Great Lakes Air Center for Integrative Environmental Research
An EPA Clean Air Research Center
Year 4 Progress Report
Period: August 1, 2013 – July 31, 2014

Introduction

This Annual Progress Report covers the activities in Year 4 of the Great Lakes Air Center for Integrative Environmental Research (GLACIER). GLACIER is a multidisciplinary center with the objective to explore one of the most prevalent and important global health-environment interfaces**:** the inter-relationships between facets of the cardio metabolic syndrome (CMS) and air pollution. CMS is among the leading causes of death and threats to worldwide health. In tandem, exposure to air pollution, most notably fine particle matter ($PM_{2.5}$), remains highly prevalent and ranks among the leading causes of global mortality. Our center's overall hypothesis is that $PM_{2.5}$ and O_3 are 1) capable of eliciting multiple important adverse cardiometabolic health effects that are dependent on 2) the local multipollutant milieu, 3) an individual's pre-existing cardiovascular (CV) and metabolic condition (susceptibility), and 4) the interactive toxicity of $PM_{2.5}$ and O_3 coexposure.

In conjunction with 3 core facilities (Administration, Exposure Characterization, and Biostatistics and Data Management) GLACIER consists of 3 controlled exposure projects that each address specific aspects of the CMS-air pollution interface. The projects are scientifically integrated and interactive which will foster synergistic insights and cohesive synthesis of conclusions. ***Project 1*** aims to elucidate in humans the mechanisms of adverse CMS responses and the concentration-response relationships of acute exposures to differing $PM_{2.5}$ mixtures. ***Project 2*** aims to determine the short-term CV, autonomic and airway toxicity in rats exposed to differing $PM_{2.5}$ mixtures. ***Project 3*** expands upon the main theme by determining the CMS toxicity of differing longer-term exposures in mice. Each project will also investigate the role of pre-existing susceptibility and the comparative effects of $PM_{2.5}$ mixtures derived from 2-3 dissimilar multipollutant milieus of regional importance (near-roadway, industrial, transported). Toxic effects of $PM_{2.5}$, O_3, each alone and in combination, will be evaluated at each location. We will address 1) temporal-response relationships to pollutant exposure and the development of CMS, 2) CMS effects of ozone and fine particle mixtures from three differing locations and their interactive toxicity, 3) the role of obesity and pre-existing cardiometabolic abnormalities in individual susceptibility, 4) concentration-response relationships for particles and O_3, and 5) mechanisms whereby air pollutants elicit CV and metabolic health effects. Our results will provide critical insights into the health effects of $PM_{2.5}$, O_3, and their coexposures in a multipollutant context.

During Year 4, there were no changes in our overall research objective or specific aims. There was, however, a change in the institutional location of the subcontract for Project 3. Dr. Rajagopalan moved to the University of Maryland where he is the Melvin Sharoky Endowed Professor in Cardiovascular Medicine, and Co-Director of the University of Maryland Heart Center. Therefore, the subcontract for his GLACIER Project was moved from his previous academic home at The Ohio State University to the University of Maryland, effective January 1, 2014. This change in location for Project 3 has not had any significant impact on the productivity from this group of investigators, since their inhalation exposure studies have continued to be performed in the Columbus, Ohio area under the leadership of Dr. Sun who remains as a faculty member at The Ohio State University. The change in the Project 3 subcontract also did not result in any changes to the Center's overall budget, operations, and objectives.

Dr. Bhramar Mukherjee, Data Management and Biostatistical Core (DMBC), has returned to her duties at the University of Michigan and GLACIER after her sabbatical year. We are excited with Dr. Mukherjee's return and that Dr. Bin Nan, who served as her replacement in the DMBC this past year, has agreed to remain as a statistician for GLACIER.

GLACIER's research projects/cores have made substantial progress since the last progress report. Our Center's research accomplishments and future endeavors from August 1, 2013 to July 31, 2014 are presented in this document. Separate written reports are presented for each of our research projects and cores. In these reports, we briefly 1) review objectives of the specific research, 2) highlight major accomplishments, and 3) discuss future plans. Please note that progress regarding our ongoing intramural CLARC collaborative project with Harvard University is found in the report of Project 2.

GLACIER PROJECT 1 (Year 4 Progress Report)

Date of Report: 7/31/14
EPA Agreement Number: RD83479701
Center Name: Great Lakes Air Research Center for Integrative Environmental Research (GLACIER)
Project Title: Cardiometabolic Effects of Exposure to Differing Mixtures and Concentrations of $Pm_{2.5}$ in Obese and Lean Adults
Investigators: Robert D. Brook, Elif Oral, Marianna Kaplan, Jesus Araujo
Institutions: University of Michigan (Brook, Oral, Kaplan), University of California at Los Angeles (Araujo)
Research Category: Air Quality and Toxics
Project Period: August 1, 2013 – July 31, 2014

Objective of Research: We have elucidated the existence of an important confluence between key facets of the cardio-metabolic syndrome (CMS) and fine particulate matter ($PM_{2.5}$). Brief exposure to concentrated $PM_{2.5}$ (fine CAP) for 2 hours has proven capable of triggering vasoconstriction, raising diastolic blood pressure (BP), and impairing vascular endothelial function (VEF) 1 day later – the latter occurring in location-dependent manner suggesting that particle constituents/sources are important determinants of the responses. Two distinct mechanistic pathways were implicated – with altered autonomic nervous system (ANS) balance responsible for the increased BP and systemic inflammatory responses for the slower impairment in VEF. Though these findings are important as they help to explain how $PM_{2.5}$ might cause acute cardiovascular (CV) events, several important issues remain to be clarified. Moreover, our studies also suggest that a more-encompassing, yet unappreciated, convergence between $PM_{2.5}$ and the CMS might exist. Not only could obesity enhance the susceptibility for adverse health effects induced by $PM_{2.5}$ exposure, but $PM_{2.5}$ might promote the development of metabolic insulin resistance (IR), a central factor in the pathogenesis of obesity and the CMS itself (i.e. reciprocal relationship). We propose to build upon our previous research on the effect of short-term $PM_{2.5}$ exposure on key facets of the CMS. The broad objectives are to investigate: 1) if exposure to fine CAP mixtures are capable of acutely instigating metabolic IR in addition to elevating diastolic BP and impairing VEF; 2) whether obesity confers enhanced susceptibility for these adverse responses; 3) details of the mechanistic pathways involved; 4) the extent and nature of the dose-response relationships even to levels below current 24-hour $PM_{2.5}$ standards; and 5) if fine CAP derived from 2 dissimilar multi-pollutant ambient $PM_{2.5}$ mixtures elicit differing CMS responses and the specific pollutants responsible. We will achieve these aims by examining the BP and VEF responses, along with additional/novel outcomes, in obese versus healthy adults induced by fine CAP exposures in 2 separate locals comprised of dissimilar $PM_{2.5}$ mixtures (industrial/urban versus a near-roadway/residential). The concentrations of fine CAP will be varied to include levels from below 35 to above100 $\mu g/m^3$. Using state-of-the-art physiological testing and novel biomarkers (including adipocytokines, HDL function, endothelial progenitor cell levels and function), the mechanisms responsible for the alterations in the CMS responses will be explored. The role of the ANS in the etiology of the BP increase and the effectiveness of a prophylactic measure, $\alpha+\beta$ adrenergic blockade, in obviating this response will also be tested. Finally, we will evaluate whether exposure to fine CAP can acutely elicit metabolic IR, the underlying cause of the CMS itself. This project addresses several RFA questions (Q) in an experimental fashion with humans exposed to real-world $PM_{2.5}$, thereby providing findings of tremendous health/regulatory importance. The expected results will elucidate pivotal new insights into: the enhanced susceptibility of obese individuals (Q#3), the extent of the concentration-response relationship (Q#4), the mixtures of $PM_{2.5}$ and their constituents /sources responsible (Q#2), and the mechanisms underlying the CV responses (Q#6). Finally, we will explore for the first time the evidence for a novel $PM_{2.5}$ health effect (Q#6) – instigation of metabolic IR by $PM_{2.5}$ mixtures - of critical health importance given the rising global epidemics of obesity and the CMS.

Progress Summary/Accomplishments: Change of co-investigators: Dr Marianna Kaplan moved from the University of Michigan Health Systems during year 4 and was replaced by Dr. Jason Knight in Rheumatology as a co-investigator. He provided the measurements of EPC levels per protocol design without change during year 4. We began urban exposures per protocol design in Dearborn after the end of winter season starting in April 2013. Randomized exposures continued until April of 2014. During this period (year 4), we completed 30 subjects having both CAP and FA exposure done at the urban Dearborn site. All health outcomes and exposures/monitoring were completed per study design without deviation from the protocol, pitfalls, or adverse events reportable to IRB. Due to concerns about performing human exposure to concentrated PM2.5 (fine particles) we chose to re-design study #2 of project 1 without using fine CAP. Though the study was approved by the IRB to perform fine CAP exposures, after discussions with our IRB and the EPA (and members of

our SAC at our annual meeting in 2013) and we re-designed the protocol for study #2 to involve only ambient level exposures in a panel study. In June of 2013, the re-designed panel study of obese and lean individuals (n=50) undergoing 2 separate non-scripted exposure window periods (7 days of local site pollution monitoring; 1 day of personal PM2.5 monitoring using pDR-1500) was approved by our IRB at the University of Michigan. The health outcomes collected have not changed from the original study design; however, they will be obtained on 2 separate mornings (from 1-4 weeks apart) and associated with daily 24-hour average PM2.5 levels measured from personal (pDR-1500) and local ambient PM2.5 levels during the prior 1 and 7 days, respectively.

Publications/Presentations:

Bard RL (2013) The hemodynamic, autonomic, and vascular effects of exposure to coarse particulate matter in a rural location. Presented at: American Society of Hypertension. 2013 Annual Meeting. May 15, 2013 - San Francisco, California.

Brook RD. (2012) GLACIER Project 1 update. Presented at: Clean Air Research Centers Annual Meeting. June 21-22, 2012 - Cambridge, Massachusetts.

Brook RD, Xu X, Bard R, Dvonch JT, Morishita M, Kaciroti N, Sun Q, Harkema J, Rajagopalan S. (2013) Reduced metabolic insulin sensitivity following sub-acute exposures to low levels of ambient fine particulate matter air pollution. Sci. Total Environ. 448:66-71. PMID:22901427.

Brook RD. American Society of Hypertension national meeting New York, May 2014. "The Components of Coarse Particulate Matter Air Pollution Associated with Alterations in Blood Pressure and Heart Rate during Controlled Exposures" (Poster)

Brook RD. Society of Toxicology Annual Meeting 2014. Pheonix Az. "*Air pollution exposures and the cardio-metabolic syndrome*" (Oral communication)

Brook RD, Bard RL, Kaplan MJ, Yalavarthi S Morishita M, Dvonch JT, Wang L, Yang H-Y, Spino C, Mukherjee B, Oral EA, Sun Q, Brook JR, Harkema J, Rajagopalan S. (2013) The effect of acute exposure to coarse particulate matter in a rural location on circulating endothelial progenitor cells: Results from a randomized controlled study. Inhal Toxicol; 25: 587-92. PMID:23919441

Brook RD, Bard RL, Morishita M, Dvonch JT, Wang L, Yang H-Y, Spino C, Mukherjee B, Kaplan MJ, Yalavarthi S, Oral EA, Ajluni N, Sun Q, Brook JR, Harkema J, Rajagopalan S. (2014) The hemodynamic, autonomic, and vascular effects of exposure to coarse particulate matter in a rural location. Environ Health Perspect; 122: 624-30. PMID:24618231

Maiseyeu A, Yang HY, Ramanathan G, Yin F, Bard RL, Morishita M, Dvonch JT, Wang L, Spino C, Mukherjee B, Badgeley MA, Barajas-Espinosa A, Sun Q, Harkema J, Rajagopalan S, Araujo JA, Brook RD. (2014) No effect of acute exposure to coarse particulate matter air pollution in a rural location on high density lipoprotein function. Inhal Toxicol; 26: 23-29. PMID:24417404 (In Process)

Morishita M, Bard RL, Dvonch JT, Wang L, Das R, Spino C, Mukherjee B, Sun Q, Harkema J, Rajagopalan S, Brook RD. (2014) The Components of Coarse Particulate Matter Air Pollution Associated with Alterations in Blood Pressure and Heart Rate during Controlled Exposures. Journal Exposure Science and Environmental Epidemiology (in press).

Morishita M, Bard RB, Kaciroti N, Fitzner C, Dvonch JT, Harkema JR, Rajagopalan S. Brook RD. (2014) Exploration of the Composition and Sources of Urban Fine Particulate Matter Associated with Same-Day Cardiovascular Health Effects in Dearborn, Michigan. Journal Exposure Science and Environmental Epidemiology; (in press). doi:10.1038/jes.2014.35. PMID:24866265

Future Activities for year 4: We will begin study #2 of project 1 (re-designed panel study) during July of 2014. The plan is to complete all 50 subjects (2 exposure periods each = 100 exposure windows) before the end of the project (year 5 end). We will complete analyses of our controlled urban Dearborn CAP exposure study outcomes by September 2014 and submit manuscripts to journals regarding these results by December 2014. We will aim to complete analyses and submit manuscripts of the study #2 results within a few months of the trial completion.

