposures to Air Pollution and Cholesterol Metabolism

ABSTRACT

Systemic effects of exposure to particulate matter (PM) air pollution can elucidate the mechanisms underlying the observed associations between acute and chronic exposure to PM and cardiovascular morbidity and mortality. We applied multilevel models to NHANES III data merged with ambient air pollution monitor data to relate between and within-county variation in PM_{10}, NO_2, and SO_2 to individual LDL and HDL levels. The bulk of the available variation in cumulative prior year exposures to these pollutants exists at the ecological (between counties) level. In random intercepts models of LDL, a mean county average increase of 10μg/dL of PM_{10} and 10 ppb of NO_2 was associated with an increase of 4.26 mg/dL (95%CI: -1.57, 10.06) and 3.61 mg/dL (95% CI: 0.98,6.30). Estimates at the individual level corroborate the county level associations although with considerably larger confidence intervals. Empirical Bayes estimates suggest that within county effects for PM_{10} and SO_2 are greater at higher county level average pollutant levels. Effects of pollutants on HDL were less apparent though the point estimates for county effect were all negative and the empirical bayes estimates suggest greater reductions associated with higher county averages of PM_{10}, NO_2 and SO_2. In addition to the previously demonstrated effects of PM on acute phase proteins, hemostatic variables and endothelial dysfunction, the current results suggest that air pollution may have a proatherogenic impact on blood lipid levels.

1. Introduction

Exposure to particulate matter (PM) air pollution is a significant cause of cardiovascular (CVD) morbidity and mortality.[1-4] The bulk of the epidemiological evidence comes from studies of acute effects, which may largely reflect effects among the vulnerable. A few studies, however, have found that living in a polluted environment is related to long-term risk of death from heart disease reflecting a far greater burden of health effects from chronic exposure to PM pollution in the general population. Studies of intermediates in the CVD pathway can provide critical linkages in establishing the biological mechanisms underlying PM health effects.

The leading hypothesis of the pathophysiological mechanism underlying these associations is that upon exposure to the lung, PM results in oxidative stress which, in turn upregulates inflammatory mediators systemically. The evolving knowledge about the initiation and progression of atherosclerosis points to critical interactions between the endothelium of the circulatory system and the inflammatory response, coagulation, and blood lipids. While studies have observed an association between acute exposures to air pollutants and oxidative stress,[5] markers of inflammation,[6-8] coagulation parameters,[9-12] and autonomic dysfunction, no studies have evaluated the effect of PM on the lipid profile.

In epidemiological studies of chronic exposures to air pollution, exposure measurement is a serious limitation. The pathogenic properties associated with PM have been tied variously to particle surface chemistry, or the specific constituents such as transition metals, elemental or organic carbon and endotoxin. In the absence of personal monitoring, epidemiological studies rely on ambient monitoring of PM that is characterized by particle size. In-vivo and in-vitro laboratory studies have found pathogenic properties associated with the course

fraction of PM ($PM_{2.5}$ to PM_{10}) and ultrafine components of PM; however, human studies primarily implicate fine particles ($PM_{2.5}$) and in particular that from automobile exhaust and diesel particles as well as their respective surrogates, NO_2 and SO_2.

Because of common climate conditions and the dispersion properties of PM, people residing in the same geographic region will have quantitatively and qualitatively similar exposures. Studies within a single area will therefore, have reduced exposure contrasts and little power to detect effects. Exposures from different regions and meteorological conditions are necessary to statistically enhance the ability to observe the outcome under a range of exposure levels. However, across different regions and meteorological conditions, particulate air pollution is likely to have different constituents and properties in relation to the measured PM, NO_2 and SO_2. Furthermore, statistical methods must account for correlation that exists among individuals within the same population and the source of variability in pollution measures from either between or within geographical area.

The current study employs multilevel analyses of the association between air pollution and cholesterol parameters (LDL & HDL) in the NHANES III data set. In this study we explicitly examine air pollution as an individual level parameter as well as a community level parameter, in relation to cholesterol that is - similar to many inflammatory and coagulation proteins associated with air pollution - produced in the liver. The clustered nature of the sampling design provides necessary variation at both county and individual levels that rather than being adjusted for, are of interest to evaluate the effect of air pollution.

2. Methods

The methods related to the NHANES sampling and the air pollution data are described in the earlier paper published on these same data which examined the relationship of same day

exposure to PM_{10} to fibrinogen levels;[10] however, these methods are repeated here with modifications to reflect the lipid outcomes and the mixed models employed in this analysis.

a. Health data (NHANES III)

The Third National Health and Nutrition Examination Survey (NHANES III) was conducted between 1989 and 1994. NHANES III is a stratified random sample of the U.S. population, with oversampling of the elderly and minority populations. Blacks and Mexican Americans each represent about 30% of the NHANES III sample. Persons older than 60 years of age (16% of the U.S. population) account for 20% of the sample population. The NHANES population is equally split by gender. The NHANES III survey was conducted during two phases, each sampling approximately the same number (20,000) subjects in 44 communities, and each representative of the general U.S. population when weighted for the oversampling. This analysis was restricted to the first phase.

Subjects were seen in their homes by trained interviewers, and extensive medical history and demographic data were collected. The subjects then visited mobile medical examination centers, where they were examined. The blood specimens for analysis of lipids was collected in a red top 5 ml tube, stored at -20° C and shipped to the analytic center for testing. The analytic methods used by each of the participating laboratories are described in laboratory Procedures Used for NHANES III.[13] The blood analysis included total cholesterol, triglycerides and HDL. LDL itself was not measured but was calculated from measured total and HDL cholesterol, and fasting triglycerides using the equation –LDL cholesterol= total cholesterol - high density cholesterol - triglyceride/5.).[14] The dependent variables of primary interest in this particular study are: LDL and HDL.

b. Air Pollution Data

Air pollution data was obtained from the Aerometric Information Retrieval System (AIRS) of the U.S. Protection Agency. AIRS contains information on all of the routine pollution monitoring in the United States. Pollution exposure was assigned by means of geocoding. Each participant in NHANES III was assigned a longitude and latitude of the population centroid of the census block group in which they lived. Block groups are collections of adjoining blocks, selected to be uniform in socio-economic status, with populations (in 1990) of about 1,000 persons. The longitude and latitude of each monitor in the United States was obtained by AIRS. Persons were assigned exposure values equal to the average of measurements from all monitors in their county of residence and adjoining counties, with the average weighted in proportion to the inverse of the square of the distance between their residence and the monitor. We created an exposure variable, derived from this measurement value subtracted from the mean of all values (the grand mean). We refer to this as the unpartitioned exposure measure to distinguish it from the partitioned exposure measure described below. Pollution monitor data is missing for some participants in counties where there were no monitors and some counties had pollution data for some pollutants but not others. Therefore, analyses for individual pollutants do not include all of the same subjects.

In the current analysis, the use of the weighted average of prior year pollutant measurements provide geographic variability in exposure to be reflected in the exposure measurement of chronic exposure to PM_{10}, SO_2 and NO_2. This variability exists from differences in exposure that exist between counties as well as within counties. To this end we parameterized the pollution exposure variables to be used in mixed models in order to arrive

at separate estimates reflecting the county level (ecological) effect and the within county effect that has a more individual level of inference.

Partitioning of air pollution exposure measures - For each pollutant we created a variable that is the county mean of the prior year exposure to the air pollutant and subtracted the grand mean of the pollutant over all counties. The result is a county level average air pollution exposure measure expressed as a deviation from the grand mean. To the extent that true variation in air pollution exposure is derived from between county variation, inference is limited to the population (ecologic or county) level; such that living in a polluted area is associated with increase/decreased average ALT levels. For each individual, we also created a variable that is the individual's prior year exposure minus the county average; this gives an individual's air pollution exposure measure expressed as a deviation from the county mean. Inference on this parameter has a specific individual level interpretation, allowing for measurement error, independent of its ecological analogue. It also tends to capture more local, rather than regional sources of pollution.

PM_j = j^{th} county mean PM- grand mean PM

PM_{ij} = i^{th} individual in county j PM – j^{th} county mean PM

We refer to this parameterization of the air pollution variable as the partitioned air pollution exposure, in reference to analogous between-county and within-county variation of the mixed model.

c. Statistical Analysis

NHANES III sampled populations within counties selected at random within strata of geographic region. Blacks, Hispanics and the elderly were oversampled to insure adequate numbers for analyses within these groups. NHANES III data have weights based on the probability of sampling. When used with software, such as SUDAAN that accounts for the particular sampling design, the weights generate estimates that are representative of the U.S. non-institutionalized population in a single level model. In this way, the variance structure from the sampling design is adjusted to derive estimates for the individual level analysis. In the analysis conducted here however, we employ these data, not as a single level model, but as a collection of populations with relevant variation at both the county and individual levels. We use this data in the a multilevel model, in order to evaluate the effect of air pollution on individual lipid parameters, understanding that air pollution exposures are shared by people in the same geographic area and therefore have a large county level component, and recognizing that health outcomes tend to cluster within geographic regions due to commonalities of local ethnic, dietary and other environmental factors.

We employed mixed modeling (Proc Mixed, SAS, Cary, NC), of individual participants clustered within counties to estimate the relationship between 3 criteria air pollutants LDL and HDL cholesterol. The analytic strategy was to specify a model (irrespective of pollutants) including the individual level variables based on subject matter knowledge and known risk factors in order to account for as much of the individual level variation from the deterministic components known to govern cholesterol levels. The inclusion of these variables as fixed effects minimized the between county variation that can be accounted for by differences in the distribution of individual level determinants of cholesterol. The model

included variables involved in the sampling design: age, ethnicity (non-Hispanic Whites, non-Hispanic Blacks, Hispanics, and others). We also included variables known to be related to cholesterol levels: sex, BMI, saturated fat, waist to hip ratio and alcohol consumption and vitamin C, smoking status (Former, Current and number of cigarettes per day) as well as cotinine (among current non-smokers). The base model also included quadratic terms for the variables age and BMI and waist to hip ratio, as well as an interaction between both the linear and quadratic term for age with sex. Additionally, we included poverty income ratio (the ratio of the household income to the city specific poverty level) to adjust for socio-economic factors that may correlate with exposure to air pollution. Mixed models were fit which included a random effect for the intercept of each county, we used the robust variance estimator and the between-within method for determining degrees of freedom.

For each pollutant-cholesterol relationship, we employed the partitioned pollution variables with the individual level independent variables described above in two types of mixed models. The first was a random intercept model with a random effect for intercept corresponding to county and the partitioned variables included only as fixed effects in order to evaluate the independent effects of county and individual level pollutants, allowing for correlated errors within county. Each parameter in the model is interpreted as the effect at the mean county and mean individual exposure (relative to their county) to air pollution. Although the effects are derived from different levels of observation, each level is measuring the same effect; therefore, the evidence is most compelling when the two levels have similar effect estimates.

We evaluated the interaction of the within county (individual level) pollutant effect and the individual effect modifier in the random intercept model. Previous studies have identified

individuals with diabetes and older age to be susceptible to acute effects of PM_{10}.[15] Smoking status was also examined to evaluate the effects where smoking is likely to overshadow air pollution effects, or where smoking may materially alter the underlying mechanistic pathways by which air pollution may inflict health effects.

For each pollutant –cholesterol relationship, we also fit a random coefficient model with an additional random effect for individual level pollutant. For these models we plotted the empirical Bayes estimates derived from the random coefficients model. Each of the random coefficient models initially included the partitioned pollutant variables with a cross-level interaction (interaction between county and individual level pollution parameters). The results from this process are presented as the random coefficients model. An example of this model for PM_{10} is as follows:

$$Y_{ij} = [\gamma_{00} + \gamma_{01}(PM_j) + \gamma_{10}(PM_{ij} - PM_j) + \gamma_{11}(PM_j)(PM_{ij} - PM_j)$$

$$+ \beta_2(Age) + \ldots \beta_p(V_k)] + [\mu_{0j} + \mu_{1j}(PM_{ij} - PM_j) + r_{ij}]$$

Where:

Y_{ij} is the cholesterol value of the ith person in the jth county.

PM_j is the mean county level PM_{10} measures, expressed as a deviation from the grand mean PM_{10}.

PM_{ij} is the individual level PM_{10} measure, expressed as a deviation from the county mean PM_{10}.

μ_{0j} is a vector of deviations about the fixed effect for the model intercept γ_{01}.

μ_{1j} is a vector of deviation about the fixed effect for the model slope of γ_{10}.

r_{ij} is a vector of errors associated with the individual-level

To plot the empirical Bayes estimates, we plotted the results from the random coefficients model for each pollutant, with a line corresponding to the fixed effect for the county level slope running through the coordinate corresponding to overall pollution mean and the adjusted mean cholesterol value. We then plotted the mean adjusted cholesterol values of the individual counties (y-coordinate) against the corresponding mean county pollution value (x-axis). The individual county slopes were plotted through these individual county points, with the length corresponding to the inter-quartile range of the within county pollution values (more specific description available in Appendix A).

3. Results

The number of counties and number of individuals for which pollution measurements were available differ by pollutant (table1). Of the 46 counties in the first phase of the NHANES III data set, PM_{10} is available in 31 counties and 4,845 people, while NO_2 is available for only 25 counties and 3,845 people. Of the variation in prior year average air pollution measures, almost all of the variation is accounted for by differences between counties. SO_2 (a regional pollutant) has the least amount of within county variation at only 2.7% of the variation being explained by differences in home location within counties while NO_2 (an indicator of mobile source pollutants) has the most (7.1%).

Given the domination of the variation at the level of the county, correlation between air pollutants in turn is primarily driven by the correlation of the county mean air pollutants (table 2a). County $PM_{10\ and}$ NO_2 are highly correlated with each other (r=0.65), while county $PM_{10\ is}$ slightly negatively correlated with SO_2 (r=-0.27). The correlation between PM_{10} and

NO_2 at the county level suggests that the geographical distribution of $PM_{10\ measured}$ is largely associated with PM from mobile source pollution. County NO_2 itself is not correlated with SO_2. Within counties, correlations between pollutants are in fact highly variable demonstrating that the remaining variation that exists within counties will provide their own unique pollution mixtures, (table 2b). The somewhat negative correlation between PM_{10} and SO_2 at both the county level and within county (on average) suggests that SO_2 is not a surrogate for PM_{10}, though it may correspond to PM at other unmeasured fractionations.

Several counties have no variation in the pollutant measures at all, while the counties with the most variation tend to be at higher mean levels of air pollution (figure 1). NO_2 in particular has three counties with materially greater variation in NO_2 levels than the rest. SO_2 also has little within county compared to between county variation; however, it has a more even distribution of variation among counties. As a consequence, the estimates of the average within county (individual level) effect, in the mixed models, will be driven by those counties with the most variation.

The mean cholesterol parameters and their standard deviations were all very similar across the subpopulations for which pollution measures were available (table 3); however, some small variability in the distribution of variables among pollution subpopulations exists. The proportion of black subjects was lowest in the counties with PM_{10} measurements (27.0%) and highest in the counties with SO_2 measurements (28.7%). The proportion of Hispanics varied from 24.4% in SO_2 counties, to 32% in the PM_{10} counties. However, these differences being subtle, they are not likely to reflect significant differences in the populations under study in the different pollution analyses.

a. Random intercepts model

LDL Cholesterol – In mixed models of LDL, an increase of 10 ppb of the average county NO_2 levels is associated with a 3.61 mg/dL (95%CI 0.98,6.25) increase of LDL and within county it is associated with a remarkably similar increase of 3.36 mg/dL (95%CI: -4.45,11.17) increase of LDL. An increase of 10 μg/dL in the county average year exposure to PM_{10} is associated with an increase 1.64 mg/dL of LDL (95%CI -0.691, 3.971) while an increase of 10 μg/dL over local differences in prior year exposure to PM is associated with a 4.26 mg/dL (95%CI –1.566, 10.059). The county level effect for a 10 ppb increase in SO_2 is virtually null (-0.06 mg/dL; 95%CI: -3.79, 3.67) while individual level SO_2 is substantially more positive (6.06 mg/dL; 95%CI:-2.13, 14.25).

HDL Cholesterol- All county level pollutant effect estimates are consistent with a negative shift in HDL levels, indicating that living in a polluted county is associated with an average decrease of HDL. The within county estimates provide at best little support for a negative (deleterious) effect (NO_2). Within county effects show approximately a 1 mg/dl increase in HDL for an increase of 10 μg/dL (PM_{10}) or 10 ppb (SO_2).

Effect Measure Modification - Evidence exists for Age being an effect measure modifier of the PM_{10} and NO_2 relationship with LDL and the SO_2 relationship with HDL (table 5). Greater adverse relationships existed in relation to the lipid levels with increasing age. Contrary to the hypothesis of susceptibility to air pollution, the joint effect of exposure to NO_2 and diabetes increased HDL levels over their respective independent effects suggesting a protective effect. Similarly, cigarettes appeared to be protective of the effect of NO_2 on LDL. However, number of cigarettes smoked appeared to be associated with higher levels of LDL with increased exposure to SO_2.

b. Random coefficients model

The results of the mixed models that allow the slopes of the within county effects to vary are presented in Table 6, and the resulting empirical Bayes estimates are presented in Figures 6 (LDL) and 7 (HDL). None of the LDL models give evidence that the association of local differences in yearly average pollutant effects differ significantly from the estimated fixed effects. However, the cross level interactions that are apparent for both PM_{10} and SO_2, indicate that the within county differences in local year average pollutant exposures are different across the range of mean county average year exposures. Deleterious increases in LDL are associated with increased local average pollutant levels only at high county average pollutant levels. Only for NO_2 is there evidence that the within county level effect is constant across the range of mean county levels of NO_2. A change in yearly average county levels of NO_2 is associated with an elevation of 3.6 mg/dL of LDL. On average within counties, a change of 10 ppb in average yearly NO_2 pollution is associated with an increase of 7.08 mg/dl of LDL levels.