Supplemental Keywords: human exposures, susceptible populations, acute cardiovascular effects, particulate matter, human exposures, cardiometabolic syndrome

Relevant Web Sites: http://greatlakesairresearchcenter.org/

GLACIER PROJECT 2 (Year 4 Progress Report)

Date of Report: 7/31/2014
EPA Agreement Number: RD83479701
Center Name and Internal Number: Great Lakes Air Research Center for Integrative Environmental Research (GLACIER)
Project Title: Cardiometabolic, Autonomic, and Airway Toxicity of Acute Exposures to $PM_{2.5}$ from Multipollutant Atmospheres in the Great Lakes Region
Investigators: Jack Harkema (PI), Greg Fink and James Wagner
Institutions: Michigan State University, East Lansing, MI
Research Category: Air Quality and Toxics
Project Period: August 1, 2013 – July 31, 2014

Objective of Research: Our objectives in Project 2 arise out of GLACIER's overarching hypothesis that the major air pollutants, fine particulate matter ($PM_{2.5}$) and ozone (O_3), are 1) capable of eliciting multiple important adverse cardiometabolic health effects that are dependent on 2) the local multipollutant milieu, 3) an individual's pre-existing cardiovascular (CV) and metabolic condition (susceptibility factors), and 4) the interactive toxicity of $PM_{2.5}$ and O_3 co-exposure. Goals of Project 2 are to determine the cardiovascular (CV), autonomic nervous system (ANS), and airway toxicity in rats acutely exposed to concentrated ambient $PM_{2.5}$ (CAP) from distinct multipollutant atmospheres commonly found in the Great Lakes Region of the United States. Our studies are extensions of our previous findings that CAP-induced alterations in heart rate variability are dependent on specific $PM_{2.5}$ emission sources in distinct locations in the Great Lakes Region. We will use a mobile air research facility (AirCARE 1) that is fully equipped with inhalation toxicology and atmospheric monitoring labs to conduct toxicology studies of rats exposed to CAP derived from real-world $PM_{2.5}$ in three distinct locations dominated by industrial/urban, transported/regional, or near-roadway/residential emission sources. Blood pressure, heart rate, heart rate variability and direct measurements of autonomic nerve activity will be continuously monitored during CAP and/or O_3 exposures in lean or obese rats with and without diet-induced facets of the cardiometabolic syndrome (CMS; hypertension, insulin resistance, endothelial dysfunction), respectively. Acute functional responses will be measured by radiotelemetry and will be correlated with specific PM constituents and their emission sources determined for the same highly resolved 30-minute timeframes, thereby making associations of exposure and health effects especially robust. Studies will feature novel real-time sympathetic nerve recordings during $PM_{2.5}$ and/or O_3 inhalation exposure. In addition, our project will highlight the unique integrative capabilities of our research team to link specific health cardiovascular effects in a sensitive obese population with PM content by a combined technological expertise that is unavailable elsewhere. Our GLACIER project will extend and complement the research of lean and obese human subjects (Project 1), conducted at the same exposure sites, by making invasive and prolonged measurements that could not be practically or ethically done in humans (e.g., repeated CAP exposures, continuous recordings of CV and autonomic nerve function, and microscopic examination of multiple organs for exposure-related pathology). Our acute animal studies will also overlap and integrate scientifically with the animal toxicology study of long-term multipollutant exposures in Project 3.

Progress Summary/Accomplishments: There have been no changes in study investigators or other personnel in year 4 of this project. Our objectives in Project 2 have also remained the same in Year 4. Our studies have been designed to address GLACIER's overarching hypothesis that the major criteria air pollutants, fine particulate matter ($PM_{2.5}$) and ozone (O_3), are 1) capable of eliciting multiple important adverse cardiometabolic health effects that are dependent on 2) the local multipollutant milieu, 3) an individual's pre-existing cardiovascular (CV) and metabolic condition (susceptibility factors), and 4) the interactive toxicity of $PM_{2.5}$ and O_3 co-exposure. Research in Year 4 addressed all four of these hypotheses by conducting both field-exposure studies and mechanism-driven experiments. These studies were extensions of our previous findings that CAP-induced alterations in heart rate variability are dependent on specific $PM_{2.5}$ emission sources in distinct locations in the Great Lakes Region. Our results in Years 1-4 have been designed to extend and complement the initial research of lean and obese human subjects (Project 1), by making invasive and prolonged measurements in laboratory rats/mice that could not be practically or ethically done in humans (e.g., repeated CAP exposures, semi-continuous cardiovascular recordings, and microscopic examination of multiple organs for exposure-related pathology). Our acute

animal studies were also designed to contrast and complement the initial animal toxicology studies of long-term multipollutant exposures in Project 3.

In Year 4, several research milestones were achieved that led directly to a better understanding of the health effects of both individual pollutant and multipollutant exposures in the face of experimentally induced facets of the cardiometabolic syndrome (CMS). Specifically these milestones included the following: 1) Our field study of rats fed normal or a high fructose (HFr) diet, and repeatedly exposed to O3 and concentrated PM2.5 aerosols in the urban/industrial community of Dearborn MI was published in the January issue of *Environmental Health Perspectives* (Jan;122:27-33, 2014); 2) We finished the telemetric, cardiovascular data analysis from rats in a similar inhalation toxicology study designed to investigate the toxicity of PM2.5, O3 and PM2.5/O3 exposures but in a rural/regional airshed (Dexter, MI); 3) We completed a laboratory study designed to determine the effects of O3 exposure on the development of insulin resistance and other health facets of the CMS in mice that are genetically prone to develop type two diabetes (T2D), 4) We initiated a study investigating the role of lymphocytes in the development of O3-induced eosinophilic rhinitis in mice; and 5) We continued our data analysis from an intraCLARC collaborative animal toxicology study with Harvard University that employed our rat model of diet-induced CMS and their inhalation exposure system to traffic emissions from the Boston Tunnel. A brief summary of specific Project 2 accomplishments in the last year and proposed future studies in Year 5 are briefly described below.

Rat Inhalation Toxicology Study in Dexter, MI. In June 2013, we initiated a rat inhalation toxicology study at our rural/regional exposure site in Dexter, MI, that was similar in design to that previously conducted in Dearborn MI. Repeated acute inhalation exposures of rats, fed normal (ND) or HFr-diets (HFrD), to O3/PM2.5, were performed for 19 consecutive weekdays, 8 h/day, in our mobile air research laboratory (AirCARE I) parked in a rural setting in Dexter, MI. In contrast to the urban/industrial site in Dearborn MI with local traffic and industrial air pollutant emission sources, this Dexter exposure site is dominated by transferred air pollution from distant regional emission sources. Like the previous Dearborn study, cardiovascular responses (e.g., blood pressure, heart rate, heart rate variability) were measured every 5 minutes by radiotelemetry during and after daily exposures. The inhalation exposures were successfully completed in early July.

Drs. Cathie Spino and Bin Nan from our Biostatistical and Data Management Core (BDM) at the University of Michigan completed a thorough quality control and statistical analysis of all the data collected from this study. We first used data from the first nine days from this study to compare the responses to O3/PM2.5 in Dexter to our previous data from rats exposed to O3 alone and to O3/PM2.5 in Dearborn. We found that inhalation of O3/PM2.5 in Dexter caused significant drops in mean arterial pressure (3-8 mmHg) in rats fed ND that were maintained for most days of the exposures (Figure A, black circles). In contrast both decreases and increases in blood pressure (3-5mmHg) were detected in HFrD rats during exposures (Fig 1A, white circles). These responses are in stark contrast to O3/PM2.5 exposure in urban/industrial Dearborn (Fig 1B) where ND rats had no exposure-related responses and HFrD quickly adapted after acute drops in blood pressure during the first day of exposure. *In both rural Dexter and urban Dearborn, coexposure to PM2.5 diminished the vascular response induced by O3 exposure alone (compare Fig 1A, B to 1C).*

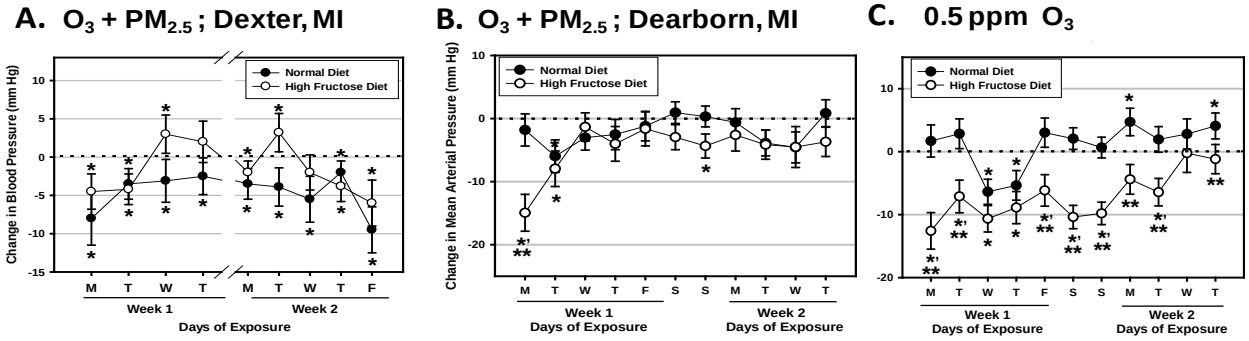
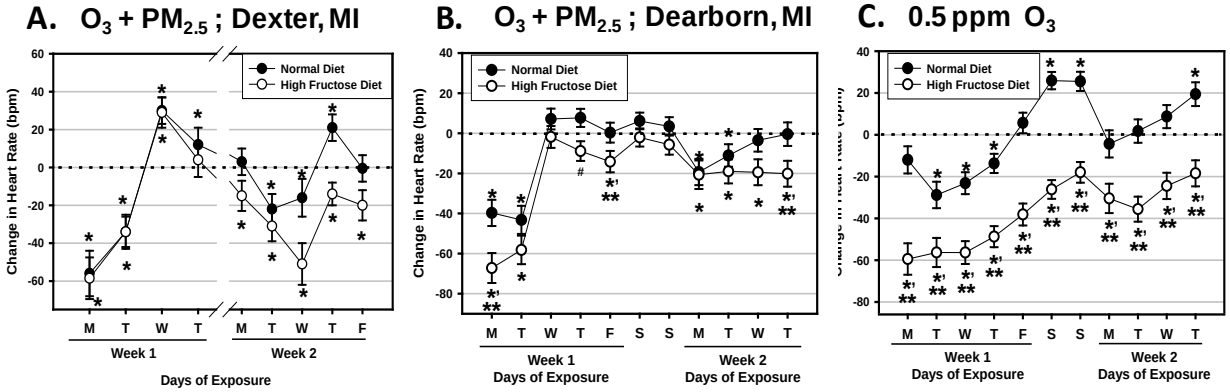


Figure 1. Daily Effect of Inhalation Exposure to O3/PM2.5 in Dexter, MI (A), O3/PM2.5 in Dearborn, MI (B) or O3 alone (C) on Blood Pressure in Rats Fed Normal (black circles) or High Fructose (white circles) Diets. Data is plotted as the effect estimate on blood pressure (mmHg) of the indicated exposure compared to filtered air (indicated by dotted line – zero axis). * -indicates significant difference from Air-exposed rats on the same Diet; * * - indicates significant difference from rats fed a Normal Diet.

Changes in heart rate were initially similar in ND- and HFrD rats exposed O3/PM2.5 in Dexter, with acute drops (40-60bpm) during the first two days, and followed by increases (10-30bpm) by end of the week (Fig 2A). During the second week of exposure diet-related responses diverged, with drops in HR consistently greater in HFrD compared to ND rats. In Dearborn, exposure to O3/PM2.5 caused drops in heart rate were that were remarkable during the first two days (decreases of 40-70 bpm), but were less dramatic with repeated exposures (0-20 bpm decreases; Figure 2B). Coexposure to PM2.5 in either Dexter or Dearborn enhanced O3-induced drops in heart rate in ND rats the first two days of exposure (compare Fig2A and 2B to 2C), but this effect diminished with repeated coexposure. Furthermore PM2.5 at both sites was associated with diminished ozone-induced cardiac responses by O3, with greater effects by urban PM in Dearborn. *Overall responses to coexposures to O3 and PM2.5 were not greater than exposure to O3 alone.*



Exposure-related responses of decreased blood pressure and heart rate persisted during a third and fourth week of exposure to O3/PM2.5 (Fig 3). Depression on blood pressure was similar in NF and HFrD rats (5 mmHg), but drops in heart rate were greater in HFrD rats (~40 bpm).

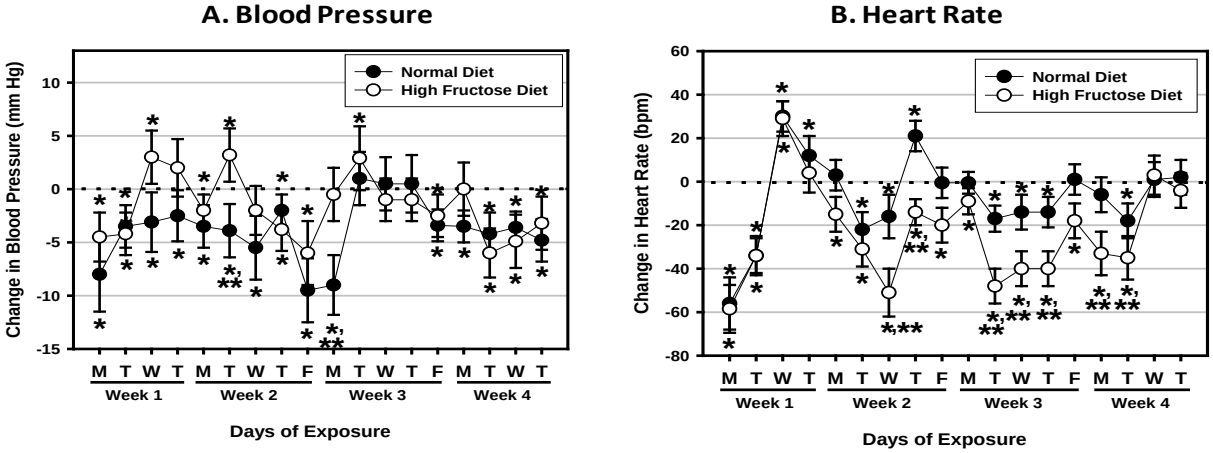


Figure 3. Daily Effect of 4-Week Inhalation Exposure to O3/PM2.5 in Dexter, MI on Blood Pressure (A) and Heart Rate (B) in Rats Fed Normal (black circles) or High Fructose (white circles) Diets. Data is plotted as the effect estimate on cardiovascular responses O3/PM2.5 exposure compared to filtered air (indicated by dotted line – zero axis). * -indicates significant difference from Air-exposed rats on the same Diet; ** - indicates significant difference from rats fed a Normal Diet.

Associations of Exposure/Health Outcomes in Rats, Dearborn, MI. With the help of Dr. Bin Nan in the Biostatistics and Data Management Core, and Dr. Morishita in the Exposure Core, cardiovascular responses and PM2.5 metrics (e.g., trace elements, gases, sources) collected over the same 30-minute timeframes were analyzed with linear mixed model approaches to determine the effect of PM2.5 emission sources on blood pressure and heart rate. Dr.Morishita determined that our field site at Salinas Elementary School in Dearborn, MI was impacted by five major sources of PM2.5: secondary, urban dust, diesel/motor vehicle, refinery, and iron/steel. Depression in blood pressure in both ND and HFrD rats was related to diesel/motor vehicle sources, while iron/steel manufacturing was associated with increased blood pressure in ND rats (Fig 4A). Traffic sources were also associated with decreased heart rate in HFrD (Fig 4B), whereas urban dust was linked to increase heart rate in these same animals. These data suggest that subjects with metabolic syndrome may be

more susceptible to traffic sources of PM2.5 than normal healthy subjects, and sensitivity for cardiovascular responses to specific PM sources is dependent on metabolic health.

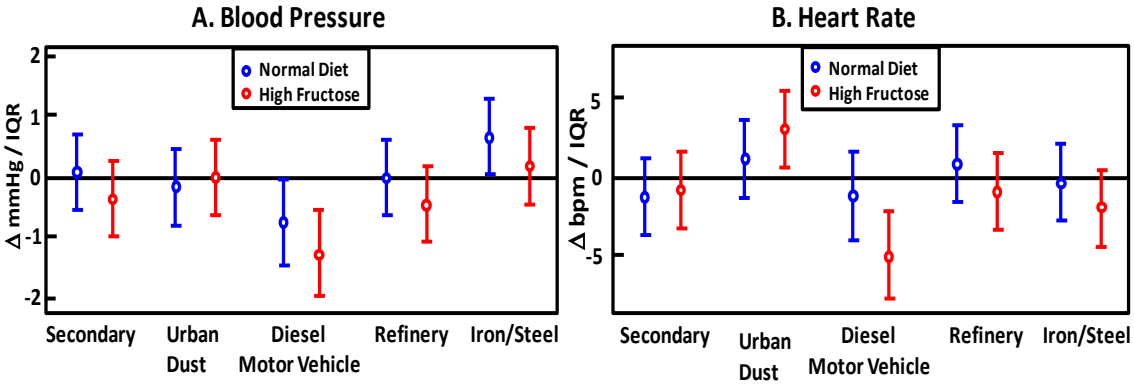


Figure 4. Effect Estimates for Cardiovascular Responses to Inhalation Exposure to $PM_{2.5}$ Derived From Specific Emission Sources. Data are expressed as change in blood pressure **(A)**, and heart rate **(B)** per IQR of $PM_{2.5}$ emission sources. Estimates with confidence intervals that do not intersect the 0-axis are significant, $p < 0.05$

Metabolic and Respiratory Effects of Ozone Exposure in Mice Genetically Prone to Type II Diabetes (T2D). Epidemiological studies suggest that diabetics may be more susceptible to the adverse health effects of air pollution. Mice chronically exposed to particulate air pollutants induce insulin resistance (IR), an early indicator of type II diabetes (T2D). In this study we tested the hypothesis that episodic inhalation exposures to a common gaseous air pollutant, ozone (O3), will induce early onset of IR in mice genetically prone to develop T2D. Male C57BL/6, KK, and KKAy mice were exposed to 0 ppm (filtered air; FA) or 0.5 ppm O3, 4h/day, for 13 weekdays. Two hours after the last exposure, mice were subjected to insulin tolerance tests (ITT) and then sacrificed 24 hours postexposure. Mice received a single intraperitoneal injection of insulin and blood glucose levels were measured at 0, 10, 20, 30, 60, 90, 120 and 130 minutes after injection. Bronchoalveolar lavage fluid (BALF) was analyzed for inflammatory cells, and lungs were processed for light microscopy.

Normoglycemic, C57BL/6 mice exposed to FA or O3 had normal ITT. Marked IR was present in O3-, but not FA-, exposed KK mice. Both FA- and O3-exposed, hyperglycemic KKAy mice developed IR.

In C57BL/6 mice, O3 caused modest increases in the number of BALF inflammatory cells and minimal to mild pulmonary pathology. In contrast, O3 induced greater increases in BALF inflammatory cells in the hyperglycemic KK mice, along with more severe pulmonary pathology. KKAy mice exposed to O3 had the greatest numbers of BALF inflammatory cells and the most severe pulmonary pathology of all the mice on

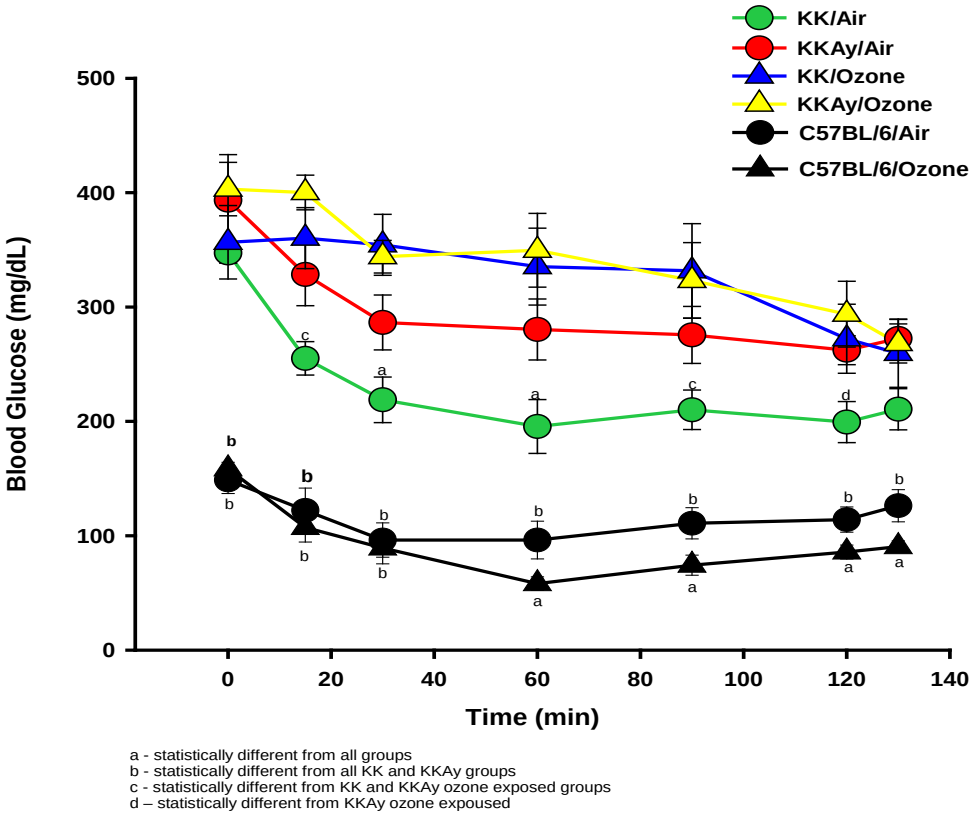


Figure 5. Insulin tolerance tests (ITT) in C57/BL/6, KK and KKAy mice after exposure to 0 or 0.5 ppm O3 for 13 consecutive weekdays.

In conclusion, episodic O3 exposures caused an early onset of IR in mice genetically prone to T2D. These hyperglycemic animals had greater O3-induced lung injury and inflammation compared to normoglycemic, insulin responsive mice. These results suggest that people at risk for T2D may be more susceptible to respiratory and metabolic health effects caused by elevated concentrations of ambient O3.

Development of Ozone-Induced Eosinophilic Rhinitis is Lymphoid-Dependent.

While nasal epithelial injury and remodeling have been reported in laboratory animals repeatedly exposed to O3, associated granulocytic rhinitis and pro-inflammatory cytokine expression have not been fully characterized. We investigated the temporal changes in granulocyte influx, cytokine gene expression, and epithelial remodeling in the nasal mucosa of mice episodically exposed to O3.

C57Bl/6 male mice were exposed to 0 or 0.5 ppm O3 for 1, 2, 4, or 9 weekdays (4h/day). Airway mucosa from nasal turbinates and lateral wall were analyzed for cytokine and epithelial gene expression. Nasal tissues were prepared for light microscopy and morphometry. Immunohistochemistry was used to identify neutrophils, eosinophils, and chitinase-like proteins (Ym1/Ym2). Epithelial mucosubstances were histochemically detected.

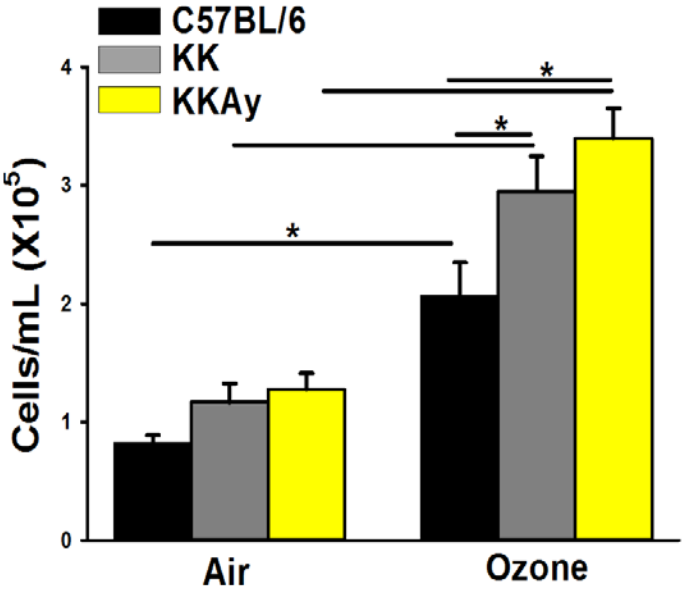


Figure 6. Total inflammatory cells in the bronchoalveolar lavage fluid of mice exposed to ozone or air for 13 consecutive weekdays**.**

1-day-O3 exposure induced a marked neutrophilic influx and concurrent epithelial necrosis (Figure 7). These responses were associated with overexpression of KC, MIP-2, IL-1 [illegible]-5, IL-6 and Eotaxin genes. After repeated O3 exposures, neutrophils waned and eosinophils increased, along with epithelial regeneration and remodeling. 9-day-O3 exposed mice had marked eosinophilic rhinitis with few neutrophils, mucous cell metaplasia and increased epithelial Ym1/Ym2 proteins (Figure 7). Concurrently there was overexpression of Gob5, Muc5AC, IL4, IL5, eotaxin and Ym2 genes. 24-day-O3 mice developed marked eosinophilic rhinitis, epithelial hyperplasia, mucous cell metaplasia, hyalinosis, and increased Ym1/Ym2 expression.

Based on these initial findings, a second study was conducted to investigate the role of lymphocytes (hypothesized cellular sources of Th1 and Th2 cytokines) in the development of eosinophilic rhinitis and associated nasal epithelial remodeling. Male Rag2 x common gamma chain (γc) - deficient [RAG2(-/-) x γc(-/-)] mice, that are lymphoid-deficient (lack both T and B lymphocytes, as well as innate lymphoid cells) were exposed to 0.5 ppm O3 for 9 consecutive weekdays (4h/day).

Unlike the O3-exposed and lymphoid-sufficient C57 Bl/6 mice that developed a marked Th2-associated eosinophilic rhinitis with nasal epithelial remodeling, the similarly exposed RAG2(-/-) x γc(-/-) mice did not develop an eosinophilic rhinitis or O3-induced nasal epithelial lesions (Figure 8). In addition, these lymphoid-depleted mice had no O3-induced overexpression of Th-2 cytokine mRNA like that found in the O3-exposed and lymphoid-sufficient C57 Bl/6 mice.

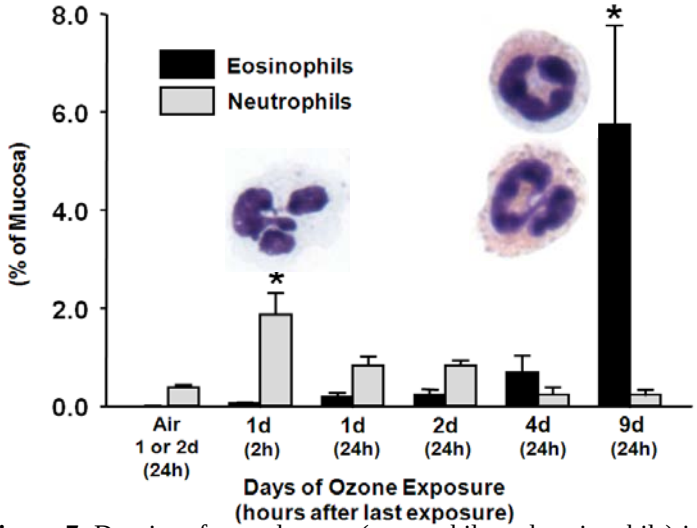


Figure 7. Density of granulocytes (neutrophils and eosinophils) in the nasal mucosa of C57 BL/6 mice exposed to 0.5 ppm O3 or filtered air (0 ppm O3, controls) for 1, 2, 4, or 9 consecutive weekdays (4h/day) and sacrificed 2 or 24h postexposure.

All tissues sections were immunohisto-chemically processed using a polyclonal antibody for mouse esosinophil-specific major basic protein (red chromagen; arrow). An influx of major basic protein-laden eosinophils is found only in lymphoid-sufficient C57 BL/6 mice exposed to O3 (C). Lymphoid-depleted RAG2(-/-) x γc(-/-) mice similarly exposed to O3 had no eosinophilic influx in the nasal mucosa (D).

These results confirmed our hypothesis that lymphocytes are a crucial component to O3-induced eosinophilic rhinitis and nasal epithelial remodelling. Furthermore, these findings in mice provide biological plausibility for previous epidemiological studies that found associations of increased ambient O3 concentrations and increased incidence of eosinophilic rhinitis in atopic and nonatopic children, suggesting that chronic exposure to this gaseous air pollutant may cause nasal lesions that mimic those of allergic rhinitis. Studies are ongoing to identify the type of lymphoid cells (e.g., T lymphocytes, innate lymphoid cells) that mediate these O3-induced inflammatory and epithelial lesions in the nasal airways of mice.

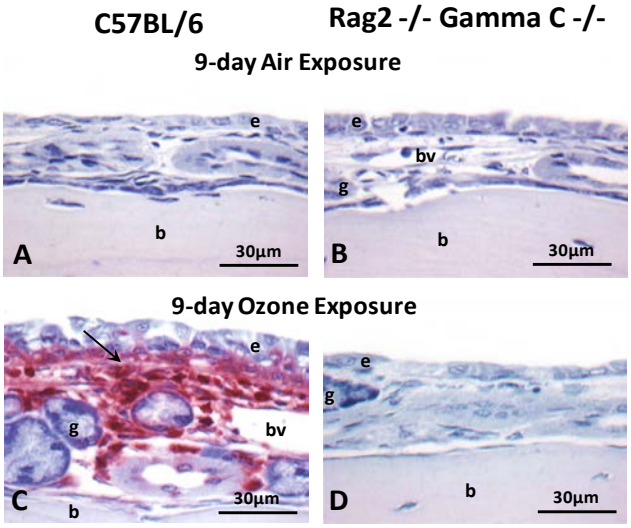


Figure 8. Light photomicrographs of the nasal mucosa of mice exposed to 0.5 ppm O3 or filtered air (0 ppm O3) for 9 consecutive weekdays (4h/day).

We further extended this line of research by examining O3-induced eosinophilic rhinitis in KK, KKAy and C57BL/6 mice exposed for 13 consecutive weekdays. Standard morphometric techniques were used to measure the density of major basic protein-laden eosinophils in the nasal mucosa (a quantitative assessment of the severity of eosinophilic rhinitis). The diabetes-prone, hyperglycemic KKAy and KK mice exposed to O3 had remarkably more severe eosinophilic rhinitis compared to similarly exposed normoglycemic C57BL/6 mice (Figure 9). Interestingly, a similar stain-dependent increase in eosinophils was also found in the lungs of these mice (Figure). These results suggest that diabetics or those suffering from facets of the metabolic syndrome (e.g., hyperglycemia) may be more susceptible to airway injury caused by repeated ambient O3 exposure.