Of the pollutant models of HDL, only PM_{10} shows evidence of heterogeneity of slopes. However, each pollutant model shows evidence of a difference in the direction of effect across the range of average county level pollutant exposures, suggesting an increase in HDL levels with local increases in pollutant at low county average year pollutant levels, and a decrease in HDL with local increases in pollutant levels at higher county mean pollutant levels.

4. Discussion

To our knowledge, this study is the first to examine the relationship between cholesterol levels and air pollution. We employed hierarchical analyses using individual-level data

nested in counties to generate separate estimates of pollutant effects at the between-county and within-county levels. These data indicate that living in a county with high PM_{10} and NO_2 pollution levels was associated with higher levels of LDL cholesterol. Though comparatively little variation of air pollution exposures exists within counties, the within county effect estimate corroborated the county level estimates of the effects of PM_{10} and NO_2 on LDL. Within county SO_2 is positively related to LDL, though not statistically significant. Furthermore, the within county estimates of the effect of PM_{10} and SO_2 were more positively associated with LDL at higher mean county levels of air pollutants that also correspond with higher within county variation of air pollutant estimates.

Effects of either between-county or within-county pollutants on HDL were not as apparent though this is not surprising since HDL is not as labile as LDL (looking for reference). In the random coefficient models allowing the within county slope to vary randomly and across mean county pollutant levels, it is curious to observe the different directions of the effect at low mean county pollutant levels compared to high county means.

The two most significant limitations of the current study are that the design is cross-sectional and exposure is measured with error. In the absence of personal monitoring, exposures were estimated by interpolation of ambient pollution to the residences of participants. However, people spend only a portion of their lives at home so these estimates may differ from personal exposure. Furthermore, the one-year interval over which the exposure is averaged ignores possibly important dimensions of exposure related to intensity and duration. Finally, PM_{10}, NO_2, and SO_2 are surrogates for different characteristics of pollutant exposures, like $PM_{2.5}$ with which health effects have been associated.

The data used here come from around the country and consequently provide greater exposure contrasts than studies in a single location. However, the vast majority of the variation in air pollutants exists between counties. Lack of variability within county leaves the county level to serve as a proxy for more specific measurements of individuals. The validity of these models is dependent on an adequate specification of the model to 1) account for the variability at the county level which arises from differences that exist in the individual level; 2) account for cluster effects that may be correlated with county mean exposures. If these conditions are met, the county level errors are independent of the individual (within-county) errors and the between-county level estimates are valid for inference on the effect at the individual level. Our results were robust to many different specifications of the individual level model and after controlling for different possible confounders, which suggests that no residual aggregation bias exists. However, inadequate sample size at the county level can result in an under-estimation of the error associated with county level parameters in proportion to how balanced the data are across counties.

To the extent that within-county variation exists, this level provides evidence for the effect of air pollution on cholesterol parameters that is not subject to aggregation bias. The random coefficient models that allow the within county effect to vary randomly and to differ over county means with the cross-level interaction, have added benefits for inference. The evidence that air pollution exposures are related to higher levels of LDL suggests an important effect measure modification that may be dose response. Alternatively, it may suggest a difference related to exposure measurement. It is plausible that low within county exposure variation may reflect common background exposure to pollutants, while in counties with greater internal variation exposures represent pollution from traffic related emissions for

which NO_2 and SO_2 measurements are surrogates. Thus cross-level interaction may reflect differences in exposure measurement error, indicating more deleterious effects (higher LDL and lower HDL) in counties where pollution is from sources that are more likely to correspond to $PM_{2.5}$.

The current study adds to the accumulating evidence of detrimental effects of air pollution. Though not as specific as other acute phase reactants, acute inflammation is known to alter lipoprotein metabolism.[16] The characteristic acute phase changes include increased triglyceride levels secondary to an increase in very low-density lipoprotein (VLDL) and reduced HDL and LDL. However, a more relevant model comes from chronic effects of cigarette smoking, which is associated with increases in plasma triglycerides, LDL, VLDL and decreases in plasma HDL cholesterol concentration.[17]

If these associations are related to inflammation, the potential consequences are under represented by alterations of cholesterol, since oxidative stress in combination with LDL results in oxidized LDL, which is far more likely to stimulate atheroma production.[18]

In conjunction with several epidemiological and laboratory studies that have observed an association between acute exposures to air pollutants and markers of inflammation,[6] and coagulation parameters,[9-12] adverse changes in the distribution of blood lipids provides a link in the list of pathophysiological changes that occur with the progression of atherosclerosis.

Direct evidence for proatherogenic changes as a consequence of PM exposure exist in animal and human studies. In Wataabe hyperlipidemic rabbits, exposure to PM_{10} caused progression of atherosclerotic lesions with greater volume, with greater lipid content and proportionate to the number of alveolar macrophages that phagocytosed PM_{10}.[19] In a study of otherwise healthy subjects with elevated LDL or homocysteine levels with baseline carotid

artery intima-medial thickness (CIMT) measures from two cohort studies,[20] long term mean ambient concentrations of 10 ug/m3 $PM_{2.5}$ were associated with a 4.2% larger CIMT, similar to that associated with exposure to ETS,[21,22] the single largest contributor to indoor air pollution.

Overall, the findings support an association between particulate air pollution and adverse distributions of cholesterol measured in the blood of participants in the NHANES study. Exposure to cigarette smoke, both primary and secondary is associated with similar changes.[17,23] Other physiological effects of air pollution, notably increases in inflammatory markers,[7,10,24] oxidation (in rats)[5] blood coagulation,[25] endothelial dysfunction,[26] and bone marrow stimulation, are shared with exposure to tobacco smoke. These finding provide further support for the hypothesis that exposure to PM air pollution may contribute to the initiation and progression of atherosclerosis representing significantly more morbidity and mortality than that which is implied by studies of acute effects of air pollution.

Table 5. 1. Prior year average pollution exposure in 46 NHANES III phase I counties

Pollutant	unit	Mean	IQR	SD	No. of locations	No. of observations	Proportion of variance explained by county
PM_{10}	ug/m3	37.2	15.8	13	31	4858	95.3%
NO_2	ppb	26.5	10.7	11.4	25	3,845	92.9%
SO_2	ppb	19.7	21	21.0	25	3,874	97.3%

Table 5. 2 Spearman correlation coefficients between county average pollution exposure (prior year)

2A. Correlations between county mean pollution levels

Pollutant	PM_{10}	N counties	NO_2	N counties	SO_2	N counties
PM_{10}	1	31	0.650	25	-0.267	23
NO_2	0.650	25	1	25	-0.095	21
SO_2	-0.267	23	-0.095	21	1	25

2B. Statistics of county-specific within-county correlations between pollutants

	PM_{10}		NO_2		SO_2	
	Min	Max	Min	Max	Min	Max
PM_{10}	-	-	-0.97	0.89	-0.99	0.92
NO_2	-0.97	0.89	-	-	-0.68	0.99
SO_2	-0.99	0.92	-0.68	0.99	-	-

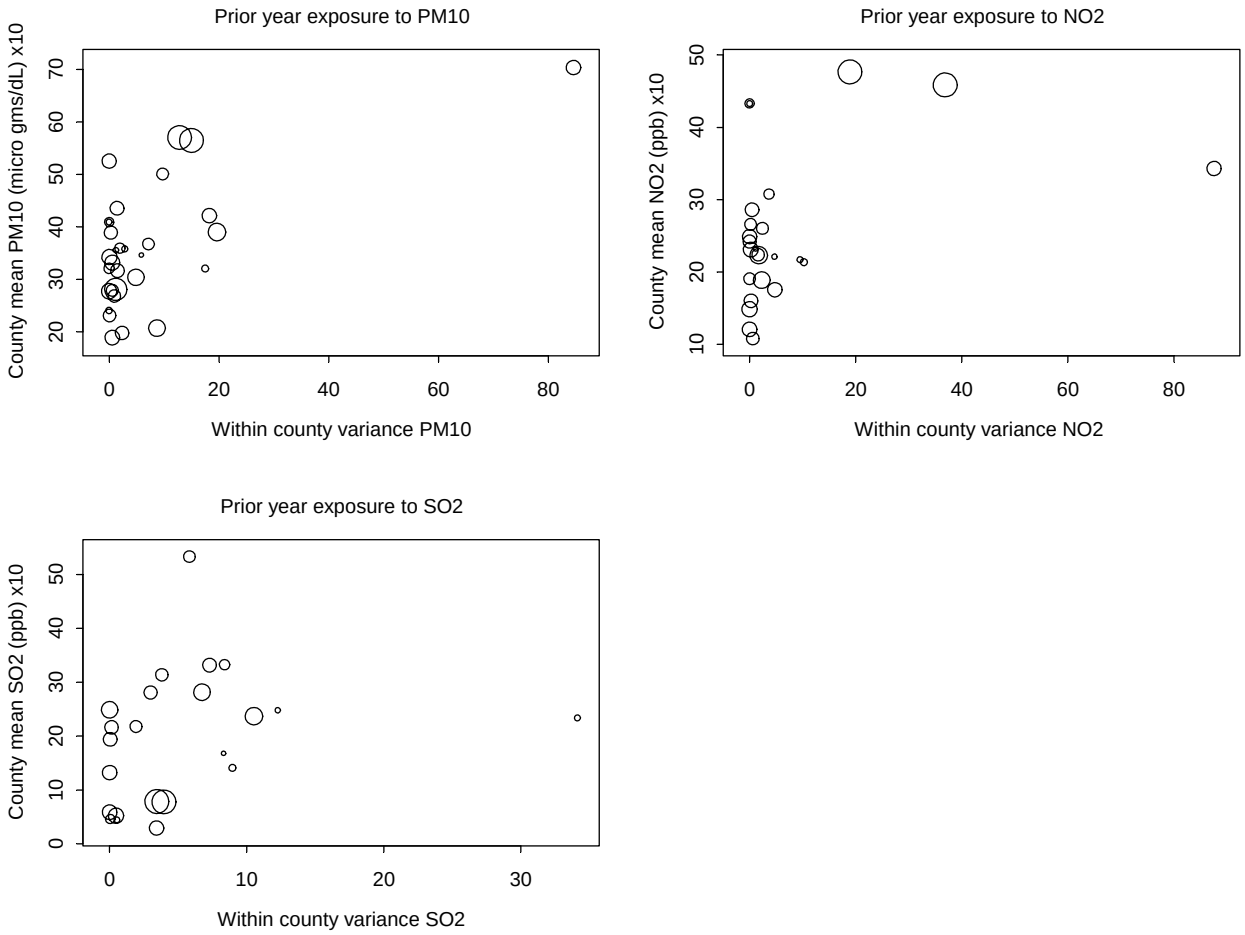

Figure 5. 1. Prior year county pollutant mean by within county variance, bubbles proportional to county sample size

Table 5. 3. Univariate characteristics of lipid parameters and covariates by sub-populations in NHANES III

	Measurements in counties with available ambient pollution measurements								
	PM_{10} (n= 31)			NO_2 (n= 25)			SO_2 (n= 25)		
Outcome	n	mean	std	n	mean	std	n	mean	std
LDL	2059	129.43	39.78	1587	128.78	39.01	1586	130.10	39.19
HDL	4839	51.97	15.34	3741	51.58	14.87	3740	51.85	15.25
Total Chol.	4856	208.05	44.78	3754	206.44	44.37	3753	206.95	44.33
LDL HDL ratio	2059	2.71	1.20	1587	2.71	1.21	1586	2.74	1.23
Covariates									
Age (yrs)	5228	47.82	18.80	4057	46.39	18.55	4042	47.60	18.78
Female	2319	49%		1815	49.0%		1908	49.3 %	
White	1740	37%		1310	35.4 %		1631	42.1 %	
Black	1267	27%		1050	28.4 %		1111	28.7 %	
Mexican American	1512	32%		1183	31.9 %		945	24.4 %	
Other	179	4%		161	4.4 %		187	4.8 %	

	Measurements in counties with available ambient pollution measurements								
	PM_{10} (n= 31)			NO_2 (n= 25)			SO_2 (n= 25)		
Outcome	n	mean	std	n	mean	std	n	mean	std
BMI	5206	26.84	5.53	4042	26.91	5.54	4029	26.70	5.56
BMPWHR	4953	0.92	0.09	3854	0.92	0.08	3830	0.92	0.09
Former smoker	1230	26%		951	25.7 %		1013	26.2 %	
Current smoker	1257	27%		1023	27.6 %		1081	27.9 %	
Cigarettes per day (current smokers)	5228	3.70	8.45	4057	3.75	8.51	4042	4.12	9.11
Potential Confounders									
Social factors									
Poverty-Income ratio	4649	2.46	1.65	3590	2.46	1.63	3592	2.62	1.66
Education (total years)	5186	10.80	4.13	4020	10.80	4.06	4005	11.33	3.83
Household size (persons)	5228	3.57	2.22	4057	3.70	2.27	4042	3.50	2.18

	Measurements in counties with available ambient pollution measurements								
	PM_{10} (n= 31)			NO_2 (n= 25)			SO_2 (n= 25)		
Outcome	n	mean	std	n	mean	std	n	mean	std
Other exposures									
Wood stove use	177	3.8 %		111	3.0 %		142	3.7 %	
Fireplace use	799	17.0%		602	16.3 %		616	15.9 %	
Gas stove use	2837	60.5 %		2355	63.7 %		2351	60.8 %	
Environmental tobacco smoke	1745	37.1 %		1437	38.8 %		1535	39.6 %	
Serum cotinine (ng/ml)	4760	66.26	133.32	3684	66.57	132.41	3680	70.34	136.74
Other factors									
Caffeine (drinks/month)	5216	34.41	51.07	4048	33.10	51.66	4028	35.03	52.81
Alcohol (g/day)	5014	9.77	27.44	3887	9.72	26.86	3866	9.94	26.48
Saturated Fat (g/day)	5032	26.40	17.31	3901	26.74	17.34	3882	26.81	17.67
Serum vitamin C (mg/dL)	4707	0.70	0.44	3644	0.71	0.43	3634	0.71	0.44
Dietary fish and shellfish	5219	6.28	7.91	4051	6.19	8.13	4034	6.53	8.43

	Measurements in counties with available ambient pollution measurements								
	PM_{10} (n= 31)			NO_2 (n= 25)			SO_2 (n= 25)		
Outcome	n	mean	std	n	mean	std	n	mean	std
(servings/week)									
Systolic BP (mmHg)	5213	125.96	19.67	4044	124.84	19.10	4028	124.86	19.16

Table 5. 4 Random Intercepts Model

Effect	Estimate	df	prob t	lower	upper	τ_{00}	SE of τ_{00}
LDL (4a)							
PM_{10} (10 $\mu g/m^3$)						14.963	11.635
County level	1.640	29	0.161	-0.691	3.971		
Within county	4.246	1460	0.152	-1.566	10.059		
NO_2 (10 ppb)						12.873	12.317
County level	3.611	23	0.009	0.977	6.245		
Within county	3.358	1148	0.399	-4.454	11.169		
SO_2 (10 ppb)						24.187	15.602
County level	-0.063	23	0.972	-3.794	3.667		
Within county	6.058	1159	0.147	-2.128	14.245		
HDL (4b)							
PM_{10} (10 $\mu g/m^3$)						2.123	1.060
County level	-0.034	29	0.927	-0.790	0.722		
Within county	0.938	3500	0.274	-0.741	2.618		
NO_2 (10 ppb)						2.364	1.261
County level	-0.169	23	0.584	-0.799	0.460		
Within county	-0.067	2763	0.961	-2.799	2.664		

Effect	Estimate	df	prob t	lower	upper	τ_{00}	SE of τ_{00}
SO_2 (10 ppb)						2.015	1.188
County level	-0.720	23	0.245	-1.966	0.527		
Within county	0.931	2800	0.489	-1.709	3.570		

Table 5. 5 Modification of effect of Cumulative prior year Exposure to Pollutants on Total Cholesterol by Age, Diabetes and Smoking (EM)

a. LDL

Effect Modifier (EM)	Age (years)			Diabetes			Number of cigarettes smoked (current smokers)		
	Estimate	p	95%CI	Estimate	p	95%CI	Estimate	p	95%CI
EM	0.398	0.000	0.256,0.541	-12.397	0.127	-28.329,3.535	-0.052	0.731	-0.348,0.244
County PM_{10} *	1.547	0.102	-0.326,3.419	1.417	0.185	-0.716,3.551	1.404	0.180	-0.688,3.496
Indiv. PM_{10} *	4.504	0.144	-1.543,10.550	4.206	0.176	-1.891,10.304	5.216	0.168	-2.193,12.625
EM*ind PM_{10} *	0.369	0.030	0.035,0.702	-4.690	0.642	-24.455,15.075	-0.353	0.397	-1.169,0.464
EM	0.376	0.000	0.206,0.547	-16.495	0.056	-33.380,0.389	-0.116	0.394	-0.384,0.151
County NO_2 †	3.457	0.004	1.196,5.718	3.443	0.006	1.097,5.789	3.479	0.005	1.142,5.816
Indiv NO_2 †	3.856	0.363	-4.452,12.164	3.854	0.297	-3.398,11.105	5.836	0.245	-4.016,15.688