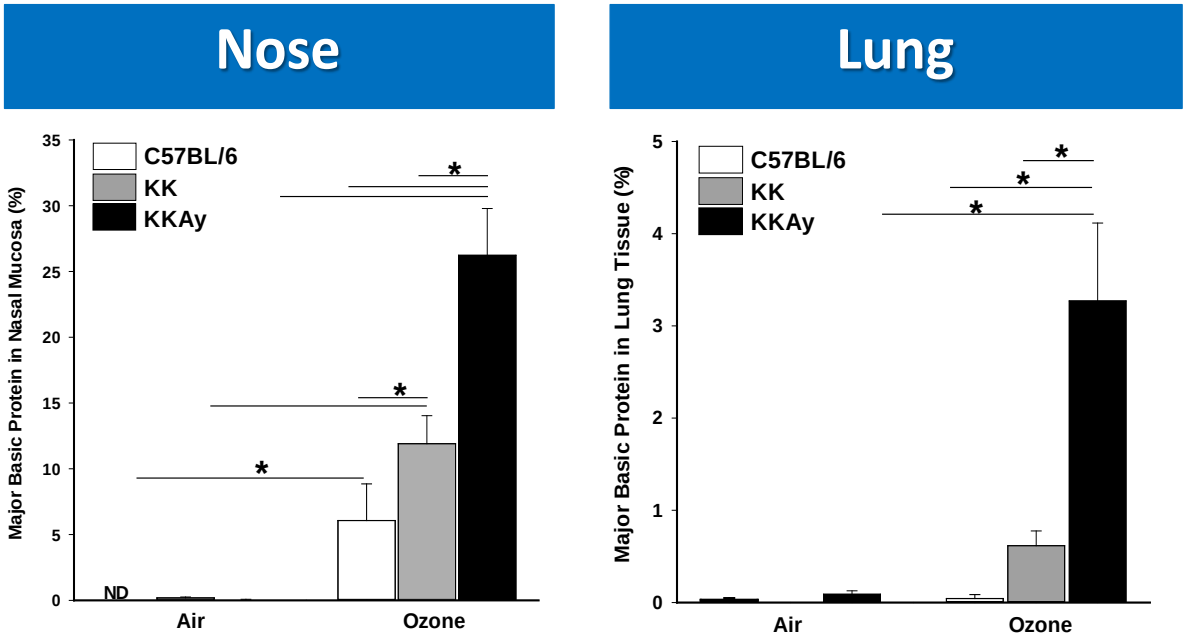


Figure 9. Density of major basic protein-laden eosinophils in the nasal mucosa and lung of mice exposed to 0.5 ppm O3 or filtered air (0 ppm O3; controls) for 13 consecutive weekdays (4h/day). O3-exposed KKAy and KK mice had a greater influx of eosinophils compared to C57BL/6 mice that were similarly exposed. KKAy mice had

Future Activities in Year 5

Our main goal in the final year of this project is to complete the ongoing studies and prepare manuscripts for peer-review publication. No new field studies will be initiated in Dearborn or Dexter, MI. Important analyses that need to be completed are listed below along with a few small, short-term laboratory studies that need to be conducted to complete the body of work necessary for manuscripts to be of publication quality.

Ongoing Analyses:

1. **Cardiotelemetry Data Analysis:** Data analysis of heart rate variability (HRV) in rats exposed to the O3/PM2.5 mixture, 5 days/wk for four weeks (Dexter inhalation exposure study) will be completed this year. A comparison of responses to O3/PM2.5 in Dexter vs. Dearborn will be conducted for HRV, heart rate and blood pressure. Lastly, we will assess exposure –related arrhythmias and ECG abnormalities in rats fed ND vs HFrD and exposed to O3, PM2.5, and O3/PM2.5 (coexposure) in all studies. These data will form the basis of two manuscripts.
2. **Associations of Exposure/Health Outcomes:** Working in close collaboration with colleagues in the Exposure Core and the Biostatistics and Data Management Core, we will continue the correlation analyses to determine associations between acute cardiovascular responses (e.g., heart rate, mean arterial pressure, heart rate variability) and PM2.5 metrics (e.g., trace elements, gases, sources). We will conduct these correlative analyses to determine modifications by ozone coexposure on the source-induced cardiovascular health effects. These data will form the basis for at least one manuscript.
3. **Morphometric, Biochemical, and Molecular Analyses:** We will complete these analyses of tissue (nose, lung, liver) and fluid (BALF and serum/plasma) samples taken from C57BL/6, KKAy, KK, RAG2(-/-) x γc(-/-) and RAG2(-/-) mice in our ongoing O3/PM2.5 studies. In addition, we will complete similar analyses taken from Sprague-Dawley rats in our collaborative study with colleagues in the Harvard CLARC (see below).

Short-term Studies:

1. **Study to identify the type of lymphoid cells responsible for the development of O3-induced eosinophilic rhinitis.** We have demonstrated that lymphoid cells play a crucial role in the development of eosinophilic rhinitis in mice exposed to O3, but the type of lymphocytes involved is yet unknown. Based on our previous data and recently published articles in the scientific literature, we hypothesize that innate lymphoid cells (e.g., innate lymphoid 2 cells) are the primary effector cells. RAG2(-/-) mice (T and B lymphocyte deficient, but innate lymphoid cell sufficient) and RAG2(-/-) x γc(-/-) mice (deficient of all lymphoid cell types) will be exposed to O3 for 9 consecutive weekdays. If innate lymphoid cells do play a role in the development of O3-induced eosinophilic rhinitis, we anticipate that eosinophilic rhinitis will be induced in O3-exposed Rag 2(-/-) mice, but not in O3-exposed RAG2(-/-) x γc(-/-) mice.
2. **Study the role of insulin resistance in the enhanced sensitivity of diabetes-prone KKAy mice to O3-induced nasal and lung toxicity.** Metformin is the most commonly prescribed drug for the treatment of type 2 diabetes, and the only anti-diabetic therapy that has been demonstrated to attenuate the cardiovascular complications of diabetes and metabolic syndrome. It also helps to reduce LDL cholesterol and triglyceride levels in the blood and improves insulin sensitivity. We will conduct a short-term inhalation study designed to determine if Metformin will protect diabetes-prone and hyperglycemic KKAy mice from O3-induced insulin resistance and O3-induced rhinitis and pneumonitis.
3. **Study to assess autonomic changes in rats exposed to ozone or PM2.5 by microneurographic recording.** We will measure direct sympathetic nerve activity in rats pre-exposed to ozone or PM2.5. In these studies we will administer sympathomimetic and vasoactive agents to determine the association of changes in sympathetic activity with blood pressure in exposed versus nonexposed rats, and in rats fed normal versus high fructose diets.

Collaborative Research Effort with Other Clean Air Research Centers.

Toxicity of Traffic-Based Air Pollution in Rats with Diet-Induced Cardiometabolic Syndrome: In the Fall of 2012, investigators from GLACIER and Harvard University CLARC initiated an intraCLARC collaborative toxicology study under the principal direction of Drs. Jack Harkema (GLACIER) and John Godleski (Harvard University). Using our established high fructose-diet-fed rat model of CMS, the collaborative study was designed to determine if this dysfunctional cardiometabolic condition predisposes to the toxic effects of traffic-related air pollution and to identify underlying toxicological modes of action by which this may occur. Our goal is to discern if CMS renders the laboratory animal more susceptible to the cardiovascular, autonomic and airway toxicity of a multipollutant mixture of primary particles and secondary organic aerosols derived from traffic emissions in the Boston Tunnel. Data analysis is still underway and results from this study will be compared to similar studies in GLACIER's Project 2. A brief description of the study design is presented below.

Animals: Twelve 200 gram Sprague-Dawley rats were obtained from Taconic Farms with implanted DSI telemeters capable of monitoring blood pressure, heart rate, and temperature. Another 36 animals were obtained from Taconic Farms without telemeters. All rats were fed a high fructose diet (Harlan TD.89247; 60% of calories comes from fructose) for 8 weeks prior to use in any experiments. The high fructose diet was provided by the GLACIER CLARC. Inhalation exposures were conducted at Harvard's Boston Tunnel site (see below). Rats were weighed weekly, and 4 hrs of continuous data was collected from the telemeters in the animals at two week intervals. Rats were without food or water during the 5 hours of daily exposure, but when returned to their housing during nonexposure hours they were fed their specified diets.

Traffic-Related Urban Aerosol Particles (TRUAP) exposure protocol: Rats were continuously exposed to TRUAP or filtered air (FA) in single-animal plethysmographs for 5 hrs/day. TRUAP inhalation exposures are derived from the real-time ventilation exhaust of a moderate traffic density tunnel (with small positive road grade, approximately 2°), in the northeast United States. TRUAP consists of primary and secondary traffic-derived fine and ultrafine particles (1nm to 2.5 μm (PM2.5)). Twelve (12) animals (6/group TRAUP; 6/group filtered air all with implanted telemeters continuously monitoring blood pressure, heart rate, and temperature) were exposed each day, four days/week for three consecutive weeks. Another group of animals without telemeters, will also be exposed to filtered air or TRAUP for studies as described below. In prior TRUAP studies, concentrations average approximately 50 μg/m3 (combined primary and secondary particles), with standard deviations less than 25% of the mean for any component; however during these Fall exposures the mass concentrations were unusually low averaging approximately 30 μg/m3. Exposures were initiated at the same time each day, limiting variability due to diurnal traffic patterns.

Outcomes: Analyses of the initial cardiopulmonary function analyses conducted by Dr. Godleski and his team are presented in detail in the Harvard CLARC annual progress report. In this coming year we at MSU will complete nose, lung, and liver histopathology and biochemical assays of serum insulin and tryglyceride levels. We will compare filtered air vs TRUAP exposures in rats on a high fructose diet which produces hypertension and other facets of the metabolic syndrome (e.g., insulin resistance, hyperglycemia, dyslipidemia). From the telemetered animals, Dr. Godleski's team will complete the following outcomes: 1) Cardiovascular parameters, including blood pressure, heart rate, heart rate variability, and facets of the ECG that are all derived from the telemetry system; Respiratory parameters including respiratory rate, times of inspiration, expiration, inspiratory pause, expiratory pause and relaxation, peak air flows during inspiration and expiration, average air flow during expiration, tidal and minute volumes, inspiratory duty cycle, and minute ventilation have been completed. In another group of non-telemetered animals, bronchoalveolar lavage fluid (BALF) was collected after 4 days of exposure and analyses were completed for total inflammatory cells and individual cell types (e.g., neutrophils) as well as total protein and ß-N-Acetyl-Glucuronidase. From the rats in which BAL was collected and analyzed, heart blood from was also collected and analyzed for complete blood counts and differentials, as well as blood chemistries (chem 17) including blood glucose, electrolytes, triglycerides, and hepatic function was assessed.

Anticipated Accomplishments in Year 5: We will finish the laboratory assays on all the rats, complete the statistical analyses, and prepare a joint report/manuscript for submission to EPA and to an appropriate scientific journal for publication, respectively.

Publications/Presentations (Cumulative Publications (**year 4 in bold)**):

Liu C, Bai Y, Xu X, Sun L, Wang A, Wang TY, Maurya SK, Periasamy M, Morishita M, Harkema J, Ying Z, Sun Q, Rajagopalan S. (2014) Exaggerated effects of particulate matter air pollution in genetic type II diabetes mellitus. Part Fibre Toxicol.;11(1):27. doi: 10.1186/1743-8977-11-27. PubMed PMID: 24886175.

Maiseyeu A, Yang HY, Ramanathan G, Yin F, Bard RL, Morishita M, Dvonch JT, Wang L, Spino C, Mukherjee B, Badgeley MA, Barajas-Espinosa A, Sun Q, Harkema J, Rajagopalan S, Araujo JA, Brook RD. (2014) No effect of acute exposure to coarse particulate matter air pollution in a rural location on high-density lipoprotein function. Inhal Toxicol. 26(1):23-9. PubMed PMID: 24417404.

Brook RD, Bard RL, Morishita M, Dvonch JT, Wang L, Yang HY, Spino C, Mukherjee B, Kaplan MJ, Yalavarthi S, Oral EA, Ajluni N, Sun Q, Brook JR, Harkema J, Rajagopalan S. (2014) Hemodynamic, autonomic, and vascular effects of exposure to coarse particulate matter air pollution from a rural location. Environ Health Perspect. 2014 Jun;122(6):624-30. doi: 10.1289/ehp.1306595. Epub. PubMed PMID: 24618231.

Morishita M, Bard RL, Kaciroti N, Fitzner CA, Dvonch T, Harkema JR, Rajagopalan S, Brook RD. (2014) Exploration of the composition and sources of urban fine particulate matter associated with same-day cardiovascular health effects in Dearborn, Michigan. J Expo Sci Environ Epidemiol. doi:10.1038/jes.2014.35. [Epub ahead of print] PubMed PMID: 24866265.

Wagner JG, Kamal AS, Morishita M, Dvonch JT, Harkema JR, Rohr AC. (2014) PM2.5-induced cardiovascular dysregulation in rats is associated with elemental carbon and temperature-resolved carbon subfractions. Part Fibre Toxicol;11(1):25. PubMed PMID: 24885999.

Ying Z, Xu X, Bai Y, Zhong J, Chen M, Liang Y, Zhao J, Liu D, Morishita M, Sun Q, Spino C, Brook RD, Harkema JR, Rajagopalan S. (2014) Long-term exposure to concentrated ambient PM2.5 increases mouse blood pressure through abnormal activation of the sympathetic nervous system: a role for hypothalamic inflammation. Environ Health Perspect. 122(1):79-86. PubMed PMID: 24240275.

Wagner JG, Allen K, Yang HY, Nan B, Morishita M, Mukherjee B, Dvonch JT, Spino C, Fink GD, Rajagopalan S, Sun Q, Brook RD, Harkema JR. (2014) Cardiovascular depression in rats exposed to inhaled particulate matter and ozone: effects of diet-induced metabolic syndrome. Environ Health Perspect;122(1):27-33. PubMed PMID: 24169565.

Liu C, Xu X, Bai Y, Wang TY, Rao X, Wang A, Sun L, Ying Z, Gushchina L, Maiseyeu A, Morishita M, Sun Q, Harkema JR, Rajagopalan S. (2014) Air pollution-mediated susceptibility to inflammation and insulin resistance: influence of CCR2 pathways in mice. Environ Health Perspect; 122(1):17-26. doi:10.1289/ehp.1306841. Epub 2013 Sep 27. PubMed PMID: 24149114.

Sun L, Liu C, Xu X, Ying Z, Maiseyeu A, Wang A, Allen K, Lewandowski RP, Bramble LA, Morishita M, Wagner JG, Dvonch JT, Sun Z, Yan X, Brook RD, Rajagopalan S, Harkema JR, Sun Q, Fan Z. (2013) Ambient fine particulate matter and ozone exposures induce inflammation in epicardial and perirenal adipose tissues in rats fed a high fructose diet. Part Fibre Toxicol;10(1):43. PubMed PMID: 23968387.

Brook RD, Bard RL, Kaplan MJ, Yalavarthi S, Morishita M, Dvonch JT, Wang L, Yang HY, Spino C, Mukherjee B, Oral EA, Sun Q, Brook JR, Harkema J, Rajagopalan S. (2013) The effect of acute exposure to coarse particulate matter air pollution in a rural location on circulating endothelial progenitor cells: results from a randomized controlled study. Inhal Toxicol. 25(10):587-92. doi: 10.3109/08958378.2013.814733. Epub 2013 Aug 6. PubMed PMID: 23919441.

Liu C, Ying Z, Harkema J, Sun Q, Rajagopalan S. (2013) Epidemiological and experimental links between air pollution and type 2 diabetes. Toxicol Pathol. 41(2):361-73. doi: 10.1177/0192623312464531. Epub 2012 Oct 26. Review.PubMed PMID: 23104765; PubMed Central PMCID: PMC3988529.

Brook RD, Xu X, Bard RL, Dvonch JT, Morishita M, Kaciroti N, Sun Q, Harkema J, Rajagopalan S. (2013) Reduced metabolic insulin sensitivity following sub-acute exposures to low levels of ambient fine particulate matter air pollution. Sci Total Environ. 448:66-71. doi: 10.1016/j.scitotenv.2012.07.034. Epub 2012 Aug 15. PubMed PMID: 22901427.

Balasubramanian P, Sirivelu MP, Weiss KA, Wagner JG, Harkema JR, Morishita M, Mohankumar PS, Mohankumar SM. (2013) Differential effects of inhalation exposure to PM2.5 on hypothalamic monoamines and corticotrophin releasing hormone in lean and obese rats. Neurotoxicology. 36:106-11PubMed PMID: 22426024.

Presentations (August 1, 2013-July 31, 2014):

1. *Oral (Invited).* Harkema JR. The Interface of Health Effects caused by Air Pollution and the Metabolic Syndrome. Fall 2013 Ohio Valley Society of Environmental Toxicology and Chemistry and the Michigan Society of Toxicology Regional Meeting, East Lansing, MI, September 20, 2013.
2. *Oral (Invited).* Harkema JR. Investigating the Health Effects of Air Pollution: Inhalation Toxicology and Animal Models of Chronic Human Disease. Henan University of Traditional Chinese Medicine, Zhengzhou, China, October 29, 2013.
3. *Oral (Invited).* Harkema JR. (Invited). The Inteface of Health Effects Caused by the Cardiometabolic Syndrome and Exposures to Air Pollutants. Seminar Series, The IEHS & Center for Urban Responses to Environmental Stressors, Wayne State University, Detroit, MI, December 5, 2013.
4. *Oral (invited).* Harkema, JR. Inhalation Studies: Challenges and Complexities. *The Toxicologist* 138(1): A5. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.
5. *Oral (invited).* Harkema, JR. Site-Specific Airway Pathology and Dosimetry of Inhaled Toxicants. *The Toxicologist* 138(1): A771. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.
6. *Oral (invited).* Allen K, Lewandowski R, Wagner JG, Harkema, JR. Inhalation Exposures to Ozone Induce Insulin Resistance and Pulmonary Pathology in Type II Diabetes-Prone Mice. *The Toxicologist* 138(1): A2324. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.
7. *Oral (invited).* Ong C, Allen K, Brandenberger C, Jackson-Humbles D, Bramble L, Lewandowski R, Wagner JG, Harkema, JR. Development of Eosinophilic Rhinitis and Nasal Tissue Remodeling in Mice Episodically Exposed to Ozone. *The Toxicologist* 138(1): A2328. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.
8. *Oral (invited).* Harkema, JR. Toxicology Pathology of the Respiratory System. Annual Meeting of the Latin American Society of Toxicologic Pathology. São Paulo, Brazil, April 13, 2014.
9. *Oral (invited).* Harkema, JR. Interface of Health Effects Caused by Air Pollution and the Metabolic Syndrome. Research Seminar, Biology Department of Calvin College, Grand Rapids, MI, April 25, 2014.
10. *Oral (invited).* Harkema, JR. The Intersection of Two Global Health Problems: Air Pollution and the Metabolic Syndrome. Meeting of the West Michigan Clean Air Action, Grand Rapids, MI, May 2, 2014.
11. *Oral (invited).* Harkema, JR. Inhalation Toxicology Studies in Air Pollution Research. Annual Meeting of the Alleghany-Erie Regional Chapter of the Society of Toxicology, Morgantown, WV, May 15, 2014.
12. *Oral (invited).* Harkema, JR. Nasal Toxicity of Inhaled Chemical Irritants. 8th Annual CounterACT Network Research Symposium, Denver, CO, June 19, 2014.
13. *Poster.* Wagner JG, Das R, Allen K, Morishita M, Nan B, Mukherjee B and JR Harkema (2014). Cardiovascular Depression During Inhalation Exposure To A Mixture Of Ozone And Rural Ambient Fine Particles (PM2.5) In Rats On A High Fructose Diet. *Am J Respir Crit Care Med* 189;2014:A1668. International Conference of the American Thoracic Society, San Diego, CA, May 2014.
14. *Poster.* Wagner JG, Kamal AS, Morishita M, Dvonch JT, Harkema JR, and AC Rohr (2014). PM2.5-induced Tachycardia and Hypertension in Rats Are Linked to Elemental Carbon and Specific Temperature-Resolved Carbon Subfractions. *The Toxicologist* 138(1): A1240. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.
15. *Oral (Invited).* Wagner JG. Cardiometabolic Interactions of Diet and Air Pollution: Field Studies with Multipollutant Atmospheres. *The Toxicologist* 138(1): A2071. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.
16. *Oral(Invited).* Wagner JG. Interface of Cardiovascular Health Effects and Exposures to Air Pollutant Mixtures caused by the Metabolic Syndrome. Invited Seminar, University of Washington, Environmental and Occupational Health Sciences Seminar Series, Seattle, WA, June 5, 2014.

Supplemental Keywords: inhalation toxicology, acute multipollutant exposures, high-fructose diet, rats, PM, ozone, cardiometabolic syndrome

Relevant Web Site: http://greatlakesairresearchcenter.org/

GLACIER PROJECT 3 (Year 4 Progress Report)

Date of Report: 7/31/14
EPA Agreement Number: RD83479701
Center Name and Internal Number: Great Lakes Air Research Center for Integrative Environmental Research (GLACIER)
Project Title: Long Term Metabolic Consequences of Exposures to Multipollutant Atmospheres in the Great Lakes Region
Investigators: Sanjay Rajagopalan (PI) and Qinghua Sun
Institution: The Ohio State University, Columbus, OH
Research Category: Air Quality and Toxics
Project Period: August 1, 2013 – July 31, 2014

Objectives of Research: We have recently demonstrated that short-term exposure to inhaled concentrated airborne particulate (CAP) matter <2.5μm ($PM_{2.5}$) results in components of cardiometabolic syndrome (CMS) including development of hypertension and insulin resistance. In this project, we hypothesize that chronic inhalation of CAP in conjunction with gaseous components such as ozone from distinct multipollutant atmospheres synergistically interacts with diet and genetic susceptibility to influence development of CMS. Project 3 is an integral component of the overarching theme of this center that primary air pollutants, fine PM ($PM_{2.5}$) and ozone (O_3), cause cardiometabolic health effects that are dependent on the local atmospheric multipollutant milieu, predisposing factors, and the interactive toxicity of multipollutant coexposure. The experiments proposed are natural extensions of human research outlined in Project 1 and acute experiments in Project 2 and will focus on conducting chronic inhalation toxicology studies in diet fed and genetic models of obesity/diabetes. In Aim 1, simultaneous chronic exposure to multipollutant CAP from two locations in Columbus, OH representing near-roadside/traffic or remotely transported/aged emissions will be examined in combination with high fat chow (HFC). The impact of CAP on glucose/insulin homeostasis, adipokines, insulin signaling, adipose and pulmonary inflammation and an analysis of dose dependence and CAP components most likely associated with these effects will be evaluated in diet sensitive (C57BL/6) and genetic models of Type II diabetes susceptibility (KKA/y). In Aim 2, we will investigate the effect of co-exposure of multipollutant CAP with ozone on the temporal development of insulin resistance and adipose/lung inflammation using the KKA/y model. We will assess dose response relationship of multipollutant-O_3 mixture on insulin resistance measures (HOMA-IR and IPGTT) and novel mediators of innate immune, pivotal in the development of metabolic derangement. Based on data from Aims 1 and 2, we will design experiments in Aim 3, which will help us assess chronic effects of multipollutant CAP in potentiating inflammatory monocyte activation and infiltration into tissue niches as a central mechanism for mediating adverse metabolic effects of CAP. Using state of the art multiple exposure systems available at OSU (OASIS-1 and OASIS-2) and MI in conjunction the resources available at the ECC including the use of several novel and novel high-time resolution exposure characterization methods, GLACIER offers an unprecedented opportunity to elucidate relevant mechanisms responsible for the effects of multipollutant CAP on the pathogenesis of insulin resistance and inflammation. The insights gleaned from the acute studies planned in Projects 1 and 2 in conjunction with chronic studies in Project 3, have significant public health ramifications and may eventually lead to policy changes to avert environmental exposure to $PM_{2.5}$.

Progress Summary/Accomplishments:
In our prior year we successfully conducted experiments to determine the temporal course of diabetes development in response to CAPS and reported these results in several manuscripts. During year 3 we began conducting detailed experiments in collaboration with Dr. Harkema on the effects of ozone exposure. We were interested in isolating the effects of ozone prior to conducting multi-pollutant exposures in combination with CAPS which we had proposed performing as part of Aim 3. We hypothesized that ozone induces rapid effects on the cardiovascular system and proceeded to investigate this in a murine model of Type II diabetes. Inhalation exposure to O3 was conducted in whole-body exposure chambers, and mice were exposed to nominal ozone concentrations of 0 (filtered room air), or 0.5 ppm, for 8 h/d, 7 d/wk for 13 wk (n = 8/group). Ozone was generated with two OREC Model OZONEV1-O ozonizers (Ozone Research and Equipment Corp., Phoenix, AZ), with compressed air used as a source of oxygen. The concentration of ozone within the chambers was monitored throughout the exposure with three Dasibi 1003 AH ambient-air ozone monitors (Dasibi Environmental Corp., Glendale, CA). The air-sampling probes were placed in the breathing zone of the rats. The chamber ozone concentration was automatically maintained through a computer-controlled closed-loop feedback system, which adjusted the amount of ozone delivered to the chamber through remotely controlled mass-flow valves.

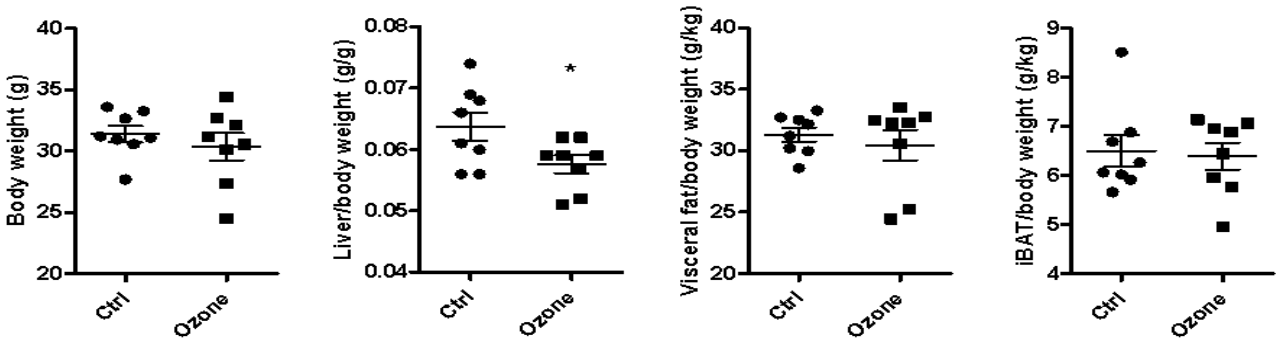


Figure 1. Depicts the effect of ozone exposure on body weight. After three weeks of inhalation exposure to control air or ozone, mice were sacrificed. The weights of whole body and organs were recorded. While the weights of whole body (A), visceral fat (C), and brown adipose tissue (D) were not significantly different between control and ozone-exposed groups, the weight of liver (B) in ozone-exposed group was significantly reduced.

Inhalational exposure to ozone over the short term resulted in infiltration of pro-inflammatory macrophages in the adipose tissue. These results are depicted in FIGURE 2.

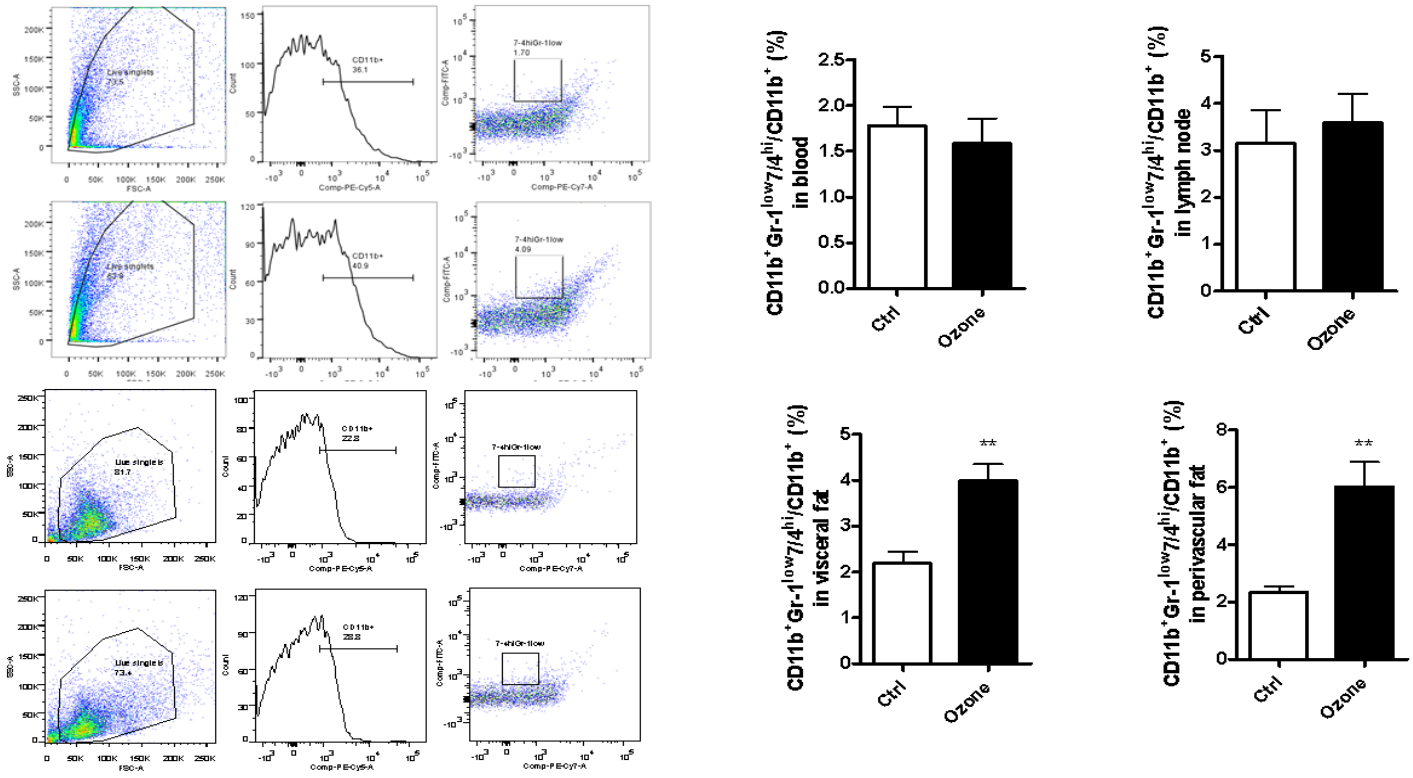


FIGURE 2. Macrophage activation in response to ozone exposure. Cells were isolated from blood (A and B), mediastinal lymph nodes (C), visceral adipose tissues (D and E), and aortic peri-vascular adipose tissues. The richness of pro-inflammatory (CD11b+Gr-1low7/4hi) macrophages were analyzed with flow cytometry. Results show that while inhalation exposure to ozone did not increase the richness of pro-inflammatory macrophages in blood (B) and lymph nodes (C), it significantly increased the infiltration of pro-inflammatory macrophages in visceral (E) and aortic peri-vascular (F) adipose tissues.