Effect Modifier (EM)	Age (years)			Diabetes			Number of cigarettes smoked (current smokers)		
	Estimate	p	95%CI	Estimate	p	95%CI	Estimate	p	95%CI
EM *ind NO_2 †	0.332	0.181	-0.154,0.819	-4.146	0.528	-17.015,8.724	-0.908	0.084	-1.938,0.122
EM	0.445	0.000	0.262,0.629	-16.451	0.052	-33.020,0.118	0.038	0.828	-0.302,0.377
County SO_2 †	0.073	0.953	-2.469,2.615	0.087	0.944	-2.452,2.626	0.071	0.954	-2.432,2.574
Indiv. SO_2 †	3.993	0.371	-4.763,12.749	4.566	0.294	-3.962,13.094	0.946	0.841	-8.313,10.204
EM *ind SO_2 †	0.024	0.921	-0.446,0.494	-22.318	0.490	-85.679,41.044	0.731	0.104	-0.150,1.613

* 10 $\mu g/m^3$

† ppb

Table 5.5 b HDL

Effect Modifier (EM)	Age (year)			Diabetes			Number of cigarettes smoked (current smokers)		
EM	0.086	0.000	0.051,0.120	-2.165	0.053	-4.360,0.031	-0.086	0.041	-0.169,-0.003
County PM_{10} *	0.225	0.456	-0.385,0.836	0.228	0.451	-0.383,0.839	0.224	0.456	-0.382,0.830
Indiv. PM_{10} *	0.822	0.308	-0.759,2.402	0.882	0.363	-1.018,2.781	0.709	0.397	-0.932,2.350
EM*ind PM_{10} *	-0.011	0.804	-0.100,0.078	-0.487	0.873	-6.436,5.463	0.045	0.267	-0.034,0.124
EM	0.071	0.000	0.032,0.111	-3.220	0.008	-5.595,-0.846	-0.103	0.027	-0.195,-0.012
County NO_2 †	0.002	0.993	-0.561,0.566	-0.005	0.986	-0.568,0.558	0.003	0.992	-0.551,0.556
Indiv NO_2 †	-0.152	0.909	-2.771,2.467	-0.366	0.804	-3.258,2.526	-0.205	0.858	-2.453,2.043
EM *ind NO_2 †	0.024	0.516	-0.048,0.096	4.578	0.112	-1.072,10.228	0.031	0.813	-0.229,0.292
EM	0.077	0.000	0.037,0.118	-2.169	0.085	-4.640,0.302	-0.097	0.026	-0.182,-0.011
County SO_2 †	-0.975	0.011	-1.700,-0.250	-0.966	0.012	-1.701,-0.231	-0.968	0.011	-1.687,-0.249
Indiv. SO_2 †	0.620	0.612	-1.780,3.021	1.217	0.407	-1.662,4.096	1.556	0.209	-0.869,3.981

Effect Modifier (EM)	Age (year)			Diabetes			Number of cigarettes smoked (current smokers)		
EM *ind SO_2†	-0.106	0.152	-0.251,0.039	-6.081	0.362	-19.167,7.005	-0.115	0.169	-0.279,0.049

* 10 μg/m^3

† ppb

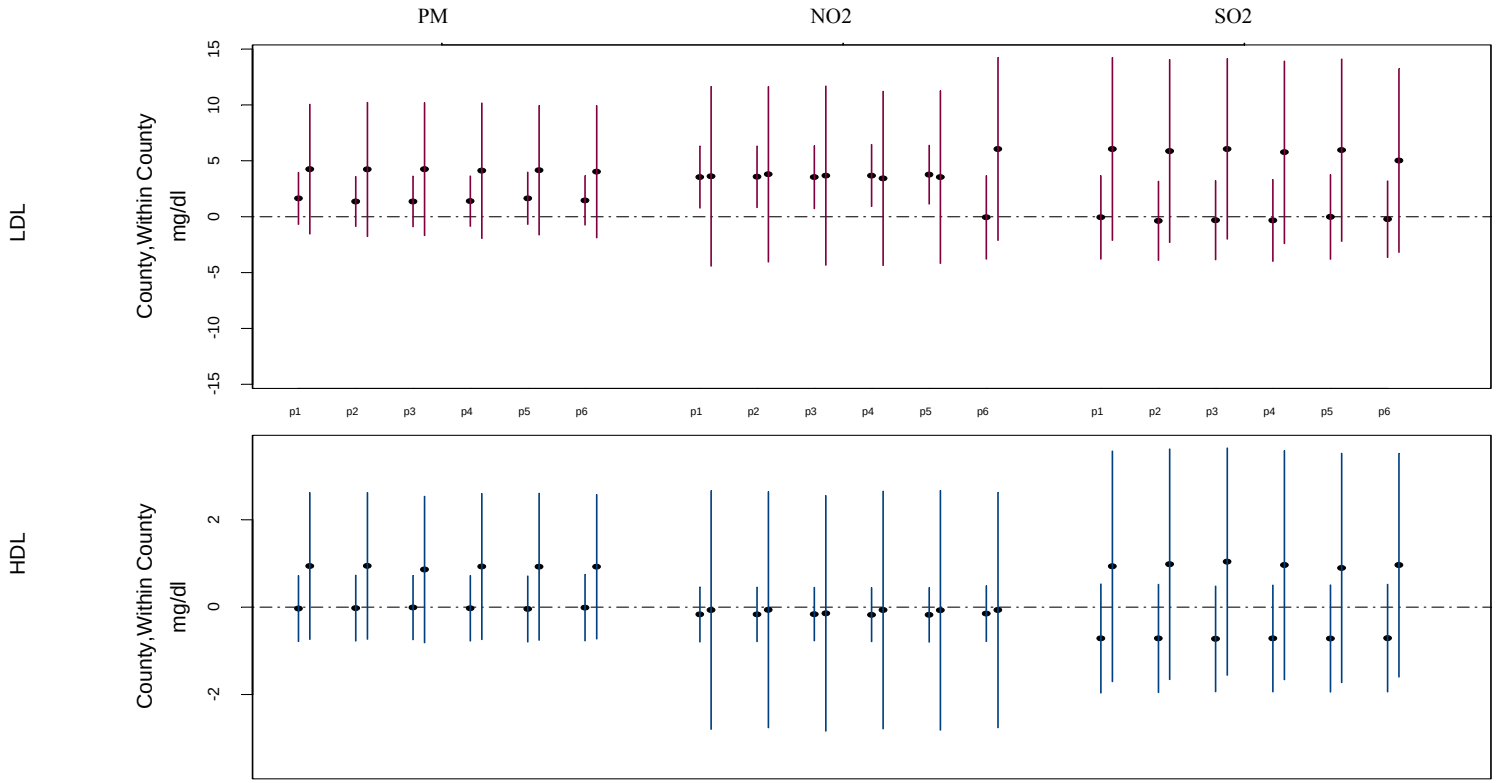

Figure 5. 2. Sensitivity analysis – Alternative parameterizations of deterministic variables

Legend: Paraterization

p1: Age (linear & quadratic), BMI (linear & quadratic), interaction of sex and age

p2: Age (linear & quadratic), BMI (linear & quadratic)

p3: Age (linear & quadratic), BMI (linear & quadratic), interaction of sex and BMI

p4: Age (piecewise linear), BMI (linear & quadratic)

p5: Age (piecewise linear), BMI (linear & quadratic), interaction of sex and piecewise age

p6: Age (linear), BMI (linear)

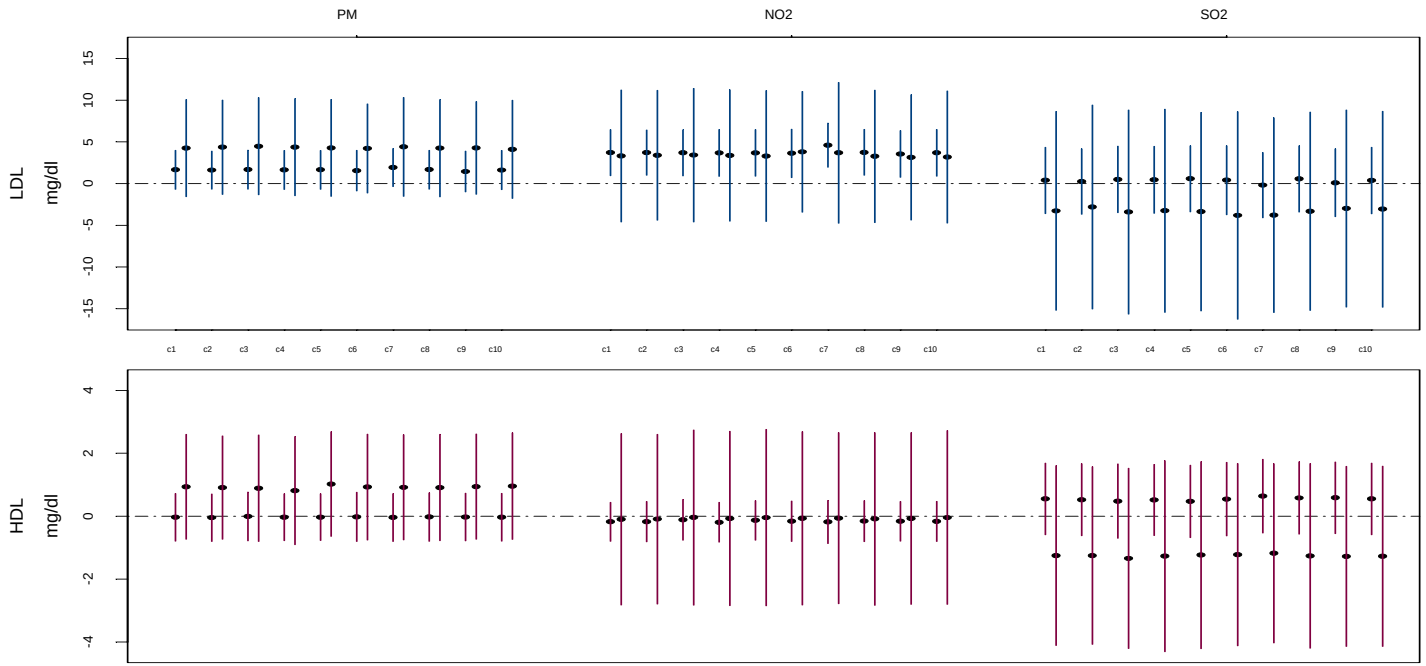

Figure 5.3 Sensitivity analysis – Adjusted for other sources of exposure and Potential confounders

Legend:

C1: At address greater than 1 year

C2: Exercise: Sum total of number of times exercised in last month (running, biking, swimming, aerobics, dancing, calisthenics, gardening, weight lifting

C3: Education – ref= college vs. high school, some high school, less than high school

C4: Household size ref= 5 or more, vs. 4,3,2, or 1.

C5: Wood stove use= Wood stove used in past 12 months.

C6: Fireplace use = Fireplace used in past 12 months.

C7: Gas stove use = Gas stove used in past 12 months.

C8: Fish/Shrimp- No times eat fish, shrimp or clams in last month

C9: Coffee – No times drink regular coffee in last month

C10: Hypertension rx – Taken prescription medicine in last month for ICD9 401.9

Table 5. 6 Random Coefficients Model

Table 5.6a Model of LDL

Effect	Estimate	df	probt	lower	upper	τ_{00}	τ_{01}	τ_{11}	S.E. τ_{00}	S.E. τ_{01}	S.E. τ_{11}
PM_{10}	Likelihood ratio test: 0.9 p=0..64										
County level (10 μg/m^3)	1.717	29	0.140	-0.596	4.030						
Within county (10 μg/m^3)	-0.655	1459	0.863	-8.074	6.764	27.597	52.038	0.000	20.250	74.397	.
Cross level Intxn (10 μg/m^3)	2.076	1459	0.110	-0.471	4.623						
NO_2	Likelihood ratio test:1.3; p=0.52										
County level (10 ppb)	3.645	23	0.010	0.953	6.336						
Within county (10 ppb)	7.083	1147	0.002	2.540	11.625	12.257	-30.38	0.000	12.507	14.537	.
SO_2	Likelihood ratio test:2.4; p=0.30										
County level (10 ppb)	-0.591	23	0.769	-4.709	3.527						
Within county (10 ppb)	-3.698	1158	0.149	-8.720	1.325	33.866	83.612	0.000	23.485	31.567	.
Cross level Intxn (10 ppb)	11.826	1158	0.000	6.036	17.616						

Table 5.6a Model of HDL

Pollutant effect	Estimate mg/dl	df	probt	lower mg/dl	upper mg/dl	τ_{00}	τ_{01}	τ_{11}	S.E. τ_{00}	S.E. τ_{01}	S.E. τ_{11}
PM_{10}	Likelihood ratio test: 5.0 ;p=0.08										
County level (10 $\mu g/m^3$)	-0.037	29	0.920	-0.783	0.709						
Within county (10 $\mu g/m^3$)	1.856	3499	0.089	-0.280	3.993	2.081	2.830	4.693	1.047	2.151	6.165
Cross level Intxn (10 $\mu g/m^3$)	-1.070	3499	0.067	-2.214	0.074						
NO_2	Random slope model did not converge										
County level (10 ppb)	-0.185	23	0.557	-0.825	0.456						
Within county (10 ppb)	2.809	2762	0.042	0.099	5.519	2.345			1.254		
Cross level Intxn (10 ppb)	-2.609	2762	0.002	-4.243	-0.976						
SO_2	Likelihood ratio test 1.5 p=0.47										
County level (10 ppb)	-0.671	23	0.259	-1.870	0.528						
Within county (10 ppb)	1.814	2799	0.200	-0.959	4.588	2.036	1.730	2.547	1.198	2.945	8.508

Pollutant effect	Estimate mg/dl	df	probt	lower mg/dl	upper mg/dl	τ_{00}	τ_{01}	τ_{11}	S.E. τ_{00}	S.E. τ_{01}	S.E. τ_{11}
Cross-level Intxn (10 ppb)	-2.299	2799	0.049	-4.585	-0.014						

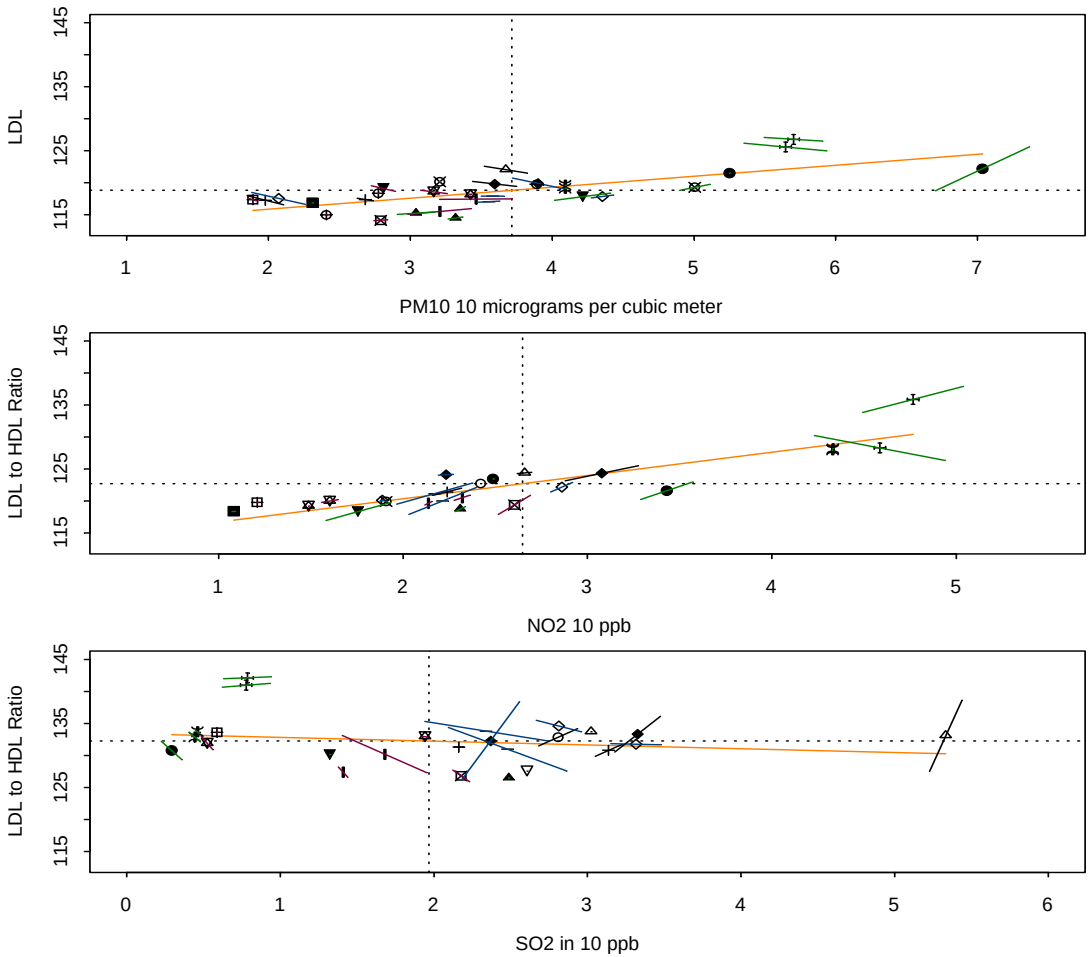

Figure 5. 4 Empirical Bayes estimates for LDL.

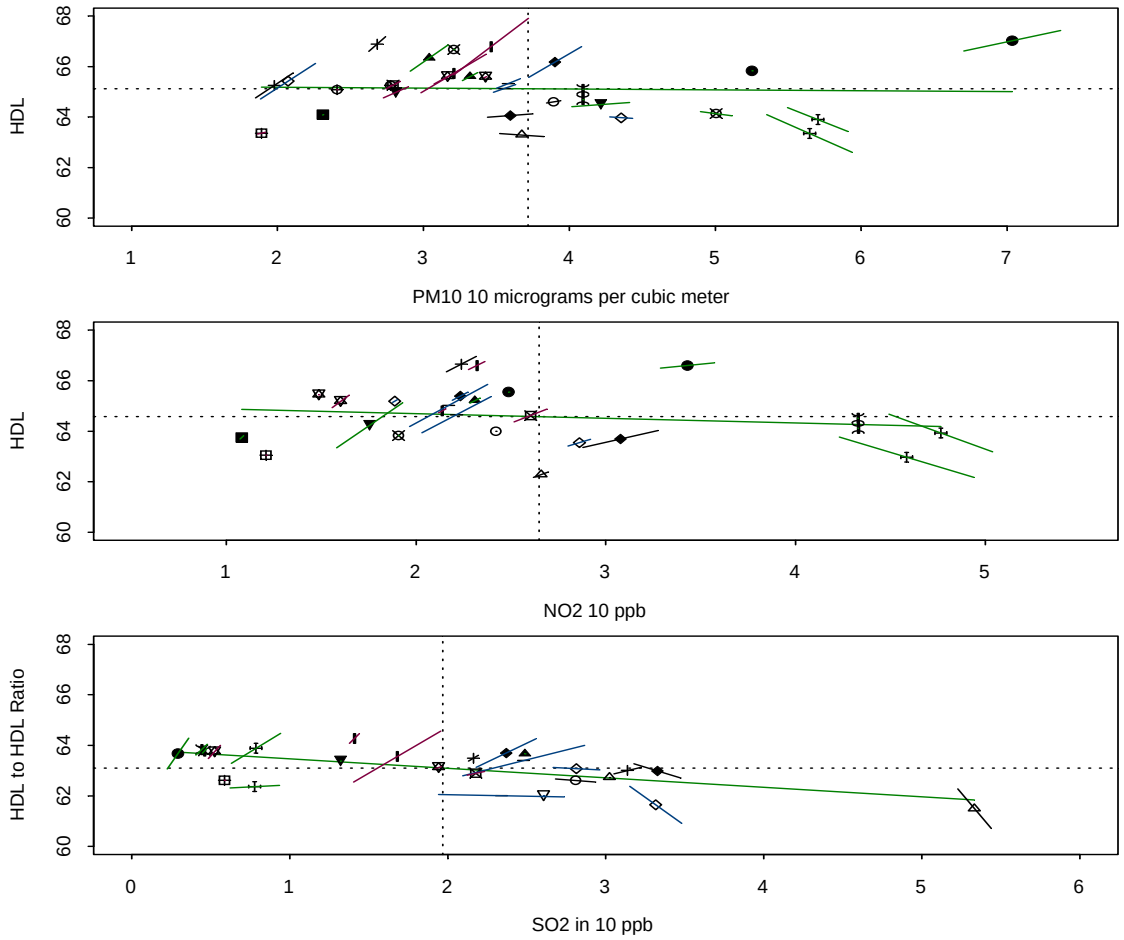

Figure 5. 5 Empirical Bayes estimates for HDL.

Table 5. 7 Two Pollutant mixed model of LDL

Pollutant (ppb)	Estimate	df	probt	lower	upper	τ_{00}	S.E. of τ_{00}
Model of LDL	mg/dl			mg/dl	mg/dl		
Random intercept						9.731	13.542
NO_2 County level	4.42	18	0.014	0.997	7.848	.	.
NO_2 Within county	2.38	939	0.482	-4.266	9.029		
SO_2 County level	-0.476	18	0.779	-3.981	3.029		
SO_2 Within county	3.181	939	0.355	-3.565	9.927		
SO_2 Cross level Intxn	10.087	939	0.001	3.957	16.217		
Model of HDL							
Random intercept	mg/dl			mg/dl	mg/dl	2.167	1.456
NO_2 County level	-0.432	18	0.319	-1.138	0.453	.	.

Pollutant (ppb)	Estimate	df	probt	lower	upper	τ_{00}	S.E. of τ_{00}
NO_2 Within county	2.989	2289	0.071	-0.245	6.223		
NO_2 Cross level Intxn	-2.516	2289	0.005	-4.284	-0.748		
SO_2 County level	-0.0672	18	0.278	-1.934	0.589		
SO_2 Within county	1.793	2289	0.232	-1.146	4.732		
SO_2 Cross level Intxn	-2.552	2289	0.025	-4.730	-0.314		

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B. Manuscript 2: Short-term exposure to air pollution and alterations in alanine aminotransferase as an indicator of involvement of the liver

ABSTRACT

Liver pathology characterized by alterations in liver metabolism and associated with elevations in alanine aminotransferase (ALT) plays a significant role in cardiovascular disease (CVD) according to contemporary research. Given that the liver is the most significant source of acute phase reaction proteins and has a unique role in detoxification, it is plausible that effects of particulate matter (PM) pollution on CVD is mediated by the liver. We applied multilevel models to NHANES III data merged with ambient air pollution monitor data in order to relate between and within county variation in pollution exposures to serum ALT levels in subjects sampled from around the country. Relative to county average exposures, prior week average air pollution exposures were associated with decreased ALT levels. Similar associations with ALT were found in relation to cigarette smoke exposures. Our findings showed that an increase of 10 $\mu g/m^3$ of PM_{10} was associated with a reduction of -0.019 log ALT. At average county pollutant levels, NO_2 and SO_2 were both negatively associated with log ALT levels. The effect of county level exposures was similarly negative for PM_{10} and SO_2, but county NO_2 was associated with increased ALT levels. These negative associations were attenuated at higher mean county levels. These results suggest metabolic alterations from short-term exposures that may be significant to the initiation and/or progression of CVD over time.

1. Introduction

The pathophysiological mechanisms underlying the associations between exposure to particulate matter air pollution (PM) and cardiovascular disease (CVD) morbidity and mortality remain to be elucidated. However, the collective evidence suggests that air pollution can promote the development of atherosclerosis and worsen its sequelae.[1] Important linkages can be found by the study of critical pathway intermediates of CVD in relation to PM exposures.

Studies have shown that the initiation and progression of atherosclerosis involve critical interactions between the endothelium and mediators of inflammation, coagulation factors, lipids and oxidative stress.[2] Furthermore, risk factors for CVD tend to cluster together. BMI, waist circumference, fasting glucose (insulin resistance), type 2 diabetes, hypertension, hypertriglyceridemia, dyslipidemia are risk factors collectively known as metabolic syndrome. Additionally, obesity and metabolic syndrome is associated with chronic inflammation characterized by abnormal cytokine production, increased acute-phase reactants and activation of inflammatory signaling pathways.[3] C-reactive protein (CRP), a marker of systemic inflammation is prospectively associated with incident CVD.[4,5] Exposure to PM has been found to be associated with elevated CRP, Fibrinogen, blood coagulation factors, oxidative stress, endothelial dysfunction, and blood pressure.[6] Few studies have evaluated the effects of PM air pollution on liver metabolism that is intimately connected to risk factors for CVD as well as the principle source of inflammatory and coagulation proteins with which air pollution has been associated.

It is currently believed that PM's effects on CVD events result from pulmonary oxidative stress and inflammation with systemic consequences from the release of pro-inflammatory cytokines, reactive oxygen species (ROS) , activation of hemostatic pathways and impair vascular function. However the evidence remains inconclusive.[1] An alternative hypothesis supports a more direct role by which components of PM, gas condensates, soluble constituents (e.g. transition metals) or nanometer sized particles (PM <= 0.1 μm) may penetrate the epithelium of the lung and pass directly into the blood to be disseminated systematically.[1] Regardless of whether the effects are indirect or direct, the liver remains pivotal for evaluating sub-clinical atherogenic stimuli from air pollution, as the liver is the origin of many inflammatory proteins (such as fibrinogen and CRP) and is the organ responsible for metabolizing foreign substances in the blood. Changes in the metabolizing capacity of the liver may be the first sign that a pathological process is beginning.[7]

Involvement of the liver is further suggested by the recent recognition of its involvement in CVD. Non-alcoholic fatty liver disease is associated with visceral adiposity and metabolic syndrome. Unexplained aminotransferase elevation --an indicator of non-alcoholic fatty liver disease and an indicator of hepatic injury --is strongly associated with male sex, BMI, various measures of adiposity, dyslipidemia, diabetes, high insulin levels, hypertension and cigarette smoking.[8-10]. Fatty liver disease is believed to involve initial steatosis that creates a necessary metabolic alteration upon which additional stimuli causes oxidative stress, ROS formation and abnormal cytokine production.[11] Elevated liver function tests have been found to be associated with serum CRP concentrations suggesting that systemic inflammation associated with CVD is from liver damage from local inflammation (in fatty liver disease) to the liver.[3]

The current study explored how the liver may be involved in mediating health effects of air pollution. We used the NHANES III data merged with Aerometric Information Retrieval System (AIRS) data from the U.S. Environmental Protection Agency (EPA). Use of these data enabled us to measure exposures to pollutants under various geographical contexts in order to evaluate the hypothesis that ambient air pollution may precipitate alterations in the levels of alanine aminotransferase (ALT) in the blood. These data have been used in previous studies to evaluate the effects of air pollution on other hematological parameters.[12] In the current study we evaluate use of ALT as the dependent variable, as it is the most specific aminotransferase associated with liver cytotoxicity. Further, elevated ALT has been found among municipal police in Rome, Italy exposed to urban traffic.[13]

2. Methods

a. Health Data

The Third National Health and Nutrition Examination Survey (NHANES III) was conducted between 1989 and 1994. NHANES III is a stratified probability sample of the U.S. population, with oversampling of the elderly and minority populations. Blacks and Mexican Americans each represent 30% of the NHANES III sample. Persons older than 60 years of age (16% of the U.S. population) account for 20% of the sample population. The NHANES population is equally split by gender. The NHANES III survey was conducted during two phases, each sampling approximately the same number (20,000) subjects in 44 counties (the primary sampling units) and each representative of the general U.S. population when weighted for the oversampling. The current analysis was restricted to 8,039 adults in the first phase.

Subjects were seen in their homes by trained interviewers who collected extensive medical history and demographic data. The subjects then visited mobile medical examination centers, where they were examined. Blood specimens were collected and analyzed. The analytic methods for aminotransferases and serum cotinine used in this analysis are described in Laboratory Procedures Used for NHANES III.[14]. Subjects were categorized into one of four ethnicity groups: 1) non-Hispanic white; 2) non-Hispanic black; 3) Mexican American; and, 4) other. Information on age, education and poverty income ratio were categorized according to the design suggested by the National Center for Health Statistics. Individuals were classified as being in the low or medium income group if the poverty income ratio was less than 1.3 and 3.5 respectively.[14] Medication use was ascertained through a series of questions related to prescription medications taken in the last month. The variable for diabetes was derived from either self-report of doctor having diagnosed diabetes, or taking medication for diabetes. Anthropometric data was acquired using standardized methods during the physical examination, which included weight, standing height and waist circumference. From these data the body mass index (BMI in kg/m^2) and waist to hip ratio were calculated. BMI categories were based on the National Heart, Lung and Blood Institute. Diagnosis of metabolic syndrome was determined if a participant had three or more of the following: 1) systolic blood pressure >= 130 mm Hg, diastolic blood pressure >=85 mm Hg, or on antihypertensive medication; 2) triglyceride >= 1.7 mmol/L 3) low HDL cholesterol <=40 mg/dL for men and <= 50 mg/dL for women 4) fasting glucose of greater than 6.1 mmol//L 5) waist circumference >102 cm for men and >88 cm for women.[15] Due to underreporting of smoking and the desire to measure the dose response of persons exposed to environmental tobacco smoke, we used serum cotinine measurements to reflect active and

passive smoking based on a validation study of reported smoke exposure in NHANES III.[16] Participants with cotinine levels >15 ng/ml were designated as active smokers. ALT was measured from serum with the Hitachi 737 Analyzer (Boehringer-Mannheim Diagnostics).

b. Air Pollution Data

Air pollution data was obtained from the Aerometric Information Retrieval System (AIRS) of the U.S. Environmental Protection Agency (EPA). AIRS contains information on all of the criteria pollutant monitoring in the United States. Pollution exposure was assigned by means of geocoding, which describes the longitude and latitude of the centroid of the census block group in which the participants lived. Block groups are collections of adjoining blocks with populations of 500 to 1,000 persons. The longitude and latitude of each monitor in the United States was obtained by AIRS. Persons were assigned exposure values equal to the weighted average of all monitors in their county of residence and adjoining counties, with weights proportionate to the inverse of the square of the distance between their residence and the monitor. Air pollutants available for this study included PM_{10} , NO_2 and SO_2.

Pollution monitor data are missing for some participants in counties where there were no monitors and some counties had pollution data for some pollutants but not others. Therefore, analyses for individual pollutants do not include all of the same subjects. Variability of pollution exposure in these data reflects temporal and spatial variability. Temporal variability is derived from the subjects being examined over the course of six weeks in each county. Geographic variability of exposure within county is driven by the spatial distribution of sources estimated by the smoothing method described above. Geographical variability in air pollution that exists between counties reflect variation of the air pollutants between counties that contribute to average health related outcomes from exposure to air pollution to residents

in the county. Some portion of exposure variation however, can arise from differences in the number of monitors in relation to the physical distance over which the participants reside, which can influence measurement error.

We parameterized the pollutant exposures to reflect the between county (ecological level) and within county (individual level) measures. To do this we created a variable for each pollutant that is the county mean of the prior week exposure to the air pollutant and subtracted the grand mean of the pollutant over all counties. The result is a county level average air pollution exposure measure expressed as a deviation from the grand mean. To the extent that true variation in air pollution exposure is derived from between county variation, inference is limited to the population (ecologic or county) level, such that living in a polluted area is associated with increase/decreased average ALT levels. For each individual, we also created a variable that is the individual's prior week exposure minus the county average; this provides a measure of an individuals air pollution exposure expressed as a deviation from the county mean. Inference on this parameter has a specific individual level interpretation, allowing for measurement error, independent of its ecological analogue. It also tends to capture more local, rather than regional sources of pollution.

$PM_j = j^{th}$ county mean PM- grand mean PM

$PM_{ij} = i^{th}$ individual in county j PM – j^{th} county mean PM

c. Statistical Analysis

The NHANES III data sampled populations within counties selected at random within strata of country region. Blacks, Hispanics and the elderly were oversampled to insure adequate numbers for analyses within these groups. NHANES III data have weights based on the probability of sampling. When used with software applications (e.g. PROC

SURVEYREG) that use between PSU within strata variation (still working this out) to account for the particular sampling design, the weights generate estimates that are representative of the U.S. non-institutionalized population in a single level model. In this way, the variance structure from the sampling design is adjusted to derive estimates for the individual level analysis. In the current analysis however, we used an alternative analytic technique. In order to evaluate the relationship between ALT and air pollution, which has both large and small-area property characteristics, we treated these data as a collection of populations with relevant variation at both the county and individual levels. We therefore performed multilevel analysis with the partitioned air pollution exposure parameters to separate the effect of the county and individual exposures to air pollution in relation to ALT. The multilevel model accounts for correlated errors from the sampling of individuals within PSUs, which can arise from the tendency of health outcomes to cluster within geographic regions due to commonalities of environmental factors, ethnicity, diet and other. For the sake of comparison we first ran models using both survey analytic software and mixed models using the single level (unpartitioned) air pollution parameter.

Because aminotransferase elevations are strongly associated with excessive alcohol use, hepatitis and iron overload, simple adjustment may be insufficient to fully control confounding. We therefore restricted the analysis to patients with normal levels or "unexplained" elevations in aminotransferases as defined in a previous study using the NHANES III dataset.[10] Subjects with elevations of aminotransferases – men with aspartate aminotransferase (AST) > 37 SI U/L or ALT > 40 ; women with AST > 31 or ALT > 31 - who had hepatitis B surface antigen, Hepatitis B antibodies, transferrin saturation >50%, or daily alcohol consumption of more than one drink (10 gms of alcohol) a day for women, or

two drinks a day for men, were considered to have explained elevations of aminotranserases in accordance with Clarke et al. These subjects (n=189) were excluded from the analysis.

Because residuals from models using ALT were highly skewed, the log of ALT was used as the dependent variable. Initial bivariate relationships between individual risk factors and ALT were evaluated using procedures that account for the study design (PROC SURVEYMEANS in SAS). In order to provide estimates of the individual pollutant effects on log ALT under the usual prescriptions for analysis using the NHANES data, we used the same covariates described for the mixed models described below in a survey model (PROC SURVEY REG in SAS) with weights that sum to the non-institutionalized population of the United States. As a way to compare the two analytic techniques, we ran both styles using the single level (unpartitioned) pollution parameter.