Interestingly these pro-inflammatory changes in adipose tissue were not paralleled by alteration in T cell subsets either in the mediastinal lymph nodes (data not shown) or in the visceral adipose tissue (FIGURE 3). In distinct contrast to the effects on macrophages in adipose, ozone exposure resulted in improvement in vascular function as evidenced by an improvement in response to endothelial dependent agonist acetylcholine and a reduction in vasoconstrictor response to phenylephrine. There results were accompanied by a reduction in pro-inflammatory gene expression in the vessel wall (aorta). The conclusion of this experiment were that inhalation exposure to O3 has minimal effects on markers of T cell activation in adipose but increases the content of CD11b+Gr-1low7/4hi cells.

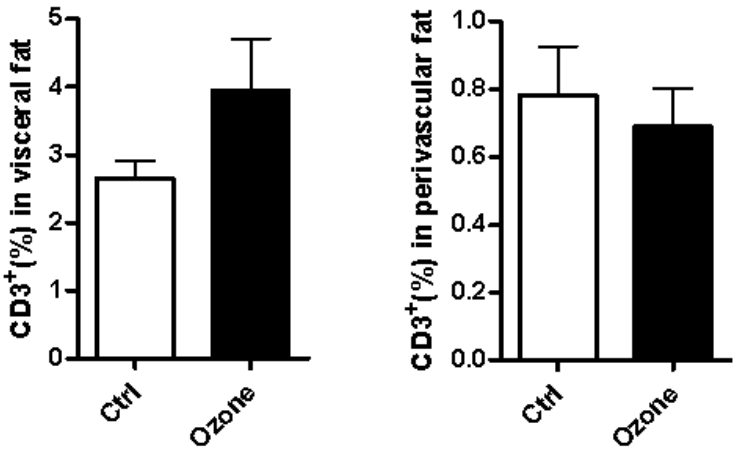


FIGURE 3. The effect of ozone exposure on T cell subsets in adipose tissue. Since T cells regulate innate immune inflammatory response, and given that ozone exposure increased pro-inflammatory macrophage infiltration in adipose we investigated CD3+ T cell content in peri-vascular fat and compared this with visceral adipose tissue. Stromal vascular cells were prepared from visceral adipose tissues and peri-vascular followed by profiling with flow cytometry.

O3 exposure appears to have effects on endothelial function that appear paradoxical to its pro-inflammatory effects in the lung. These results suggest complex effects of O3 systemically, further suggesting that combination of O3 with PM may have effects that may be hard to predict based on their individual effects on vascular and inflammatory phenotype. There was minimal evidence of inflammatory gene expression in the vasculature when we assessed this by RT-PCR (data not shown).

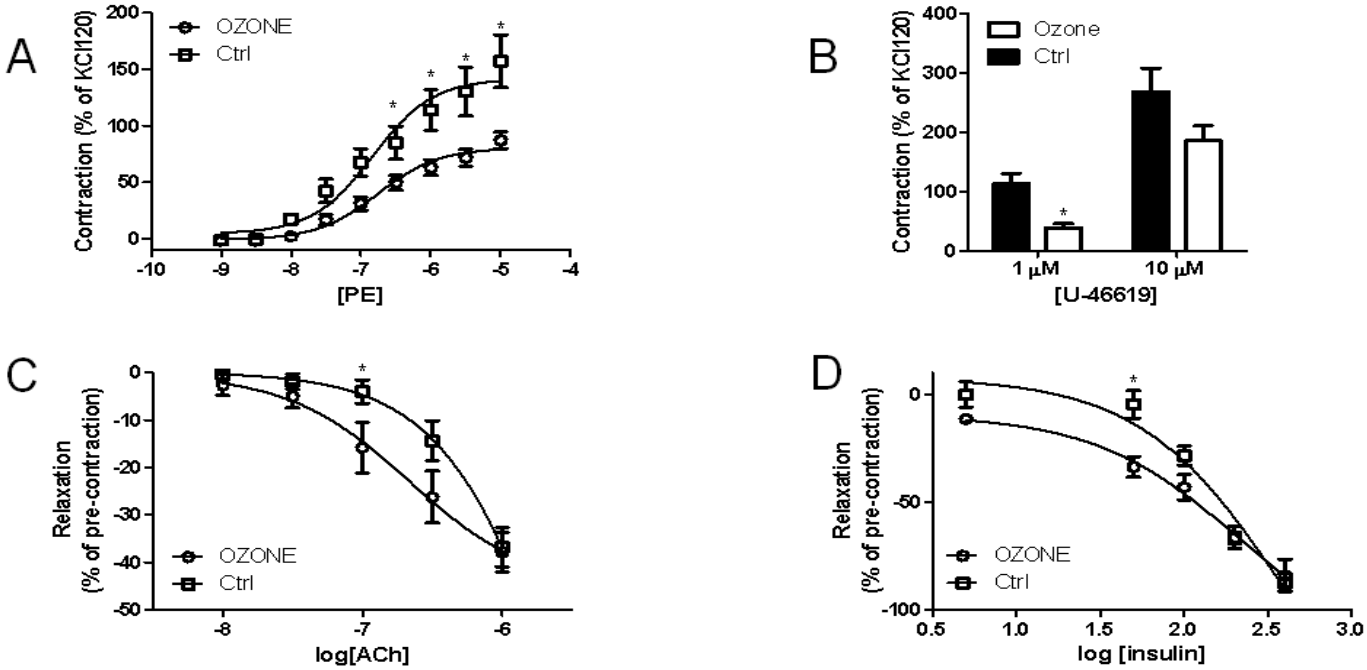


FIGURE 4. The effect of ozone exposure on vascular function. Thoracic aorta were isolated and mounted onto myograph. A, Dose response to phenylephrine; B, Dose response to U-46619; C, Dose response to Ach following pre-constriction with aphenylephrine (1 mM), D, Dose response to acetylcholine of aortic rings pre-contracted with phenylephrine (1 vs Ctrl. Twoway ANOVA.

Publications/Presentations:

Liu C, Bai Y, Xu X, Sun L, Wang A, Wang TY, Maurya SK, Periasamy M, Morishita M, Harkema J, Ying Z, Sun Q, Rajagopalan S. (2014) Exaggerated effects of particulate matter air pollution in genetic type II diabetes mellitus.. Part Fibre Toxicol. 11:27. PMID: 24886175.

Liu C, Xu X, Bai Y, Wang TY, Rao X, Wang A, Sun L, Ying Z, Gushchina L, Maiseyeu A, Morishita M, Sun Q, Harkema JR, Rajagopalan S. (2014) Air pollution-mediated susceptibility to inflammation and insulin resistance: influence of CCR2 pathways in mice. Environ Health Perspect. 122(1):17-26. PMID: 24149114.

Sun L, Liu C, Xu X, Ying Z, Maiseyeu A, Wang A, Allen K, Lewandowski RP, Bramble LA, Morishita M, Wagner JG, Dvonch J, Sun Z, Yan X, Brook RD, Rajagopalan S, Harkema JR, Sun Q, Fan Z. (2013) Ambient fine particulate matter and ozone exposures induce inflammation in epicardial and perirenal adipose tissues in rats fed a high fructose diet. Part Fibre Toxicol. 10:43. PMID: 23968387.

Mendez R, Zheng Z, Fan Z, Rajagopalan S, Sun Q, Zhang K. (2013) Exposure to fine airborne particulate matter induces macrophage infiltration, unfolded protein response, and lipid deposition in white adipose tissue. Am J Transl Res. 5(2):224-34. PMID: 23573366

ABSTRACTS

Ying Z, Xu X, Zhong, J, Bai, Y, Morishita M, Sun Q, Spino C, Brook RD, Harkema JR, and Rajagopalan, S. The Effects of Inhalation Exposure to Ozone on Inflammation and Vascular Function. Presented at the Annual CLARC meeting in Detroit, MI.

Future Activities

All aspects of the study protocol are approved by our IACUC. We anticipate beginning our CAPS + Ozone exposure this year.

Summary

During this project cycle we have generated the following new findings and contributed to further understanding of the link between PM2.5 and inflammation

1. Began exposures in a near roadway site and performed characterization of the exposure milieu.
2. CAP exaggerated Type II DM development in a genetic model with effects evident within 5 weeks including decreased thermogenesis, increased peripheral inflammation.
3. PM2.5 exposure caused rapid effects (in KKay) of hypothalamic inflammation.
4. Centrally inhibition of IKKβ but not TNFα blockade showed improvement in insulin resistance and metabolism.

Supplemental Keywords: Ozone (O_3) $PM_{2.5}$, Type II Diabetes Mellitus (DM), Insulin Resistance (IR)

Relevant Web Sites: http://greatlakesairresearchcenter.org/

GLACIER EXPOSURE CORE (Year 4 Progress Report)

Date of Report: 7/31/14
EPA Agreement Number: RD83479701
Center Name: Great Lakes Air Research Center for Integrative Environmental Research (GLACIER)
PROJECT TITLE: Exposure Characterization Core
Investigators: J. Timothy Dvonch, Masako Morishita
Institutions: University of Michigan
Research Category: Air Quality
Project Period: August 1, 2013 – July 31, 2014

Objective of Research: The GLACIER Air Pollution Center includes three projects to assess the impacts of air pollution exposure at field study locations with specific types of air pollution emission source impacts. Our Center's overall hypothesis is that primary air pollutants, fine particulate matter ($PM_{2.5}$) and ozone (O_3), are 1) capable of causing important adverse health effects that are 2) dependent on the local combinations of air pollutants, 3) a person's pre-existing health condition, and 4) the interactive adverse effects of exposure to both $PM_{2.5}$ and O_3. As part of GLACIER, our Exposure Characterization Core (ECC) coordinates with and supports GLACIER Projects 1-3 to provide measurements of air pollutant exposure.

Our previous work in human population studies, as well as human and animal studies of exposure to concentrated ambient particles (CAP) and ozone have demonstrated the critical importance of complete exposure characterization. Several of these studies have identified adverse health effects related to metabolic syndrome such as development of high blood pressure due to specific components of air pollution, as well as specific sources of air pollution. Because ambient PM is currently regulated on a mass basis, assuming all particles equally impact health, it is clear that in order to determine the most effective way in which to regulate PM and ensure that reductions in PM do in fact improve human health, additional studies are required.

The proposed ECC is designed to investigate the components and sources of air pollution prominent across the Great Lakes region that are responsible for adverse health effects. The ECC is highly innovative in design by virtue of the use of mobile ambient particle concentrators coupled with mobile toxicological laboratories to evaluate the acute health effects of air pollution dominated by different chemical components and sources. These mobile labs will be stationed in three communities in Michigan (Detroit, Dearborn, and Dexter) for short-term exposure studies conducted in Projects 1 and 2, as well as two locations in Columbus, OH, for long-term exposure studies in Project 3. The ECC will specifically utilize these five exposure sites in Michigan and Ohio primarily impacted by (1) near-roadway motor vehicle emissions (two sites), (2) industrial point sources (one site), and (3) regionally transported air pollution (no local emission sources, two sites). The primary objectives of the ECC are to: characterize the chemical components of air pollution exposure, identify the sources of air pollution exposure, and assess the air pollution components and sources responsible for the adverse cardiometabolic responses observed for each of Projects 1-3.

Progress Summary/Accomplishments: There have been no changes in ECC core investigators. To date in Year 3 of the ECC core, after successfully completing the human exposures for Project 1 at our rural Dexter, MI site for all subjects (including 2 exposures per subject, coarse CAP and filtered air), we have transition our mobile exposure facility to the urban/industrial Dearborn, MI site, and now continue with human exposures as part of Project 1 at this location. In addition to the field studies, sample laboratory analysis and data analyses for PM mass and associated components are ongoing.

After completing the first phase of acute animal exposures for Project 2, which took place at the Dearborn, MI site, we have now begun the next phase of studies for Project 2 at our Dexter, MI site. These field component of these studies are ongoing May through August, 2013. Exposure characterization for these exposure studies includes operation of the SEAS aerosol slurry sampler mobile laboratory, as well as the EPA collaborative components of GLACIER: the EPA Chemvol Tox mobile lab and Tisch samplers for organic speciation. Laboratory analysis and data analyses for PM mass and associated components for these exposure studies are ongoing.

Last, chronic animal exposures and associated exposure characterization as part of Project 3 continue at the Columbus, OH exposure sites. Data and sample collection and analysis are ongoing as part of these efforts.

Publications/Presentations:

Brook RD, Bard RL, Kaplan MJ, Yalavarthi S Morishita M, Dvonch JT, Wang L, Yang H-Y, Spino C, Mukherjee B, Oral EA, Sun Q, Brook JR, Harkema J, Rajagopalan S. (2013) The effect of acute exposure to coarse particulate matter in a rural location on circulating endothelial progenitor cells: Results from a randomized controlled study. Inhal Toxicol. 25(10):587-92.doi: 10.3109/08958378.2013.814733. Epub 2013 Aug 6. PMID:23919441.