Using the mixed models (Proc Mixed, SAS, Cary, NC), we used a random intercept model as the base model of ALT with covariates and the two-level partitioned pollutant parameters with the random intercept corresponding to each county. Later we extended the model to create random coefficient models that allowed the slope of the individual level pollution parameter to vary across counties. These models were fit using restricted maximum likelihood (REML) and between-within degrees of freedom.

The regression models included variables involved in the sampling design: age, ethnicity (non-Hispanic Whites, non-Hispanic Blacks, Hispanics, and others). Smoking status (indicator variable for former smoker) was used to account for the effect of prior smoking history. Instead of using the self-reported smoking status and exposure to ETS, we used measured levels of serum cotinine– a metabolite of nicotine- as measures of cigarette smoking (as a continuous variable among those with cotinine levels > 15 ng/ml); and passive

smoking (as a continuous variable among those with cotinine levels less than 15 ng/ml). Each individual has a 0 referent for one or the other tobacco exposure variable to account for the competing exposures of active smoking or exposure to environmental tobacco smoke (ETS). We then included covariates based on subject matter knowledge of biology and risk factors in order to account for as much of the individual level variation from the deterministic components known to regulate ALT. These included sex, BMI, waist to hip ratio (WHR), and alcohol consumption. We also included a quadratic term for alcohol consumption and WHR to allow for potential non-linear associations with ALT. Indicator variables for low and middle income (high income being the referent category) were included to adjust for socio-economic. Additionally, to account for seasonal variation in ALT levels, an indicator variable was included for winter months if the subject was surveyed between the months of November to April.

In order to evaluate the consistency of the pollutant effects within and between pollutant models, we generated empirical Bayes estimates from a random coefficient model for each pollutant model. This model included the partitioned pollutant variable with an interaction between county and individual level pollution parameters, and a random slope for individual pollution. If the interaction was not significant ($p>0.20$) it was not included in the model. To facilitate comparisons of the county level and individual level in relation to each other and across pollutants, we plotted the resulting models, with a line corresponding to the county level slope. We plotted the mean adjusted log ALT values (posterior county means, y-coordinate) against the corresponding mean county pollution value (x-axis). The individual county slopes (posterior county slopes) were plotted through these individual county points,

with the length along the x-axis corresponding to the inter-quartile range of the within county pollution values (details in appendix).

3. Results

Air pollutants were monitored in urban areas only; furthermore, not all air pollutants were monitored in each area of the United States during the study period. Thus, the analytic data is a subset of counties and individuals within counties. The mean, interquartile range (IQR), standard deviation, number of counties, and number of persons for whom the measure is available for each pollutant is presented in Table 1. For each pollutant, variation that occurs within a county will be variation around a particular latent quality of pollution as it relates to the particular county characteristics of the pollutant. Variability in air pollution exposure that exists at the aggregate level and that motivates this multi-level model is demonstrated to different degrees for each pollutant (last column of Table 1). The amount of exposure variation that is at the aggregate (county) level is highest for NO_2 at almost 90%. Less clustering of exposure exists for SO_2 (70%), ozone (67%) and PM_{10} (46%). The individual level variation (within-county) therefore is smallest for NO_2 (10%) and greatest for PM_{10} (54%).

The variation of exposure to pollutants is different across counties (Figure 1). For the pollutants PM_{10} and SO_2, the within-county variation tends to increase at higher county pollutant averages. For NO_2, four counties have distinctly higher variation than the remaining counties.

County level PM_{10} is highly correlated with NO_2 (top table 2), and its correlation with SO_2 is slightly negative. County level NO_2 is negatively correlated with SO_2. The average county correlation between PM_{10} and NO_2 was 0.28 and ranged from -0.49 to 0.74 (bottom

table 2). The average county correlation between PM and SO_2 was 0.27 and ranged from -0.42 to 0.82.

The sub-populations of the NHANES III data for which each pollutant measure is available do not differ greatly from each other (Table 3). However, the mean age and the proportion of whites are lower in each of the pollution sub-samples than in the entire NHANES III study population (data not shown). This reflects differences that correspond to largely urban rather than populations. Generalizability of the relationships from analysis of these data to the U.S. population would be affected to the extent by which variables involved in the sampling design of NHANES are effect modifiers of the pollutant ALT relationship.

After adjusting for variables included in the base model without air pollutants, the amount of variation in the outcome that is accounted for by the clustering of ALT levels within counties (the intraclass correlation) was 4.65%. Limited to the subpopulations with available pollution measures for PM_{10}, NO_2, and SO_2, the intraclass correlation coefficient was 4.49%, 4.67% and 4.43%, respectively (data not shown). In spite of this relatively small amount of clustering within county, all statistics for variation in the adjusted mean county ALT levels (random intercepts) were significant (based on p for the variance of the random intercepts- the τ_{00} statistic- from the mixed model being less than 0.05), reflecting statistically meaningful residual geographic clustering of log ALT after adjusting for individual level predictors of ALT.

Males have higher ALT levels than women (Table 3). ALT levels are highest among those in their 30s and are decrease over each successive age group. ALT levels are highest among Mexican Americans and those classified as “other” while the levels are lowest among the non-Hispanic Blacks. Consistent with the literature, ALT levels are highest among the

most obese either defined by BMI or WHR. Notably, levels of ALT in the underweight category were higher than those in the normal BMI category. Former smokers had higher ALT levels compared to current and never smokers. Consistent with the clustering of conditions associated with CVD in the literature, ALT levels were higher among those categorized as having metabolic syndrome and diabetes.

In the survey style regression analysis, both PM_{10} and SO_2 pollution exposures are associated with lower levels of ALT, while NO_2 was associated with higher levels of ALT (top of Table 4). However, in the mixed models analysis with the same unpartitioned parameterization of pollutant exposures, NO_2 was unrelated to ALT levels, indicating that the association is lost in allowing the county level intercepts (representing the adjusted mean ALT levels) to vary randomly. However, these estimated effects from these two types of analysis do not take into account the level from which the variation of air pollution is derived. Of the pollutants in the mixed model analysis, SO_2 has the strongest effect showing an increase of 10 ppb exposure to be associated with –0.021 (95% CI: –0.57, 0.15) change in log ALT. An increase of 10 μg of PM_{10} is associated with a –0.017 (95% CI: -.029,-0.005) change in log ALT.

The partitioned pollution effects from the simple random intercepts model on log ALT are shown at the bottom of Table 4. None of the county level pollutant estimates show statistically meaningful associations with log ALT, with only the NO_2 coefficient as positive. Each individual level effect for the pollutants associated with particles - PM_{10}, NO_2 and SO_2 - is negative; statistically the negative ALT effect is most strongly associated with PM_{10}. NO_2 has a negative association with log ALT but is not as significant owing to a smaller N and

less within county variability noted above. The effects for PM_{10} and SO_2 are consistent (the same negative association) between the county and individual level effects.

The partitioned pollutant effects from the random intercepts model are consistent when the models are adjusted for other possible confounders that include competing exposures (wood stove, fireplace and gas stove use), exercise over the last month, other socioeconomic status (SES) related variables (education and household size), and other dietary variables, such as -coffee and seafood intake that indicated omega-3 fatty acid intake (data not shown). Controlling for hypertension or diabetes medications did not change the effects. Also, the inclusion of whether or not the patient had lived in the household for at least a year or if the ALT specimen was collected under fasting conditions did not change the effect estimate of either between or within county air pollution.

To evaluate the effects across the range of exposures that exist among the counties sampled in NHANES III the final models resulting from the backward elimination from the random coefficient models with cross-level interactions described above are shown in Table 5. Evidence that the individual level effects of PM_{10} and SO_2 differed across counties exists based on the criteria of a p-value of 0.20 or less. The likelihood ratio test for the inclusion of the random slope of PM_{10} and SO_2 had a p-value of 0.004 and 0.045 respectively. The random coefficient model for NO_2 did not converge due to the lack of within county variability so only the fixed effects from the random intercepts model could be presented. However, interactions for NO2 as well as SO_2 were included in the model as evidence exists that the individual level slopes differed across the range of county level exposures (based on the criterion of 0.20).

The fixed county level effect and the empirical Bayes estimates of the county specific individual level results from these models were plotted in Figure 2. Though there is some variability, the individual level slopes for PM_{10} are mostly negative, the average slope being -0.013 log-ALT/per 10 μg per micrometer (95%CI -0.032,0.013;). The effect of NO_2 on log ALT is different across mean county levels of NO_2 (cross-level interaction), being negative at low county means and approximately null at higher county means. Similarly, the effect of SO_2 on log ALT is negative at low county means and on average approximately null at high county means. Of the pollutant models, due to the absence of a cross-level interaction and the consistency of the slopes, it is justifiable to model the use the simple random intercepts model in interpreting the effects of PM_{10} - where the exposure is simply an individual effect independent of the county level.

Finally, we performed the analysis on the data that included those with "explained" elevations in ALT and the results were not materially different (data not shown).

4. Discussion

We found consistent negative relationships among the county specific estimates of the relationship between exposure to PM_{10} and ALT levels. Similarly, at average county mean pollution levels for NO_2 and SO_2, these pollutants were also negatively associated with ALT. The evidence suggests that the associations became less negative in counties with higher mean levels of air pollution. The implications of the county level effects are discussed below in relation to the interpretation of partitioned effects from mixed models. To our knowledge, this is the first population based (nonoccupational) study of ALT levels in relation to air pollution.

Consistent with the literature, metabolic syndrome, diabetes, BMI, and in particular, visceral fat, are all strongly related to elevations in ALT levels. The relationship between air pollution measures and ALT, however, are not consistent with the Rome Constables Study , or with what would be expected if air pollution is toxic to the liver. Nonetheless, it is significant that the association with cigarette smoking among current smokers - known to stimulate oxidative stress and is associated with elevations in CRP -is similarly associated with a decrease in log ALT levels in these data, ranging from a decrease of 0.0033 to 0.0047 log ALT per cigarette smoked ($p=0.002$) and NO_2 ($p=0.0001$) respectively. Therefore, while not showing evidence of liver damage, exposure to air pollution is associated with a particular metabolism shared with cigarette smoking.

A few studies provide support for the observation of decreases in ALT in relation to environmental pollutant exposure. A previous study of Japanese workers has shown a negative association between ALT and number of cigarettes smoked per day.[17] In an animal study, exposure to combustion exhaust gases containing a high percentage of SO_2 caused decreases in ALT activity in guinea pig livers[18] and exposure to wood smoke was noted to cause a decrease in ALT activity in rodents.[19]

The principal limitation of this study is that these are cross-sectional data and therefore are limited in establishing causal relationships. Furthermore, serum aminotransferase levels can fluctuate in individuals. Another limitation is that exposure measurement relied upon interpolation from ambient monitors to residence. This is likely to result in misclassification of personal exposure to the measured pollutants. $PM_{2.5}$, which is a more relevant measure of exposures that have been associated with health effects, was not available in these data that were collected before ambient monitoring of $PM_{2.5}$ began. However, in a study of the

surrogacy of ambient air pollution levels for personal exposure, ambient levels of NO_2, SO_2 and ozone were poor indicators of personal exposures to the gases themselves; however, they were good surrogates for exposure to $PM_{2.5}$.[20] This study suggests that ambient NO_2 and SO_2 may stand for personal exposure to their associated fine particles from urban traffic and sulfites respectively.

Background concentrations in parts of the country are higher for NO_2 (in the south) and SO_2 (in the north). In the parameterization of pollutants utilized in this analysis, background concentrations would be reflected by low variability within county and small values for the individual level pollution measure, with the magnitude of the exposure represented by the mean county level. Local sources of pollutants such as that from urban traffic will be reflected by substantial variation in these pollutants within county and the magnitude of the differences relative to the county mean are reflected in the individual level parameter. In these data, rather than reflecting exposure to SO_2 specifically, we interpret individual level exposures as a surrogate measure for sulphite particles from either point source (power plants) or combustion products from mobile diesel sources. Similarly individual level NO_2 is a marker for traffic related particulate ($PM_{2.5}$) air-pollution. Individual level PM_{10} is likely to encompass particle related air pollution from both though still encompassing the less coarse fraction of PM that is less relevant as an exposure.

Individual level relationships are more reliable for causal inference than ecological associations. In these data however, the association at the county level contributes significantly to the interpretation from the mixed model. In counties with little variation in exposure to an air pollutant, the county level measurement reflects relevant exposure to levels that are shared across the county. Furthermore, county average exposure over the prior

week is correlated with the same over longer periods of time and therefore, the county level effect has causal value as a reflection of consequences of living in a polluted county.

As a consequence of the ambiguity in the inference derived from the county level pollution parameter, the cross-level interactions may reflect two dynamics: 1) The less negative individual level slopes at higher mean county levels may reflect either dose-response of the health effects; or, 2) the cross-level interaction may relate to qualitatively different exposures with respect to ALT of either NO_2 or SO_2 corresponding to different county mean levels of the pollutant. A third possibility is that the exposure measurement error is different across mean county pollution levels.

The observation of decreased levels of ALT in relation to exposure to air pollution suggests alterations in metabolism in response to exposure to particulate air pollution and that is shared with cigarette smoke exposure. These results are not consistent with hepatocyte cytotoxicity from direct exposure to components of ultrafine particulate matter in the circulation via exposures to the lung. While contrary to evidence of hepatotoxicity, these results may reflect a paradoxical toxicity arising from oxidative or inflammatory stimuli originating in the lung. Under the two hit hypothesis suggested in the literature, hepatocyte injury that results in necrosis or apoptosis and higher levels of ALT, would be apparent at high exposure levels and/or in livers that are susceptible livers. However, at low levels and/or in healthy livers, such exposures may result in a proliferation of hepatocytes instead of cell death.[11] We hypothesize that at lower exposures among people with healthy livers, exposure to air pollution may reflect a proliferation rather than destruction of liver cells (reflected by reduced levels of ALT), ultimately making livers more vulnerable to future environmental exposures.

Table 6. 1 Prior week average pollution exposure in 46 NHANES III phase I counties

Pollutant	unit	Mean	Interquartile range	std dev	No.of locations	No. of observations	Proportion of variance explained by county
PM_{10}	ug/m3	35.8	21.4-45.7	19.1	30	4698	46.00%
NO_2	ppb	27.6	14.3-32.5	14.3	24	3704	89.00%
SO_2	ppb	17.5	9.4-25.0	10.7	25	3874	69.50%

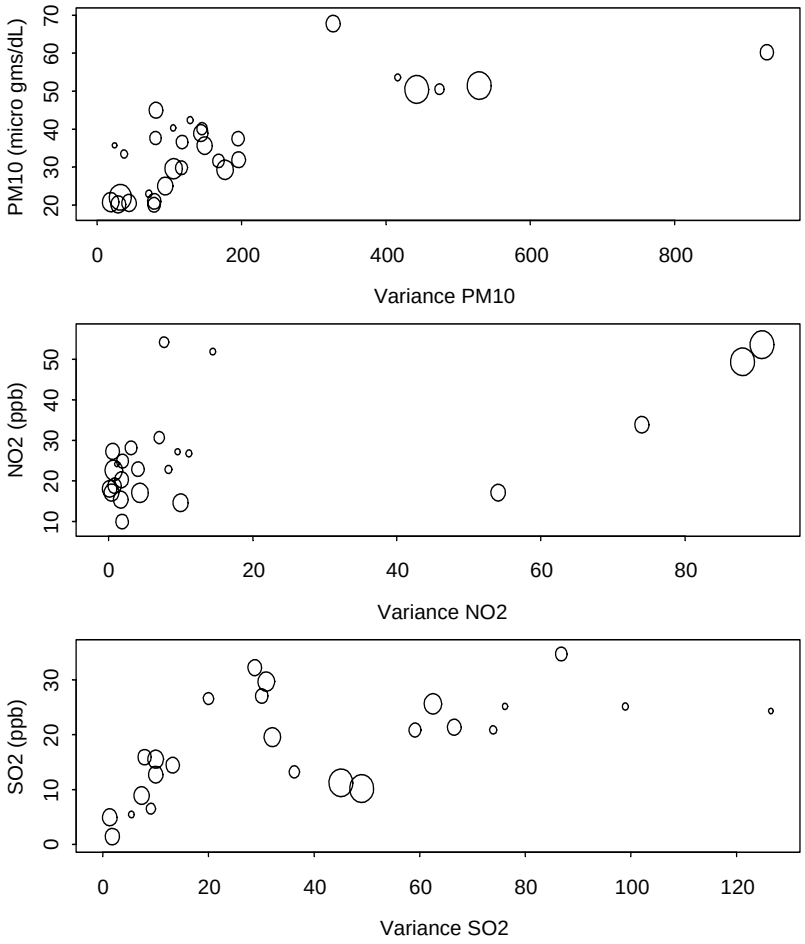

Figure 6. 1 County pollutant mean by variance, bubbles proportional to county sample size.

Table 6. 2 Spearman Correlation coefficients between county average Pollution exposure (prior week)

	Correlations between pollutants								
Variable	PM_{10}	p	N counties	NO_2	p	N counties	SO_2	p	N counties
PM_{10}	1		30	0.48	0	24	-0.07	0	23
NO_2	0.48	0	24	1		24	-0.15	0	21
SO_2	-0.07	0	23	-0.15	0	21	1		25

	Average within county correlations between pollutants								
	PM_{10}			NO_2			SO_2		
	Mean Corr	Min	Max	Mean Corr	Min	Max	Mean Corr	Min	Max
PM_{10}	1			0.28	-0.5	0.74	0.27	-0.4	0.82
NO_2	0.28	-0.5	0.74	1			0.31	-0.1	0.71
SO_2	0.27	-0.4	0.82	0.31	-0.1	0.71	1		

Table 6. 3 Univariate Characteristics of Lipid Parameters and covariates by sub-populations

	Measurements in counties with available ambient pollution measurements								
	PM_{10} (n= 30)			NO_2 (n= 24)			SO_2 (n= 25)		
	n	mean	std	n	mean	std	n	mean	std
Outcome									
ALT (SI u/l)	3964	14.54	9.71	3090	14.57	9.48	3275	14.46	9.43
Elevated ALT	186	4.70%		146	4.70%		153	4.70%	
Covariates									
Age (years)	4368	48.05	18.87	3444	47.16	18.76	3628	48.11	18.89
Female	2184	50%		1711	49.70%		1814	50.00%	
White	1665	38.10%		1252	36.40%		1562	43.10%	
Black	1186	27.20%		986	28.60%		1044	28.80%	
Mexican Amer.	1348	30.90%		1054	30.60%		849	23.40%	
Other	169	3.90%		152	4.40%		173	4.80%	
Body Mass Index (kg/m^3)	4349	26.74	5.51	3430	26.74	5.52	3615	26.6	5.51

	Measurements in counties with available ambient pollution measurements								
	PM_{10} (n= 30)			**NO_2 (n= 24)**			**SO_2 (n= 25)**		
	n	mean	std	n	mean	std	n	mean	std
Outcome									
Waist/Hip ratio	4138	0.92	0.09	3260	0.92	0.08	3431	0.92	0.09
Former smoker	1159	26.50%		899	26.10%		964	26.60%	
Current smoker	1160	26.60%		941	27.30%		997	27.50%	
Cigarettes/day (Current smoker)	4368	3.78	8.66	3444	3.86	8.78	3628	4.08	9.14
Potential Confounders									
Social factors									
Poverty/Income ratio	3885	2.53	1.66	3069	2.52	1.63	3233	2.64	1.66
Education (years)	4333	10.96	4.08	3412	10.95	3.99	3596	11.38	3.82
Household size (persons)	4368	3.52	2.16	3444	3.59	2.17	3628	3.46	2.11

	Measurements in counties with available ambient pollution measurements								
	PM_{10} (n= 30)			**NO_2 (n= 24)**			**SO_2 (n= 25)**		
	n	mean	std	n	mean	std	n	mean	std
Outcome									
Other exposures									
Wood stove use	165	3.80%		102	3.00%		134	3.70%	
Fireplace use	738	16.90%		550	16.00%		568	15.70%	
Gas stove use	2615	59.90%		2177	63.30%		2192	60.50%	
Environmental Tobacco Smoke	1595	36.50%		1312	38.10%		1409	38.80%	
Serum cotinine (ng/mL)	4068	0.9	5.67	3191	0.84	5.06	3374	0.86	5.45
Dietary factors									
Caffeine (drinks/month)	4356	35.28	53.44	3435	34.02	53.35	3615	35.86	54.36
alcohol (g/day)	4176	10.03	27.16	3291	9.86	26.19	3463	9.95	26.02
Saturated fat (g/day)	4191	26.4	17.03	3303	26.84	17.29	3478	26.67	17.36
Serum Vitamin C (mg/dL)	3918	0.71	0.44	3059	0.71	0.43	3240	0.72	0.43

	Measurements in counties with available ambient pollution measurements								
	PM_{10} (n= 30)			**NO_2 (n= 24)**			**SO_2 (n= 25)**		
	n	mean	std	n	mean	std	n	mean	std
Outcome									
Fish and shellfish (portions/week)	4360	6.27	7	3439	6.23	7.13	3622	6.46	7.34

Table 6. 4 Bivariate relationships between variables and ALT levels in all Phase I NHANES data

Variable	N	Mean (SI U/L)	Std Error	95% Confidence Interval
Sex				
Total	7137	15.2651	0.3042	14.6358, 15.8944
Males	3562	17.9038	0.3033	17.2764,18.5313
Females	3575	12.8888	0.3523	12.1601,13.6174
Age				
20 to 29	1365	15.0941	0.5342	13.9889,16.1992
30 to 39	1355	16.6242	0.496	15.5981,17.6502
40 to 49	1136	16.1089	0.4543	15.1690,17.0487
50 to 59	863	15.601	0.4923	14.5826,16.6195
60 to 79	1056	14.2813	0.4494	13.3516,15.2110
70 to 79	793	12.5803	0.2369	12.0902,13.0704
80 & above	569	10.3952	0.477	9.4084,11.3819
Race/Ethnicity				
White non-H	3240	14.8007	0.3652	14.0451,15.5562
Black non-H	1727	13.4735	0.3065	12.8394, 14.1076
Mexican Amer.	1945	20.0975	0.653	18.7468, 21.4483
Other	225	20.1349	1.209	17.6340, 22.6359

Variable	N	Mean (SI U/L)	Std Error	95% Confidence Interval
BMI Category				
Underweight	133	15.138	3.2427	8.4299,21.8462
Normal	2786	13.1629	0.2802	12.5832,13.7426
Overweight	2536	16.1781	0.3443	15.4658,16.8904
Class I obese	1113	17.3087	0.4146	16.4511,18.1663
Class II and III obese	541	20.4766	1.2231	17.9464,23.0068
WHR quartiles				
4^{th}- smallest	1750	12.5208	0.4322	11.6266, 13.4150
3rd	1849	15.0855	0.3637	14.3332,15.8377
2nd	1673	17.2731	0.4612	16.3190,18.2272
1^{st} largest	1505	18.2344	0.4149	17.3762,19.0926
Smoking Status				
Never	3306	15.0516	0.3268	14.3755,15.7276
Former	1909	15.7707	0.5142	14.7069,16.8345
Current	1922	15.1296	0.4204	14.2598,15.9993
Metabolic Syndrome				
Yes	1544	17.9998	0.6457	16.6641,19.3355
No	5593	14.7071	0.3437	13.9962,15.4181

Variable	N	Mean (SI U/L)	Std Error	95% Confidence Interval
Diabetes				
Yes	363	18.8149	1.1976	16.3375,21.2923
No	6774	15.1443	0.3083	14.5066,15.7819

Table 6. 5 Regression of Prior 1 week average exposures and ALT

Survey Regression (PROC SURVEYREG)							
Pollutant Effect	**Estimate (SI u/l)**	**S.E.**	**Denom df.**	**prob t**	**95% Confidence Interval**		
PM_{10} ($\mu g/m^3$)	-0.016	0.008	17	0.07	-0.033, 0.001		
NO_2 (ppb)	0.031	0.012	15	0.027	0.004, 0.057		
SO_2 (ppb)	-0.043	0.034	15	0.223	-0.116, 0.029		
Mixed Model (Random Intercepts)							
Pollutant Effect	**Estimate (SI u/l)**	**S.E.**	**d.f.**	**prob t**	**95% Confidence Interval**	**τ_{00}**	**S.E. of τ_{00}**
Unpartitioned							
PM_{10} ($\mu g/m^3$)	-0.017	0.006	3118	0.007	-0.029, -0.005	0.011	0. 004
NO_2 (ppb)	-0.001	0.01	2436	0.885	-0.020,0.018	0.009	0.004
SO_2 (ppb)	-0.021	0.018	2555	0.245	-0.057,0.015	0.012	0. 004
Partitioned							
PM_{10} ($\mu g/m^3$)						0.011	0.004
County level	-0.011	0.014	28	0.426	-0.040, 0.017	.	.
Individual level	-0.019	0.007	3350	0.008	-0.032, -0.005		

Mixed Model (Random Intercepts)							
Pollutant Effect	**Estimate (SI u/l)**	**S.E.**	**d.f.**	**prob t**	**95% Confidence Interval**	**τ_{00}**	**S.E. of τ_{00}**
NO_2 (ppb)						0.009	0.004
County level	0.008	0.013	22	0.525	-0.018, 0.035	.	.
Individual level	-0.014	0.016	2625	0.387	-0.045, 0.017		
SO_2 (ppb)						0.012	0.004
County level	-0.019	0.037	23	0.619	-0.095, 0.058	.	.
Individual level	-0.023	0.019	2759	0.215	-0.059, 0.013		

Table 6. 6 Random Coefficient model of log ALT by pollutant

effect	Estimate (SI u/l)	Std err	df	probt	95% Confidence Interval	τ_{00}	τ_{01}	τ_{11}	S.E. of τ_{00}	S.E. of τ_{01}	S.E. of τ_{11}
PM_{10} ($\mu g/m^3$)	Likelihood ratio test for random 11.2; p=0.004										
County level	-0.009	0.011	28	0.389	-0.032, 0.013	.	.	.	.	.	.
Within county	-0.013	0.010	3350	0.172	-0.032, 0.006	0.010	0.002	0.001	0.003	0.001	0.001
NO_2 (ppb)	Random intercept model only										
County level	0.008	0.013	22	0.525	-0.019, 0.035						
Within county	-0.037	0.026	2624	0.157	-0.089, 0.014	0.010			0.004		
Cross level Intxn	0.017	0.010	2624	0.095	-0.003, 0.037						
SO_2 (ppb)	Likelihood ratio test for random slope 6.2 ; p=0.045										
County level	-0.018	0.037	23	0.625	-0.095, 0.058	.	.	.	.	.	.
Within county	-0.046	0.018	2758	0.011	-0.081, -0.011	0.012	0.002	0.002	0.004	0.002	0.003
Cross level Intxn	0.036	0.018	2758	0.039	0.002, 0.071						

†log ALT modeled as a continuous dependent variable, using linear mixed models.

$\boldsymbol{\tau_{00}}$ = Variance of random intercepts from mixed model

$\boldsymbol{\tau_{01}}$ = Covariance of random intercepts and random slope from mixed model

$\boldsymbol{\tau_{11}}$ = Variance of random pollutant coefficient from mixed model

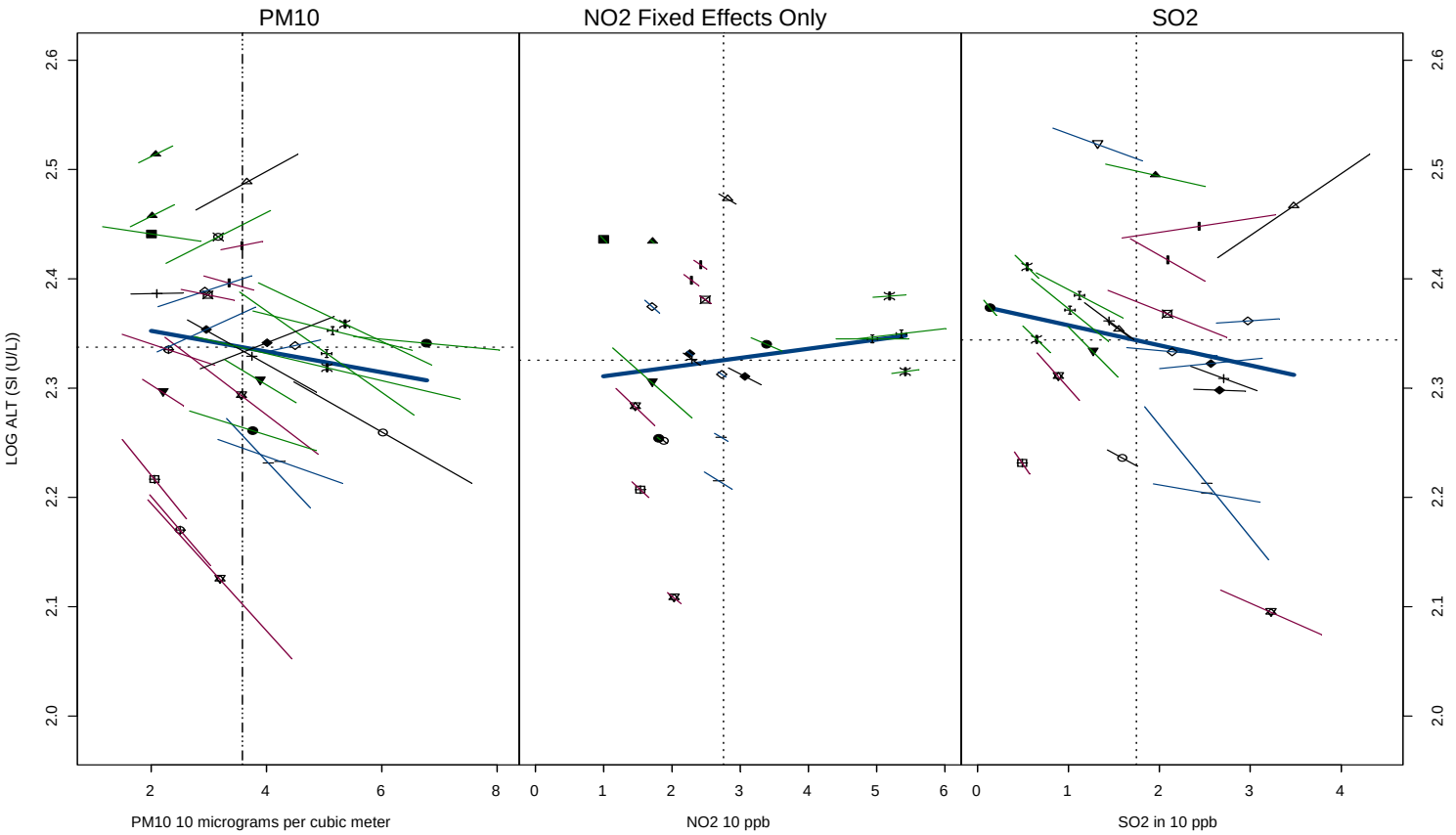

Figure 6. 2 County level slope of pollutant effect empirical Bayes estimates of log ALT.

Northeast=Black, Midwest=Blue, South=Red, West=Green ,

BC=Between county WC=Within county (individual)

Vertical is grand pollutant mean. Horizontal is mean adjusted county level average log ALT (grand intercept)

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CHAPTER VI

CONCLUSIONS

"There is no true interpretation of anything; interpretation is a vehicle in the service of human comprehension. The value of interpretation is in enabling others to fruitfully think about an idea" --Andrea Buja

While it is well established that there are cardiovascular effects of air pollution, it is not known what the full extent of the effects are. The reason for this is that the particular mechanisms by which PM air pollution affects the cardiovascular system are not well understood. This knowledge is necessary to understand the full impact that these ubiquitous yet modifiable exposures have on the population. This is particularly important at this time due to increased industrialization in developing countries with the consequences for obesity and CVD. Furthermore as the urgency related to green house emissions from the combustion of fossil fuels and foreign dependency for energy is now being recognized, the answer to this question could provide additional impetus for change and inform policy in development of alternative fuels.

Major gaps exist in understanding the pathophysiological mechanisms underlying the observed health effects. Among the questions that remain to be answered are: Are the health effects limited to a particular vulnerable population?; and are they limited to acute exposures or do acute exposures over time lead to significant health effects in the general population? Epidemiology is relied upon to demonstrate the health burdens under the circumstances of real world exposures. However, the study of air pollution has been hampered by poor

exposure measurement and a lack of understanding of coherent mechanisms including what are the particular characteristics and components of PM that affect pathological changes

Inconsistencies may occur for a number of reasons: 1) the characteristics of particle air pollution with pathogenic potential vary over space and time; 2) the pertinent comorbid conditions and potentiating exposures are not understood; 3) The time lags in epidemiological studies are not characterized well to measure the effects appropriately; and 4) Where exposures are measured they may not correspond to the personal exposures and or the burden to the host. The studies in this dissertation redress some of these limitations by exploring a novel meditative mechanism and by applying methodology in a unique way to mitigate the difficulties of exposure assessment.

Atherosclerosis underlies much of the CVD with which the health effects of PM have been associated. The studies in this dissertation fill an obvious gap in the extant literature regarding the effect of PM in initiating and promoting atherosclerosis. Alterations in liver metabolism that occur in conjunction with visceral fat and is indicated by elevations in ALT has been found to be a critical component involved in the host of conditions that lead to ischemic disease. Other epidemiological and human exposure studies have examined other manifestations of these conditions in relation to PM. These include increased blood pressure, lymphocytes, fibrinogen, blood coagulation factors (platelets), arterial vasoconstriction (blood pressure), endothelial dysfunction and systemic inflammation as indicated by CRP and fibrinogen. The studies in this dissertation examine two consequences that are implied by the involvement of the liver in mediating health effects.

Exposure assessment that relies on ambient monitoring has a number of limitations. Fine particles have a high diffusion capacity and are a significant component of indoor air

pollution. Epidemiological studies that include subjects all within the same area are limited due to common exposures. Furthermore, exposures in these areas are correlated over time. For these reasons PM is to a large extent an ecological level exposure. As a consequence the exposure contrasts within a particular region are limited. Where exposures to PM do vary within a region, additional variation is valuable as an exposure gradient within that exposure milieu. The current studies are predicated on the observation that exposure to air pollution in free living populations is both an ecological and individual level exposure. This multi-level perspective is applied in this dissertation to standardized data from around the country with a nested structure that is analogous to meaningful exposure contrasts.

A. Findings

The results of these studies point to adverse alterations in LDL, which is compatible with associations observed with cigarette smoking. The alterations while being subtle are portentous for the reason that such changes which may occur in association with oxidative stress would suggest even greater deleterious changes due to the oxidized LDL.