Brook RD, Bard RL, Morishita M, Dvonch JT, Wang L, Yang H, Spino C, Mukherjee B, Kaplan M, Yalavarthi S, Oral E, Ajluni N, Sun Q, Brook J, Harkema J, Rajagopalan S. (2014) The Hemodynamic and Vascular Effects of Acute Exposure to Coarse Particulate Matter Air Pollution in a Rural Location. Inhal Toxicol. 25.(10):587-92. PMID:23919441

Brook RD, Xu X, Bard R, Dvonch JT, Morishita M, Kaciroti N, Sun Q, Harkema J, Rajagopalan S. (2013) Reduced metabolic insulin sensitivity following sub-acute exposures to low levels of ambient fine particulate matter air pollution. Sci. Total Environ. 448:66-71. PMID:22901427

Pancras JP, Landis MS, Norris GA, Vedantham R, Dvonch JT. (2013) Source apportionment of ambient fine particulate matter in Detroit, Michigan, using hourly resolved PM chemical composition data. Sci. Total Environ. 448:2-13. PMID:23302684

Sun K, Liu C, Xu X, Ying Z, Maiseyeu A, Wang A, Allen K, Bramble LA, Morishita M, Wagner JA, Dvonch JT, Sun Z, Yan X, Brook RD, Rajagopalan S, Sun Q, Fan Z, Harkema JR. (2013) Ambient fine particulate matter and ozone exposures induce inflammation in epicardial and perirenal adipose tissues in rats fed a high fructose diet. Part Fibre Toxicol. 10(1):43. PMID:23968387

Maiseyeu A, Yang H-y; Ramanathan G, Yin F, Bard RL, Morishita M, Dvonch JT, Wang L; Spino C, Mukherjee B, Badgeley MA, Barajas-Espinosa A, Sun MD Q, Harkema J, Rajagopalan S, Araujo JA, Brook RD. (2014) No effect of acute exposure to coarse particulate matter air pollution in a rural location on high density lipoprotein function. Inhal Toxicol; 26: 23-29. PMID:24417404

Morishita M, Bard RB, Kaciroti N, Fitzner C, Dvonch JT, Harkema JR, Rajagopalan S. Brook RD. (2014) Exploration of the Composition and Sources of Urban Fine Particulate Matter Associated with Same-Day Cardiovascular Health Effects in Dearborn, Michigan. Journal Exposure Science and Environmental Epidemiology; doi:10.1038/jes.2014.35. PMID:24866265

Morishita M, Dvonch T. (2012) Source identification of ambient PM2.5 for inhalation exposure studies in Dearborn, Michigan using highly time-resolved measurements. Presented at: Clean Air Research Centers Annual Meeting. June 21-22, 2012 - Cambridge, Massachusetts.

Hotchkiss IP, Allen K, Wagner JG, Morishita M, Lewandowski RP, Bramble LA, Dvonch JT, Harkema JR. (2012) Effects of high-fructose diet on nasal epithelial and inflammatory responses to inhaled ozone and ambient fine particles in rats. The Toxicologist. 126(S2):A229.

Allen K, Kopec AK, Zacharewski TR, Wagner JG, Morishita M, Dvonch JT, Harkema JR. (2012) Metabolic changes from high-fructose feeding are altered by air pollutant exposure. The Toxicologist. 126(S2):A235.

Wagner JG, Kamal AS, Morishita M, Dvonch JT, Harkema JR, Rohr AC. (2014) PM2.5-induced cardiovascular dysregulation in rats is associated with elemental carbon and temperature-resolved carbon subfractions. Part Fibre Toxicol. 11(1):25. PubMed PMID: 24885999.

Wagner JG, Allen K, Yang HY, Nan B, Morishita M, Mukherjee B, Dvonch JT, Spino C, Fink GD, Rajagopalan S, Sun Q, Brook RD, Harkema JR. (2014) Cardiovascular depression in rats exposed to inhaled particulate matter and ozone: effects of diet-induced metabolic syndrome. Environ Health Perspect. 122(1):27-33. PubMed PMID: 24169565.

Wagner JG, Kamal AS, Allen K, Morishita M, Dvonch JT, Lewandowski RP, Fink GD, Harkema JR. (2012) Hypotensive and bradycardic responses to inhaled O3 and ambient fine particles are enhanced in rats on a high-fructose diet. The Toxicologist. 126(S2):A863.

Morishita M, Wagner J, Dvonch T, Keeler G, Harkema J, Rohr A. (2011) Source identification of ambient PM2.5 for inhalation exposure studies in Dearborn, Michigan using highly time-resolved measurements. Presented at: International Society of Exposure Science, 21st Annual Conference - Advancing Exposure Science for Environmental Health. October 23-27, 2011 - Baltimore, Maryland.

Future Activities: Exposure characterization in support of human exposures for Project 1 will continue at the urban/industrial Dearborn, MI through 2013. Exposure characterization activities in support of animal exposures for Project 2 are ongoing at the rural Dexter site in and are scheduled for completion in August 2013, with associated lab activities continuing through Year 3 and into Year 4 of the project. Chronic animal exposures and associated exposure characterization as part of Project 3 are ongoing at the Columbus sites, and will continue through 2013 and into Year 4 of the project. Data and sample collection and analysis are ongoing as part of these efforts. In the current Year 3 and more so into Year 4, the large lab-based analytical effort currently underway with specific focus on chemical characterization of collected exposure samples and associated data processing and QA will develop into further drafting of additional conference presentations and associated publications.

Supplemental Keywords: air toxics, metals exposure, SEAS

Relevant Web Sites: http://greatlakesairresearchcenter.org/

GLACIER BIOSTATISTICS AND DATA MANAGEMENT (Year 4 Progress Report)

Date of Report: 7/31/14
EPA Agreement Number: RD83479701
Center Name: Great Lakes Air Research Center for Integrative Environmental Research (GLACIER)
Project Title: Biostatistics and Data Management Core
Investigators: Bhramar Mukherjee and Cathie Spino
Institutions: University of Michigan
Research Category: Air Quality and Toxics
Project Period: August 1, 2013 – July 31, 2014

Objective of Research: The overarching thesis of the EPA Great Lakes Air Center for Integrated Environmental Research (GLACIER) is that the primary air pollutants, fine particulate matter ($PM_{2.5}$) and ozone (O_3), cause cardiometabolic health effects that are dependent on 1) the local multipollutant atmospheric milieu, 2) the individual's pre-existing cardiovascular and metabolic condition, and 3) the interactive toxicity of $PM_{2.5}$ and O_3 coexposure. The objectives of the GLACIER Biostatistics and Data Management Core (BDMC) are to provide guidance for the statistical design and analysis of studies and data management services that allow for the integration of the data into a single platform that facilitates timely analysis for GLACIER investigators and projects. In the modern era of environmental sciences research, research investigators are generating a vast amount of data on multiple pollutants, health outcomes with the goal of studying the health effects caused by an ensemble of pollutants. Although our understanding of the mechanisms in which multipollutant mixtures cause/affect/mediate chronic diseases remains in its infancy, a major goal of this core is to equip the investigators of GLACIER to face the challenges in exploring the intricacies of statistical modeling for mixtures of pollutants. The Core, consisting of biostatisticians, experienced with working on problems relevant to environmental health sciences, will equip the investigator with state of the art statistical modeling techniques to understand the generated data.

In addition, essential attention to data collection and management activities prior to and during the conduct of studies in a platform that allows collaborative interchange between the scientists at the source of the data and statisticians at the analytic end of the data provides the ability to analyze complex and rich data more fully and efficiently at the close of each study.

Progress Summary/Accomplishments: The Biostatistics and Data Management Core continues to collaborate with investigators from Projects 1 to 3 to QC their data and analyze studies. Five papers have been published during this reporting period, including a methodological paper partially supported by GLACIER and led by Biostatistics and Data Management Core PI, Dr. Bhramar Mukherjee. During this project period, we have incorporated exposure data from the Exposure Core for several of the projects. The Core statisticians met with members of the Exposures Core on several occasions to discuss the intricacies of the data and to learn about past techniques that have been used in the analyses of these data. Our goal is to have common methods to deal with the composition of particulate matter data across all projects in GLACIER. These efforts, led by Dr. Lu Wang of the Biostatistics and Data Management Core and Dr. Masako Morishita of the Exposure Core, have resulted in a manuscript that incorporates exposure data with other Project 2 outcomes; it is in revision at the Journal of Exposure Science and Environmental Epidemiology. Dr. Bin Nan of our Core and the Core's graduate assistant have also analyzed the exposure trace element and particulate matter composition data for Project 2's 815-816 study. A manuscript is in preparation. Dr. Bhramar Mukherjee returned from her sabbatical and provided statistical support for the investigators on Project 3.

Given the additional efforts needed for analyses, a decision was made to transfer support of the public access website (http://greatlakesairresearchcenter.org/) from this Core to the Administrative Core and to cease efforts on the internal website deposition of study information and data. The Core continues to perform QC efforts of datasets received from investigators and the Exposure Core, including assessment of outliers, data errors and distributions via graphical and numeric summaries of all data. QCed data and summaries are stored in a shared drive that is used by all Biostatistics and Data Management Core statisticians.

Publications/Presentations:

Maiseyeu A, Yang HY, Ramanathan G, Yin F, Bard RL, Morishita M, Dvonch JT, Wang L, Spino C, Mukherjee B, Badgeley MA, Barajas-Espinosa A, Sun Q, Harkema J, Rajagopalan S, Araujo JA, Brook RD. (2014) No Effect of Acute Exposure to Coarse Particulate Matter Air Pollution in a Rural Location on High-density Lipoprotein Function. Inhalation Toxicology, 26: 23-9.

Brook R, Bard R, Morishita M, Dvonch JT, Wang L, Yang H, Spino C, Mukherjee B, Kaplan M, Yalavarthi S, Oral E, Ajluni N, Sun Q, Brook J, Harkema J and Rajagopalan S. (2014) The Hemodynamic and Vascular Effects of Acute Exposure to Coarse Particulate Matter Air Pollution in a Rural Location. Environmental Health Perspectives, 122:624-30.

Brook RD, Bard RL, Kaplan MJ, Yalavarthi S, Morishita M, Dvonch JT, Wang L, Yang HY, Spino C, Mukherjee B, Oral EA, Sun Q, Brook JR, Harkema J and Rajagopalan, S. (2013) The effect of acute exposure to coarse particulate matter air pollution in a rural location on circulating endothelial progenitor cells: results from a randomized controlled study. Inhalation Toxicology, 10:587-92.

Morishita M, Bard R, Wang L, Das R, Dvonch T, Spino C, Mukherjee B, Sun Q, Harkema J, Rajagopalan S and Brook, R. (2014) The Characteristics of Coarse Particulate Matter Air Pollution Associated With Alterations in Blood Pressure and Heart Rate During Controlled Exposures. Journal of Exposure Science and Environmental Epidemiology, in revision.

Wagner JG, Allen K, Yang HY, Nan B, Morishita M, Mukherjee B, Dvonch JT, Spino C, Fink GD, Rajagoplan S, Sun Q, Brook RD and Harkema JR. (2014) Cardiovascular Depression Caused by Exposures to Inhaled Particulate Matter and Ozone is Augmented in Rats Fed a High Fructose Diet, Environmental Health Perspective, 122:27-33.

Methodological research partially supported by GLACIER:

Sun Z, Tao Y, Li S, Ferguson KK, Meeker JD, Park SK, Batterman SA, Mukherjee B. (2013) Statistical Strategies for Constructing Health Risk Models with Multiple Pollutants and Their Interactions: Possible Choices and Comparison. Environmental Health, 12:85

Future Activities:

Below we summarize ongoing methodological and analytic activities targeted for the next reporting period.

Project 1 (PI Brook, Statistician Wang):
The investigators and Dr. Wang are working to complete all analyses of the Dexter data, resulting in 4 publications. We explored the element data as well. We are collecting and organizing data from Dearborn, and will analyze the Dearborn coarse CAP results in September.

Project 2 (PI Harkema, Statistician Nan): Dr. Nan and the Core graduate student have completed the analysis of diet-by-exposure factorial design in Dexter on the same endpoints related to cardiometabolic syndrome, using identical linear mixed effects models as done for Dearborn in the published paper by Wagner et al., EHP, 2014. The team then completed analysis of elements and source data for Dearborn and compared model selection techniques like adaptive LASSO and elastic net. The results are being included in a manuscript led by Dr. Wagner.

Project 3 (PI Rajagopalan, Statistician Mukherjee): The paper on Central IKKβ Inhibition Prevents Air Pollution Mediated Peripheral Inflammation and Exaggeration of Type II Diabetes is in submission. It is a diet-by-exposure interaction paper with endpoints related to cardiometabolic syndrome.

In addition, Dr. Spino will work with the Core data analyst to ensure that all analysis datasets are properly archived during the next reporting period.

Supplemental Keywords: biostatistics, data management, modeling of multipollutant mixtures, high dimensional correlated data

Relevant Web Sites: http://greatlakesairresearchcenter.org/

CENTER PUBLICATIONS:

Project 1

Brook RD, Bard RL, Morishita M, Dvonch JT, Wang L, Yang H-Y, Spino C, Mukherjee B, Kaplan MJ, Yalavarthi S, Oral EA, Ajluni N, Sun Q, Brook JR, Harkema J, Rajagopalan S. (2014) The hemodynamic, autonomic, and vascular effects of exposure to coarse particulate matter in a rural location. Environ Health Perspect; 122: 624-30. PMID:24618231

Maiseyeu A, Yang H-y; Ramanathan G, Yin F, Bard RL, Morishita M, Dvonch JT, Wang L; Spino C, Mukherjee B, Badgeley MA, Barajas-Espinosa A, Sun MD Q, Harkema J, Rajagopalan S, Araujo JA, Brook RD. (2014) No effect of acute exposure to coarse particulate matter air pollution in a rural location on high density lipoprotein function. Inhal Toxicol; 26: 23-29. PMID:24417404

Morishita M, Bard RB, Kaciroti N, Fitzner C, Dvonch JT, Harkema JR, Rajagopalan S. Brook RD. (2014) Exploration of the Composition and Sources of Urban Fine Particulate Matter Associated with Same-Day Cardiovascular Health Effects in Dearborn, Michigan. Journal Exposure Science and Environmental Epidemiology; doi:10.1038/jes.2014.35. PMID:24866265

Brook RD, Bard RL, Kaplan MJ, Yalavarthi S Morishita M, Dvonch JT, Wang L, Yang H-Y, Spino C, Mukherjee B, Oral EA, Sun Q, Brook JR, Harkema J, Rajagopalan S. (2013) The effect of acute exposure to coarse particulate matter in a rural location on circulating endothelial progenitor cells: Results from a randomized controlled study. Inhal Toxicol. 25(10):587-92. PMID:23919441

Brook RD, Xu X, Bard R, Dvonch JT, Morishita M, Kaciroti N, Sun Q, Harkema J, Rajagopalan S. (2013) Reduced metabolic insulin sensitivity following sub-acute exposures to low levels of ambient fine particulate matter air pollution. Sci. Total Environ. 448:66-71. PMID:22901427.