The alterations observed with ALT from acute exposures were not consistent with hepatocyte cytotoxicity. If PM does actually translocate into the general circulation, one would expect adverse effects from intimate contact between toxic components and hepatocytes reflected in increases in ALT. The negative associations refute this hypothesis.

The consistent negative effects beg post hoc hypotheses that would be suited for laboratory investigation. It may be that ALT is down regulated as a secondary effect from other oxidative and inflammatory stimuli from PM exposures. Alternately, under the two hit hypothesis that has been proffered by investigators of liver disease, decreases in ALT may indicate a stimulus that causes a proliferation of hepatocytes. In its capacity as the organ

responsible for metabolizing xenobiotics, liver cells have characteristics that make them more robust and resistant to damage. It is plausible that hepatocytes respond differently to stimuli such that it may alter the balance between hepatocyte proliferation and apoptosis in favor of apoptosis. This is portentous for future vulnerability and CVD given that such changes may cause alterations that make the liver prone to future insults and contribute to the proinflammatory milieu associated with CVD.

Taken together these studies are suggestive of alterations in liver metabolism with consequences for other alterations related to metabolic syndrome, atherogenesis and thrombosis. While the particular study questions are novel and the methodology has advantages in addressing the study questions, these studies are far from being definitively positive studies.

B. Limitations

Reliance on ambient monitoring for exposure assessment remains a limitation as in most observational studies without personal monitoring. The interpolation from monitoring stations to the home residence is not an adequate substitute for individual level exposures. People do not spend all their lives at home. Nor is the air pollution outside the home identical to that inside the home. Other sources of air pollution exist indoors and outdoors. In particular carbon monoxide is not included in the air pollution data. Some indoor exposures are measured but with varying degrees of precision such as use of fireplace or wood stove in the past month. Much of this measurement error is likely to be unsystematic, however systematic differential exposure may also be present in these data. Nonetheless, these data have an abundance of variables that either directly or indirectly correlate with determinants of exposure. It is likely that on average, the misspecification of exposure from the use of

ambient monitoring in these data is unsystematic and will likely result in noise (random misclassification) that simply attenuates the effect estimates.

One potentially systematic misclassification may occur as a result of people crossing through different micro-environments such that measurements associated with home exposure may differ significantly from their real exposures. Occupation and commuting that may correlate with the outcomes in these studies may correlate with home exposure. It is not a tenable assumption that such misclassification is similar across all people in all counties and that it is not systematically related to characteristics associated with risk factors for the outcomes studied here.

Related to this, the following thought experiment includes a worst case scenario: Affluent people (with low CVD risk) live in less polluted areas are characterized as low exposure, but commute, exposing them to traffic relate air pollution. If the converse is also true that less advantaged people travel from their residence in high exposure neighborhoods to low exposure neighborhoods, then for part of their day, their measured exposures would be the complete reverse of their true exposure. While this is possible, it would also have to be a consistent scenario across all counties and variation was only geographical and not temporal. The results in these studies provide an estimate of the within county effect that is the average of the county effects. If the worst case scenario is infrequent, this would result in a bias toward the null.

The NHANES data were collected in the 1980s when PM_{10} was the only mass based metric collected by the EPA. Consequently, a significant limitation is that these pollution data do not include $PM_{2.5}$. Fine particulates are more representative of fossil fuel combustion with which health effects are associated. Ultrafine particulates that are associated with

transition metals and particles that are capable of unique toxicological properties are also not available. The available pollution measures are correlated with the more relevant $PM_{2.5}$. Indeed the Sarnat study demonstrated that ambient monitor data of NO_2 and SO_2 was a good surrogate for personal exposure to $PM_{2.5}$. However, each measured pollutant may represent different correlates of the toxicological properties of fine PM.

Finally, the lack of within county variation limits the contribution of this level of evidence. In particular is the fact that the variation is smallest at the lowest mean county levels. As a consequence it is difficult to make much out of the particular direction, much less strength of the within county effects at these low county levels. The cross-level interaction that is demonstrated in many of the pollutant-outcome models is ambiguous. It is possible that this does reflect a true dose-response such that the effect really does differ across the range of average county level effects. However, it may also be the result of differential misclassification related to county mean or variance of pollution levels.

C. Strengths

The current research fills an obvious gap in the extant body of evidence that links PM exposure to CVD. Alterations in liver metabolism may provide a coherent explanation for many of the associations between PM and CVD that have been observed, such as systemic inflammation with related consequences for atherosclerosis and death from CVD. Furthermore, it may suggest specific reasons why some people are more vulnerable to these effects than others. People with existing CVD, diabetes and older people will have alterations in liver metabolism that are likely to be more susceptible to the effects from PM. These two studies are reinforcing as representing near and long term consequences of PM exposure in relation to liver metabolism and risk of future morbidity and mortality.

The methodology employed in these analyses provides some methodological advantages in evaluating health effects of PM in observational studies that rely on ambient monitoring. The principle advantage comes from the partitioning of the exposure between the county level and within county level. It provides two relevant effect estimates corresponding to levels of exposure, each with relevance for statistical inference. To the extent that people within the same county share common exposures and that these exposures are correlated over time, the county level exposure is a meaningful measure that simply captures the ecological nature of PM exposure. This is estimated independent of the pollution relationship at the within-county level. If the models are adequately specified at the individual level, the county level effects will control for confounding at the individual level that may arise from differences in aggregate characteristics of measured variables being correlated with county mean pollution levels.

In the situation where the majority of the variation occurs between counties, then this parameter captures this aggregate level effect. The variation within counties where it occurs is additional evidence that is conditional on the county effect, but provides valuable evidence that arises from this level. To the extent that they both agree provides support for the single hypothesis that PM is related to the outcome, as occurs with the effect of prior year exposures on LDL levels.

While it is possible that misclassification of within-county exposure due to individuals crossing over microenvironments may be systematically related to the outcome, where the source of variability within county is not due to geography alone, this bias would not be as relevant. In the ALT study, there is also temporal variability over the course of the 6 weeks during which the samples were collected by the mobile examination center. The time at

which their blood was collected is unlikely to be related to temporal variations in air pollution exposure. Thus the individual level parameter is likely to be derived from variation that does not systematically correlate with exposure. Furthermore, the people crossing micro-environments during the day are almost certainly in the same region, and thus their macro exposure remains the same.

In evaluating the effects of pollutants on the outcome parameters, I was able to compare the effects with that of active and passive smoking that, having a similar pathway mechanism provides an internal validation of the observed pollutant effects. This is more particularly relevant in the analysis of ALT. Though the effect for pollution on ALT levels at the individual level was counterintuitive, a similar effect was seen for passive smoking. This provides support that the observed pollutant effect is not spurious.

In the absence of adequate personal exposure data, I exploited data that has large exposure gradients available in data from around the country. Furthermore it specifically addresses the latent qualities that exist in exposure monitor data. These latent qualities relate to: 1) SO_2 and NO_2 as surrogates of personal exposure relate to particulate matter from sulphites and urban traffic respectively; 2) Measured ambient levels of PM_{10}, SO_2, or NO_2 variously represent common pathogenic potential such as precipitation inflammation in the lung; 3) The unique properties of particulates that these correspond to may differ regionally in relation to their measurements; 4) Exposures are correlated in a region and over time such that effects of PM reasonably include a valid etiological inference from living in a polluted area. In this study, the separation of the effects from air pollution exposures into within and between county allow a more realistic statistical representation of the nature of exposure. In spite of the cross-sectional nature of these data, the analysis has advantages over traditional

Epidemiology studies using ambient monitor data that do not treat exposures in the relevant contextual framework.

D. Summary

The results of the study of cholesterol were consistent with the hypothesis and provide a link in the evidence that exists between PM exposures and atherosclerosis, which has previously not been examined. In the other study, the results were opposite from the expectation that systemic effects of PM would be represented by an increase in ALT reflecting hepatocyte cytotoxicity. Although to a large degree the alternative explanations were post-hoc, these provide, I believe, a valuable new hypothesis by which the liver may mediate the effects of particles. Specifically, those alterations in liver metabolism that correspond with short term decreases in ALT may signify a paradoxical response with relevance to stimuli that affect the future metabolizing ability of the liver.

APPENDICES

A. County slope plotting

B. Coding of covariates

C. Manuscript 1. Additional Results

D. Manuscript 2. Additional Results

E. Manuscript 2. Results including subjects with "explained" ALT elevations

A. County slope plotting

Details of the empirical Bayes estimates resulting from the random coefficients model: First we included a single slope running through the national average pollution mean (x-axis) and intercept (y-axis) to reflect the between county estimate. In addition we plot the individual slopes for each of the counties. For these, the position on the y-axis was a function of the overall intercept, plus the individual random intercept, plus the increment predicted from the between county slope based on the county average pollutant exposure. The position on the x-axis corresponded to the county average pollutant exposure. The slopes surrounding each county are derived from the fixed effect for the within-county slope, plus the increment from the cross-level interaction (if significant), plus the random slope (if significant). The length of the within county slope is determined by the individual county IQR for the pollutant.

B. Coding of covariates

1. Personal level covariates

a. Stratification variables used in NHANES sampling methodology:

Sex : Male=index value; Female=referent

Age (In whole years) continuous

Age categorical:1-2, 3-5, 6-11, 12-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80+

Ethnicity: Non-Hispanic; white (referent value); non-Hispanic black; Mexican American; other (includes other Hispanics, Asians and Native Americans.

b.Social-Economic variables

Education: 0-8 years, 9-11 years, 12 years, 13 plus years

Poverty income ratio (PIR)- continuous

PIR – Categorical: High (referent)= ge 3.5; Middle income 1.3 + to 3.5; Low income lt 1.3

Household size continuous: Categorical: 1 (referent), 2, 3,4, 5 and up

c. Biological risk factors

BMI in kg/m continuous

BMI as categorical:

'1=UNDERWGHT' BMPBMI<18.5

'2=NORMAL' 18.499<BMPBMI<25

'3=OVERWGHT' 24.999<BMPBMI<30

'4=CLASS I' 29.999<BMPBMI<35

'5=CLASS 2,3' 34.999<BMPBMI

Waist to hip ratio:

1st quartile lt -0.07

2nd quartile if -0.07 to 0.00

3rd quartile 0.00 to 0.06

4th quartile gt 0.06

Metabolic syndrome (Yes if 3 or more of the following):

Systolic blood pressure greater than 130;

diastolic blood pressure greater than 85;

taking medication for hypertension;

serum triglycerides greater than 1.7 mmol/L;

HDL less than 40 (male) or 50 (female) ;

fasting glucose greater than 6.0;

waist circumference grater than 102 cm (males) and 88 cm (females)

Diabetes mellitus (where available): glucose tolerance test (OGTT)

Smoking Current/Past/Never

Current Smoking Packs/day (smokers only)

Environmental Tobacco Smoke (yes/no)

Cotinine-A laboratory measure of Nicotine metabolite in (ng/ml)

d. Dietary risk factors

Alcohol Consumption (g/day)

Saturated Fat consumption

e. Competing exposures

Wood stove use

Fireplace use

Gas stove use

2. Group level risk covariates:

Season: Dichotomized to Winter (Index value: November through April) vs Summer (May through October).

C. Manuscript 1. Additional Results

Table A5. 1 Random Intercepts Total Cholesterol and LDL/HDL ratio

Effect	Estimate	df	probt	lower upper	τ_{00}	S.E. of τ_{00}
Total Cholesterol (4a)						
PM_{10}						
County level	0.963	29	0.173	-0.446, 2.371	.	.
Within county	1.974	3512	0.498	-3.739, 7.687	17.444	9.249
Survey reg						
NO_2						
County level	2.229	23	0.012	0.544, 3.914	.	.
Within county	0.186	2772	0.954	-6.146, 6.518	3.393	5.640
Survey reg						
SO_2						
County level	0.560	23	0.650	-1.961, 3.081	.	.
Within county	3.410	2809	0.385	-4.280, 11.099	11.404	7.416
LDL to HDL Ratio (4b)						
PM_{10}						
County level	0.030	29	0.476	-0.054, 0.113	.	.
Within county	0.070	1460	0.084	-0.009, 0.149	0.023	0.013

Effect	Estimate	df	probt	lower upper	τ_{00}	S.E. of τ_{00}
Survey reg						
NO_2						
County level	0.068	23	0.134	-0.022, 0.157	.	
Within county	0.111	1147	0.153	-0.041, 0.263	0.023	0.016
Survey reg						
SO_2						
County level	0.101	23	0.167	-0.046, 0.247	.	.
Within county	0.195	1159	0.197	-0.101, 0.492	0.014	0.012

Table A5. 2 Modification of effect of Prior year Exposure to Pollutants on Total Cholesterol by Age, Diabetes and Smoking

Effect Modifier (EM)	Age (year)			Diabetes			Number of cigarettes smoked (current smokers)		
	Estimate	p	95%CI	Estimate	p	95%CI	Estimate	p	95%CI
Total Cholesterol (5a)									
County PM_{10}*	1.154	0.069	-0.094, 2.402	1.558	0.017	0.299, 2.816	1.141	0.079	-0.139, 2.421
Indiv. PM_{10} *	2.791	0.299	-2.482, 8.064	1.538	0.577	-3.874, 6.950	3.427	0.253	-2.444, 9.298
EM*ind PM_{10} *	0.323	0.003	0.112, 0.534	-1.871	0.788	-15.481, 11.740	-0.481	0.002	-0.792, -0.169
County NO_2 †	2.402	0.002	0.986, 3.817	2.033	0.006	0.644, 3.422	2.42	0.002	1.025, 3.815
Indiv NO2 †	0.214	0.939	-5.268, 5.697	-0.65	0.806	-5.844, 4.544	1.956	0.438	-2.984, 6.895
EM *ind NO_2 †	0.067	0.663	-0.235, 0.369	0.46	0.961	-18.008, 18.927	-0.736	0.018	-1.348, -0.125

Effect Modifier (EM)	Age (year)			Diabetes			Number of cigarettes smoked (current smokers)		
	Estimate	p	95%CI	Estimate	p	95%CI	Estimate	p	95%CI
County SO_2 †	-0.253	0.774	-2.060, 1.553	0.22	0.819	-1.752, 2.192	-0.231	0.796	-2.055, 1.593
Indiv. SO_2 †	2.171	0.564	-5.214, 9.557	1.546	0.723	-6.995, 10.087	5.127	0.106	-1.084, 11.338
EM *ind SO_2 †	-0.321	0.047	-0.637, -0.005	-2.425	0.886	-35.475, 30.626	-0.37	0.256	-1.009, 0.268
LDL to HDL Ratio (5b)									
County PM_{10*}	0.013	0.685	-0.053, 0.080	0.017	0.641	-0.057, 0.092	0.01	0.772	-0.061, 0.082
Indiv. PM_{10*}	0.079	0.05	0.000, 0.159	0.079	0.043	0.002, 0.155	0.106	0.022	0.015, 0.197
EM*ind $PM*_{10}$	0.008	0.009	0.002, 0.014	-0.144	0.603	-0.688, 0.400	-0.011	0.235	-0.030, 0.007
County NO_2 †	0.062	0.095	-0.012, 0.137	0.061	0.123	-0.018, 0.141	0.064	0.093	-0.011, 0.139
Indiv NO_2 †	0.125	0.138	-0.040, 0.291	0.125	0.159	-0.049, 0.300	0.201	0.03	0.020, 0.382
EM *ind NO_2†	0.007	0.314	-0.006, 0.019	-0.096	0.74	-0.661, 0.469	-0.033	0.074	-0.069, 0.003

Effect Modifier (EM)	Age (year)			Diabetes			Number of cigarettes smoked (current smokers)		
	Estimate	p	95%CI	Estimate	p	95%CI	Estimate	p	95%CI
County SO_2 †	0.073	0.138	-0.025, 0.172	0.068	0.181	-0.034, 0.169	0.072	0.138	-0.025, 0.168
Indiv. SO_2 †	0.19	0.254	-0.137, 0.518	0.184	0.241	-0.123, 0.490	0.115	0.501	-0.221, 0.451
EM *ind SO_2 †	0.01	0.295	-0.009, 0.028	0.407	0.668	-1.455, 2.269	0.014	0.465	-0.024, 0.052

* 10 $\mu g/m^3$

† ppb

Table A5. 3 Random Coefficients Model for air pollutants on Total Cholesterol and LDL to HDL ratio

A. **Total Cholesterol**

effect	Estimate	df	probt	lower upper	τ_{00}	τ_{01}	τ_{11}	S.E. of τ_{00}	S.E. of τ_{01}	S.E of τ_{11}
PM_{10} ($\mu g/m^3$)				Likelihood ratio test 1 p=0.61						
County level	1.062	29	0.102	-0.226, 2.349						
Within county	0.876	3512	0.752	-4.562, 6.314	16.821	-12.61	14.199	9.123	19.176	25.527
NO_2 (ppb)				Likelihood ratio test: 1.2; p=0.55						
County level	2.560	23	0.004	0.919, 4.200	.	.	.	.	.	.
Within county	-2.280	2772	0.538	-9.534, 4.974	3.704	18.917	65.375	5.750	29.012	114.48
SO_2 (ppb)				Likelihood ratio test: 1.4; p=0.50						
County level	0.062	23	0.959	-2.395, 2.519	.	.	.	.	.	.
Within county	-0.446	2808	0.908	-8.007, 7.115	11.325	32.336	44.924	7.526	28.256	108.52
Cross level Intxn	5.487	2808	0.033	0.450, 10.523						

B. LDL to HDL ratio

effect	Estimate	df	probt	lower	upper	τ_{00}	τ_{01}	τ_{11}	S.E. of τ_{00}	S.E. of τ_{01}	S.E of τ_{11}
PM_{10}					Likelihood ratio test: 0 ;p=1.0						
County level	0.030	29	0.468	-0.054	0.114	.	.	.	.	.	.
Within county	0.064	1460	0.114	-0.015	0.142	0.023	-0.003	0.000	0.042	0.850	.
NO_2					Likelihood ratio test: 0 p=1.0						
County level	0.068	23	0.127	-0.021	0.157	.	.	.	.	.	.
Within county	0.109	1147	0.143	-0.037	0.254	0.023	0.006	0.000	0.068	0.829	.
SO_2					Likelihood ratio test 2.5 p=0.29						
County level	0.082	23	0.267	-0.067	0.232	.	.	.	.	.	.
Within county	0.001	1158	0.994	-0.226	0.228	0.018	0.050	0.000	0.105	0.050	.
Cross-level interaction	0.358	1158	0.001	0.154	0.563						

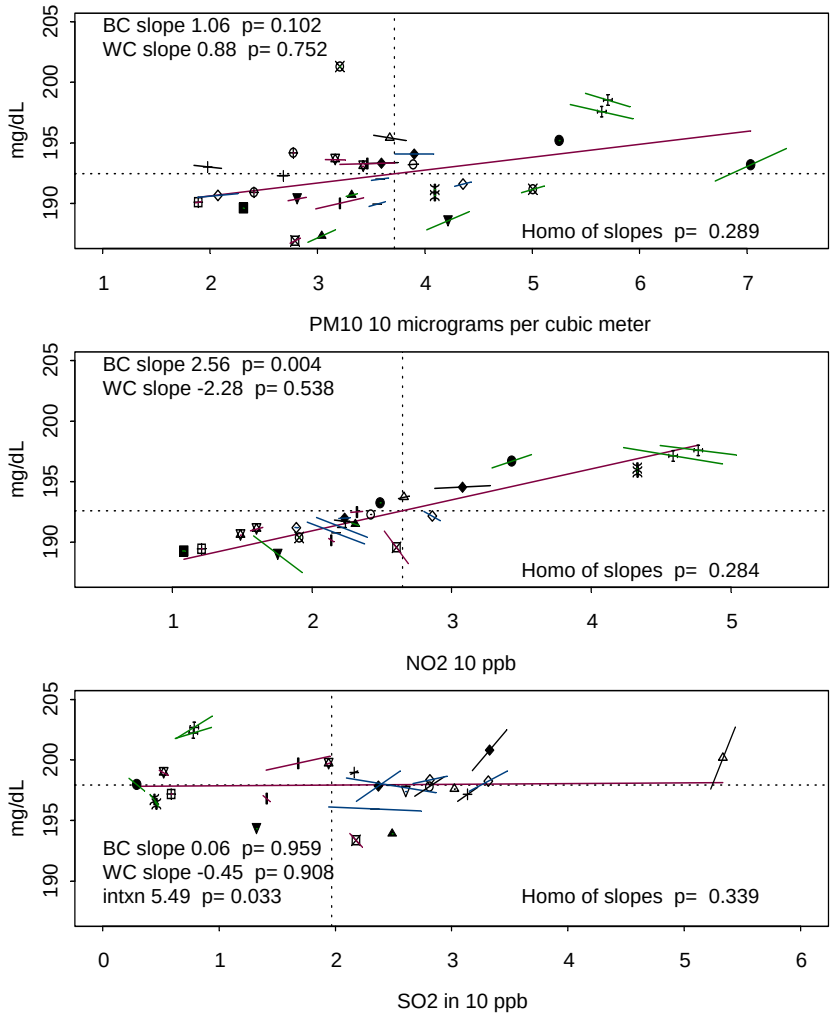

Figure A5. 1 Empirical Bayes estimates for Total Cholesterol

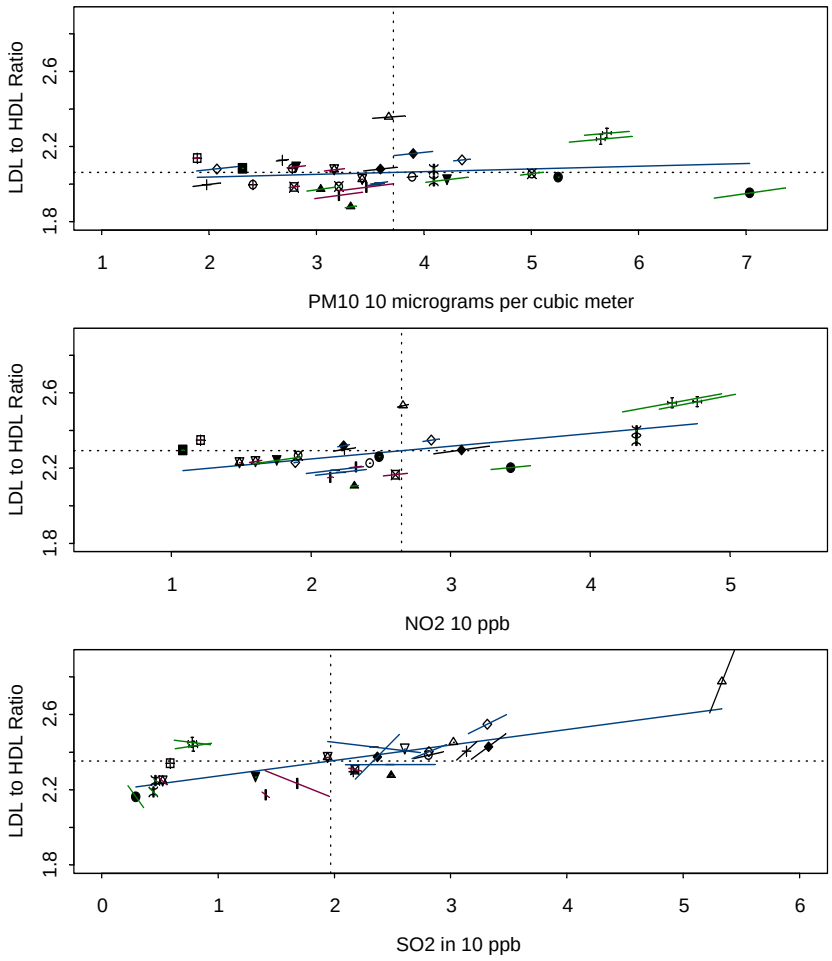

Figure A5. 2: Empirical Bayes estimates for LDL to HDL ratio

D. Manuscript 2. Additional Results

Table A6. 1 Partitioned estimates -county and individual (grey) – Adjusted for Potential confounders

	PM_{10}			NO_2			SO_2		
Adjustment Variable	**Est**	**SE**	**p**	**Est**	**SE**	**p**	**Est**	**SE**	**p**
Fasting sample	-0.011	0.014	0.426	0.008	0.013	0.529	-0.019	0.037	0.614
	-0.019	0.007	0.008	-0.013	0.016	0.398	-0.023	0.019	0.217
Times exercised in last month	-0.011	0.014	0.447	0.008	0.013	0.522	-0.020	0.037	0.599
	-0.018	0.007	0.010	-0.013	0.016	0.405	-0.020	0.019	0.292
Education	-0.011	0.014	0.417	0.008	0.013	0.545	-0.019	0.037	0.614
	-0.019	0.007	0.008	-0.014	0.016	0.377	-0.023	0.018	0.214
Household size	-0.011	0.014	0.437	0.009	0.013	0.516	-0.019	0.037	0.604
	-0.019	0.007	0.008	-0.015	0.016	0.347	-0.023	0.019	0.221
Wood Stove last 12 months	-0.011	0.014	0.423	0.008	0.013	0.534	-0.019	0.037	0.622

Adjustment Variable	PM_{10} Est	PM_{10} SE	PM_{10} p	NO_2 Est	NO_2 SE	NO_2 p	SO_2 Est	SO_2 SE	SO_2 p
	-0.019	0.007	0.007	-0.013	0.016	0.407	-0.023	0.019	0.205
Fireplace last 12 months	-0.012	0.014	0.396	0.008	0.013	0.551	-0.016	0.037	0.681
	-0.018	0.007	0.009	-0.014	0.016	0.390	-0.022	0.019	0.244
Gas stove used for cooking	-0.013	0.014	0.353	0.008	0.013	0.546	-0.016	0.037	0.677
	-0.018	0.007	0.010	-0.014	0.016	0.383	-0.022	0.019	0.238
Vitamin C (mg/dL)	-0.012	0.014	0.389	0.009	0.013	0.492	-0.018	0.037	0.638
	-0.018	0.007	0.010	-0.015	0.016	0.358	-0.022	0.019	0.236
Seafood	-0.012	0.014	0.418	0.008	0.013	0.535	-0.018	0.037	0.635
	-0.018	0.007	0.012	-0.014	0.016	0.357	-0.020	0.019	0.284
Coffee	-0.011	0.014	0.430	0.008	0.013	0.532	-0.019	0.037	0.612
	-0.018	0.007	0.009	-0.013	0.016	0.402	-0.023	0.018	0.218

	PM_{10}			NO_2			SO_2		
Adjustment Variable	**Est**	**SE**	**p**	**Est**	**SE**	**p**	**Est**	**SE**	**p**
Hyper tension medication	-0.011	0.014	0.422	0.008	0.013	0.530	-0.019	0.037	0.623
	-0.018	0.007	0.009	-0.015	0.016	0.352	-0.024	0.018	0.197
Diabetes rx	-0.012	0.014	0.405	0.009	0.013	0.507	-0.018	0.037	0.632
	-0.019	0.007	0.007	-0.014	0.015	0.356	-0.023	0.018	0.196

Table A6. 2 Effect Modification of selected variables with within county air pollutant parameter

	Age			Sex			BMI		
Parameter	**Estimate**	**Prob t**	**95%CI**	**Estimate**	**Prob t**	**95%CI**	**Estimate**	**Prob t**	**95%CI**
Effect Modifier	-.006	0.000	-.008, -.005	0.132	0.009	0.033, 0.231	0.011	0.000	0.007, 0.016
County PM_{10}	-.011	0.424	-.040, 0.017	-.011	0.426	-.040, 0.017	-.011	0.425	-.040, 0.017
Individual PM_{10}	-.019	0.010	-.033, -.004	-.017	0.026	-.033, -.002	-.039	0.009	-.068, -.009
EM * ind PM_{10}	-.000	0.655	-.001, 0.000	-.002	0.785	-.019, 0.014	0.002	0.119	-.000, 0.004
Effect Modifier	-.007	0.000	-.008, -.005	0.124	0.016	0.023, 0.225	0.011	0.000	0.007, 0.016
County NO_2	0.008	0.528	-.019, 0.035	0.008	0.526	-.018, 0.035	0.009	0.516	-.018, 0.035
Individual NO_2	-.013	0.413	-.044, 0.018	-.004	0.863	-.044, 0.037	0.009	0.672	-.034, 0.053
EM * ind NO_2	0.000	0.647	-.001, 0.002	-.021	0.475	-.078, 0.037	-.002	0.395	-.006, 0.002
Effect Modifier	-.006	0.000	-.008, -.005	0.163	0.002	0.059, 0.267	0.012	0.000	0.007, 0.017

	Age			Sex			BMI		
Parameter	**Estimate**	**Prob t**	**95%CI**	**Estimate**	**Prob t**	**95%CI**	**Estimate**	**Prob t**	**95%CI**
County SO_2	-.018	0.626	-.095, 0.058	-.019	0.613	-.096, 0.058	-.019	0.616	-.095, 0.058
Individual SO_2	-.027	0.144	-.062, 0.009	-.011	0.612	-.055, 0.032	-.079	0.071	-.165, 0.007
EM * ind SO_2	-.001	0.249	-.003, 0.001	-.023	0.401	-.076, 0.030	0.004	0.161	-.002, 0.011

EM= Effect modifier

	Metabolic Syndrome			Vitamin C			WHR		
Parameter	**Estimate**	**probt**	**95%CI**	**Estimate**	**probt**	**95%CI**	**Estimate**	**Probt**	**95%CI**
Effect Modifier	0.116	0.000	0.070 ,0.161	0.016	0.347	-.017, 0.049	0.679	0.000	0.331, 1.027
County PM_{10}	-.011	0.431	-.040, 0.017	-.013	0.380	-.042, 0.016	-.011	0.431	-.040, 0.017
Individual PM_{10}	-.016	0.046	-.031, -0.000	-.010	0.398	-.033, 0.013	-.019	0.008	-.033, -.005
EM * ind PM_{10}	-.009	0.624	-.043, 0.026	-.011	0.470	-.041, 0.019	-.081	0.082	-.172, 0.010
Effect Modifier	0.113	0.000	0.058, 0.167	0.002	0.933	-.036, 0.039	0.738	0.000	0.325, 1.151
County NO_2	0.009	0.502	-.017, 0.035	0.009	0.487	-.018, 0.036	0.008	0.535	-.019, 0.035
Individual NO_2	-.007	0.686	-.040, 0.026	-.041	0.164	-.098, 0.017	-.014	0.388	-.045, 0.017
EM * ind NO_2	-.025	0.522	-.103, 0.052	0.034	0.183	-.016, 0.084	0.076	0.722	-.344, 0.496
Effect Modifier	0.092	0.000	0.041 ,0.143	0.017	0.362	-.019, 0.052	0.632	0.003	0.220, 1.045

	Metabolic Syndrome			Vitamin C			WHR		
Parameter	**Estimate**	**probt**	**95%CI**	**Estimate**	**probt**	**95%CI**	**Estimate**	**Probt**	**95%CI**
County SO_2	-.017	0.660	-.095, 0.061	-.018	0.636	-.094, 0.058	-.019	0.619	-.095, 0.058
Individual SO_2	-.042	0.094	-.091, 0.007	0.012	0.770	-.071, 0.096	-.023	0.202	-.058, 0.012
EM * ind SO_2	0.060	0.302	-.054 ,0.175	-.050	0.341	-.153, 0.053	-.005	0.983	-.438, 0.428

E. Manuscript 2. Results including subjects with "explained" ALT elevations

Table A6.3 Regression of Prior 1 week average exposures and ALT - all patients

Random Intercepts Mixed Model								
Pollutant								
Effect	**Estimate**	**S.E.**	**d.f.**	**prob t**	**Lower**	**Upper**	**τ_{00}**	**S.E. of τ_{00}**
Unpartitioned								
PM_{10}	-0.018	0.006	3408	0.003	-0.030	-0.006	0.010	0. 004
NO_2	-0.006	0.010	2667	0.520	-0.025	0.013	0.009	0.004
SO_2	-0.020	0.018	2812	0.272	-0.056	0.016	0.012	0. 004
Partitioned								
PM_{10}							0.011	0.004
County level	-0.010	0.014	28	0.461	-0.039	0.018	.	.
Individual level	-0.019	0.007	3408	0.004	-0.032	-0.006		
NO_2							0.009	0.004
County level	0.009	0.013	22	0.462	-0.017	0.035	.	.
Individual level	-0.026	0.016	2667	0.099	-0.057	0.005		

Random Intercepts Mixed Model

Pollutant								
Effect	**Estimate**	**S.E.**	**d.f.**	**prob t**	**Lower**	**Upper**	**τ_{00}**	**S.E. of τ_{00}**
SO_2							0.012	0.004
County level	-0.026	0.037	23	0.497	-0.103	0.052	.	.
Individual level	-0.019	0.019	2812	0.32	-0.056	0.018		

Table A6. 4 Random Coefficient model of log ALT by pollutant – all patients

Effect	Estimate	Std err	df	probt	95%CI	τ_{00}	τ_{01}	τ_{11}	S.E. of τ_{00}	S.E. of τ_{01}	S.E. of τ_{11}
PM_{10}	Likelihood ratio test for random 6.5; p=0.038										
County level	-0.012	0.013	28	0.361	-0.038, 0.014	.	.	.	.	.	.
Within county	-0.011	0.011	3407	0.343	-0.033, 0.011	0.010	0.002	0.001	0.003	0.001	0.001
Cross level Intxn	-0.006	0.006	3407	0.350	-0.018, 0.006						
NO_2	Likelihood ratio test for random slope 7.7 ; p=0.021										
County level	0.011	0.013	22	0.415	-0.016, 0.038						
Within county	-0.053	0.055	2666	0.334	-0.160, 0.054	0.009	-0.020	0.038	0.004	0.010	0.034
Cross level Intxn	0.023	0.026	2666	0.390	-0.029, 0.074						
SO_2	Likelihood ratio test for random slope 0.9 ; p=0.0.63										
County level	-0.024	0.038	23	0.522	-0.102, 0.053	.	.	.	.	.	.
Within county	-0.037	0.016	2811	0.025	-0.069, -0.005	0.012	0.001	0.002	0.005	0.002	0.003

Cross level Intxn	0.036	0.018	2758	0.039	0.002, 0.071

†log ALT modeled as a continuous dependent variable, using linear mixed models.

$\boldsymbol{\tau_{00}}$ = Variance of random intercepts from mixed model

$\boldsymbol{\tau_{01}}$ = Covariance of random intercepts and random slope from mixed model

$\boldsymbol{\tau_{11}}$ = Variance of random pollutant coefficient from mixed model

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