Project 2

Wagner JG, Kamal AS, Morishita M, Dvonch JT, Harkema JR, Rohr AC. (2014) PM2.5-induced cardiovascular dysregulation in rats is associated with elemental carbon and temperature-resolved carbon subfractions. Part Fibre Toxicol. 11(1):25. PubMed PMID: 24885999.

Wagner JG, Allen K, Yang HY, Nan B, Morishita M, Mukherjee B, Dvonch JT, Spino C, Fink GD, Rajagopalan S, Sun Q, Brook RD, Harkema JR. (2014) Cardiovascular depression in rats exposed to inhaled particulate matter and ozone: effects of diet-induced metabolic syndrome. Environ Health Perspect. 122(1):27-33. PubMed PMID: 24169565.

Balasubramanian P, Sirivelu MP, Weiss KA, Wagner JG, Harkema JR, Morishita M, Mohankumar PS, Mohankumar SM. (2013) Differential effects of inhalation exposure to PM (2.5) on hypothalamic monoamines and corticotrophin releasing hormone in lean and obese rats. Neurotoxicology. 36:106-111. PMID:22426024

Sun K, Liu C, Xu X, Ying Z, Maiseyeu A, Wang A, Allen K, Bramble LA, Morishita M, Wagner JA, Dvonch JT, Sun Z, Yan X, Brook RD, Rajagopalan S, Sun Q, Fan Z, Harkema JR. (2013) Ambient fine particulate matter and ozone exposures induce inflammation in epicardial and perirenal adipose tissues in rats fed a high fructose diet. Part Fibre Toxicol. 10(1):43. PMID:23968387

Project 3

Liu C, Bai Y, Xu X, Sun L, Wang A, Wang TY, Maurya SK, Periasamy M, Morishita M, Harkema J, Ying Z, Sun Q, Rajagopalan S. (2014) Exaggerated effects of particulate matter air pollution in genetic type II diabetes mellitus. Part Fibre Toxicol. 11(1):27. doi: 10.1186/1743-8977-11-27. PubMed PMID: 24886175.

Liu C, Xu X, Bai Y, Wang TY, Rao X, Wang A, Sun L, Ying Z, Gushchina L, Maiseyeu A, Morishita M, Sun Q, Harkema JR, Rajagopalan S. (2014) Air pollution-mediated susceptibility to inflammation and insulin resistance: influence of CCR2 pathways in mice. Environ Health Perspect. 122(1):17-26. doi:10.1289/ehp.1306841. Epub 2013 Sep 27. PubMed PMID: 24149114.

Ying Z, Xu, X, Bai Y, Zhong J, Chen M, Zhao J, Liu D, Morishita M, Sun Q, Harkema J, Rajagopalan S. (2013) Long-Term Concentrated Ambient PM2.5 Exposure Increases Blood Pressure through Abnormal Activation of Sympathetic Nervous System: A Role for Hypothalamic Inflammation. Environmental Health Perspectives. PMID:24240275.

Blazek A, Rutsky J, Osei K, Maiseyeu A, Sanjay Rajagopalan. (2013) Exercise-mediated changes in high-density lipoprotein: Impact on form and function. American Heart Journal. 166(3):392-400. PMID:24016485

Liu C, Ying Z, Harkema J, Sun Q, Rajagopalan S. (2013) Epidemiological and experimental links between air pollution and type 2 diabetes. Toxicol Pathol. 41(2):361-373. PMID:23104765.

Ying Z, Xu X, Chen M, Liu D, Zhong M, Chen LC, Sun Q, Rajagopalan S. (2013) A Synergistic Vascular Effect of Airborne Particulate Matter and Nickel in a Mouse Model. Toxicol Sci. [Epub] June 20, 2013. PMID:23788629.

Exposure Core

Pancras JP, Landis MS, Norris GA, Vedantham R, Dvonch JT. (2013) Source apportionment of ambient fine particulate matter in Detroit, Michigan, using hourly resolved PM chemical composition data. Sci. Total Environ. 448:2-13. PMID:23302684

CENTER PUBLICATIONS SUBMITTED AND IN REVIEW:

Morishita M; Bard RL; Dvonch JT; Wang L; Das R; Spino C; Mukherjee B; Sun Q; Harkema J; Rajagopalan S; Brook RD. (2014) The Components of Coarse Particulate Matter Air Pollution Associated with Alterations in Blood Pressure and Heart Rate during Controlled Exposures. Journal Exposure Science and Environmental Epidemiology (in press).

ABSTRACTS/ORAL PRESENTATIONS:

Allen K, Lewandowski R, Wagner JG, Harkema, JR. Inhalation Exposures to Ozone Induce Insulin Resistance and Pulmonary Pathology in Type II Diabetes-Prone Mice. The Toxicologist 138(1): A2324. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.

Brook RD. American Society of Hypertension national meeting New York, May 2014. "The Components of Coarse Particulate Matter Air Pollution Associated with Alterations in Blood Pressure and Heart Rate during Controlled Exposures".

Brook RD. Society of Toxicology Annual Meeting 2014. Pheonix Az. "Air pollution exposures and the cardio-metabolic syndrome".

Harkema JR. The Interface of Health Effects caused by Air Pollution and the Metabolic Syndrome. Fall 2013 Ohio Valley Society of Environmental Toxicology and Chemistry and the Michigan Society of Toxicology Regional Meeting, East Lansing, MI, September 20, 2013.

Harkema JR. Investigating the Health Effects of Air Pollution: Inhalation Toxicology and Animal Models of Chronic Human Disease. Henan University of Traditional Chinese Medicine, Zhengzhou, China, October 29, 2013.

Harkema JR. (Invited). The Inteface of Health Effects Caused by the Cardiometabolic Syndrome and Exposures to Air Pollutants. Seminar Series, The IEHS & Center for Urban Responses to Environmental Stressors, Wayne State University, Detroit, MI, December 5, 2013.

Harkema, JR. Inhalation Studies: Challenges and Complexities. The Toxicologist 138(1): A5. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.

Harkema, JR. Site-Specific Airway Pathology and Dosimetry of Inhaled Toxicants. The Toxicologist 138(1): A771. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.

Harkema, JR. Toxicology Pathology of the Respiratory System. Annual Meeting of the Latin American Society of Toxicologic Pathology. São Paulo, Brazil, April 13, 2014.

Harkema, JR. Interface of Health Effects Caused by Air Pollution and the Metabolic Syndrome. Research Seminar, Biology Department of Calvin College, Grand Rapids, MI, April 25, 2014.

Harkema, JR. The Intersection of Two Global Health Problems: Air Pollution and the Metabolic Syndrome. Meeting of the West Michigan Clean Air Action, Grand Rapids, MI. May 2, 2014.

Harkema, JR. Inhalation Toxicology Studies in Air Pollution Research. Annual Meeting of the Alleghany-Erie Regional Chapter of the Society of Toxicology, Morgantown, WV, May 15, 2014.

Harkema, JR. Nasal Toxicity of Inhaled Chemical Irritants. 8th Annual CounterACT Network Research Symposium, Denver, CO, June 19, 2014.

Ong C, Allen K, Brandenberger C, Jackson-Humbles D, Bramble L, Lewandowski R, Wagner JG, Harkema, JR. Development of Eosinophilic Rhinitis and Nasal Tissue Remodeling in Mice Episodically Exposed to Ozone. The Toxicologist 138(1): A2328. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.

Wagner JG, Das R, Allen K, Morishita M, Nan B, Mukherjee B and JR Harkema. Cardiovascular Depression During Inhalation Exposure To A Mixture Of Ozone And Rural Ambient Fine Particles (PM2.5) In Rats On A High Fructose Diet. Am J Respir Crit Care Med 189;2014:A1668. International Conference of the American Thoracic Society, San Diego, CA, May 2014.

Wagner JG, Kamal AS, Morishita M, Dvonch JT, Harkema JR, and AC Rohr. PM2.5-induced Tachycardia and Hypertension in Rats Are Linked to Elemental Carbon and Specific Temperature-Resolved Carbon Subfractions. The Toxicologist 138(1): A1240. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.

Wagner JG. Cardiometabolic Interactions of Diet and Air Pollution: Field Studies with Multipollutant Atmospheres. The Toxicologist 138(1): A2071. Annual Meeting of the Society of Toxicology, Phoenix, AZ, March 2014.

Wagner JG. Interface of Cardiovascular Health Effects and Exposures to Air Pollutant Mixtures caused by the Metabolic Syndrome. Invited Seminar, University of Washington, Environmental and Occupational Health Sciences Seminar Series, Seattle, WA, June 5, 2014.

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Carey SA, Ballinger CA, Plopper CG, McDonald RJ, Bartolucci AA, Postlethwait EM, Harkema JR. (2010) Persistent rhinitis and epithelial remodeling induced by cyclic ozone exposure in the nasal airways of infant monkeys. Am J Physiol Lung Cell Mol Physiol. 300(2):L242-54. PMCID:PMC3043815.

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Great Lakes Air Center for Integrative Environmental Research
An EPA Clean Air Research Center
Annual Center Financial Report
Period: August 1, 2013 to July 31, 2014

Overall Center Financial Overview

The Great Lakes Air Center for Integrative Environmental Research (GLACIER) has expended approximately 72% of the proposed budget as of July 31, 2014 leaving 28% unexpended to date. The planned use of anticipated carry-forward is described in the individual Project and Core reports below.

Research Project 1: Cardiometabolic Effects of Exposure to Differing Mixtures and Concentrations of $PM_{2.5}$ in Obese and Lean Adults (University of Michigan)
PI: Robert Brook, MD

Project 1 has expended approximately 52% of the proposed budget through July 31, 2014. Increased spending on research at the UM and by our sub-award, UCLA, will take place later in the year. We estimate that the project will be approximately 70% expended through December 31, 2014. Carry forward funds will be utilized as originally budgeted.

Research Project 2: Cardiometabolic, Autonomic, and Airway Toxicity of Acute Exposures to $PM_{2.5}$ from Multipollutant Atmospheres in the Great Lakes Region (Michigan State University)
PI: Jack Harkema, DVM, PhD

Project 2 has expended 85% of the proposed budget through July 31, 2013. Approximately 40% of the budget has gone towards personnel, with the remaining 60% being expended on travel and supplies/services. This Project has not incurred any other major unexpected expenses. We anticipate expending 100% by December 31, 2013.

Research Project 3: Long Term Metabolic Consequences of Exposures to Multipollutant Atmospheres in the Great Lakes Region (University of Maryland)
PI: Sanjay Rajagopalan, MD

Project 3 has expended approximately 35% of the proposed budget as of July 31, 2014. These expenses relate to personnel and other research materials and supply expenses. Along with the PI, Dr. Qinghua Sun, the co-investigator, works 5% in this project. This Project has not incurred any other major unexpected expenses and research spending should increase with the move to the University of Maryland final. By the end of the fiscal year we do not anticipate to have unspent funds exceeding 15%.

Exposure Characterization Core (University of Michigan)
PI: J. Timothy Dvonch, PhD

Since a significant amount of project funds were carried forward in the Exposure Characterization Core (ECC) from Year 2-3 to Year 4, the following financial expenditure information represents a combination of the ECC budgets for Year 1-4. The ECC was 80% expended through the period June 31, 2014. We project the ECC to be 92% expended through the period December 31, 2014, and anticipate carry-forward amounts for the Exposure Characterization Core into Year 5. It is anticipated that these carry-forward amounts will be distributed proportionately across the budget categories in Year 5, and will cover the increased ECC core laboratory analysis expenses and continued field-related expenses expected in Year 5.

Biostatistics and Data Management Core (University of Michigan)
PIs: Bhramar Mukherjee, PhD and Cathie Spino, ScD

Spending for this reporting period has been below funded levels, because public website support has been transferred to the GLACIER Administrative Core and the decision was made to cease updating the internal website with the derived datasets and descriptive statistics. The Core is approximately 80% expended through June 30, 2014 and we anticipate expending 95% by December 31, 2014.

Administrative Core (MSU)
PI: Jack Harkema, DVM, PhD

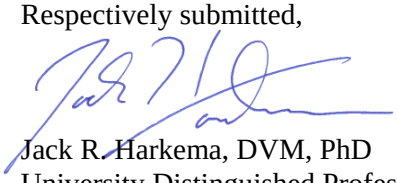
The Administrative Core has expended 67% of its budget through July 31, 2014. All personnel have been appointed and are working on the Core as proposed. A Scientific Advisory Committee (SAC) meeting is scheduled for October 2014 for which 9% of our budget has been set aside. Travel funds for investigators to attend the September 2014 Clean Air Research Center (CLARC) Annual Meeting in Atlanta, GA represents an additional 5% yet to be expended. By the end of the fiscal year, we anticipate having approximately 25% unspent, primarily in travel and meeting related expenses. The carry-forward will be used in Year 5 to offset anticipated increased costs in these categories.

Collaborative Research (MSU)
PI: Jack Harkema, DVM, PhD

Approximately $23,000 has been expended for a collaborative study with the Harvard Clean Air Research Center (Project 1; Dr. Godleski). This collaborative effort titled "**Toxicity of Traffic-Based Air Pollution in Rats with Diet-Induced Cardiometabolic Syndrome"** is focused on determining health effects of rats with and without diet-induced cardiometabolic syndrome that are exposed to traffic from the Boston Tunnel. MSU GLACIER funds for this effort have been supplies/services only. We have budgeted a total of $25,000 for this specific project that will be completed in year 4. In the remaining months of year 4 and in year 5, GLACIER will also continue collaborative efforts with the Center for Clean Air Research (CCAR) at the University of Washington titled **"Pulmonary and Systemic Inflammatory Potentials of Inhaled Ozone and Fine PM in Mice"**. We will budget $75,000 for this collaboration among Dr. Harkema at GLACIER and Dr. Campen at CCAR. For this effort, blood serum samples from both human subjects and laboratory rodents, exposed to various gaseous and particulate air pollutants, will be sent to Dr. Campen for in vitro analysis of blood-borne mediators of inflammation and specific endothelial responses. GLACIER funds for this effort will be for both personnel and supplies/services. In addition, Dr. Brook at GLACIER and Dr. Campen at CCAR will continue a collaborative study titled "**Circulating Inflammatory Potential of Inhaled Coarse PM"**. We will budget $25,000 towards this study.

For further information or clarification concerning this report, please contract Dr. Jack Harkema, GLACIER Center Director, at 517-353-8627 or harkemaj@msu.edu.

Respectively submitted,



Jack R. Harkema, DVM, PhD
University Distinguished Professor
Michigan State University
GLACIER, Center Director