Great Lakes Air Center for Integrative Environmental Research
An EPA Clean Air Research Center
Year 3 Progress Report
Period: August 1, 2012 - July 31, 2013

Introduction

This Annual Progress Report covers the activities in Year 3 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 cardiometabolic 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 3, there were no changes in our overall research objective or specific aims. There were two personnel changes in our Administrative Core. Our Center Administrator, Ms. Carol Chvojka, retired on January 2, 2013 and Ms. Amy Swagart was appointed as our new Center Administrator. Ms. Kasey Baldwin was hired to fill Amy's vacant position as our Center's Administrative Assistant. These staff changes did not result in any changes to the Center's overall budget, operations, and objectives. GLACIER's research projects/cores have made substantial progress since the last progress report. Oral and poster presentations on GLACIER research accomplishments and future endeavors were presented at the EPA CLARC Annual Meeting in Seattle, WA on July 25-26, 2013. In this document, separate written reports follow 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 on our intramural CLARC collaborative project with Harvard University is found in the report of Project 2.

GLACIER PROJECT 1 (Year 3 Progress Report)

Date of Report: 7/31/13
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, 2012 - July 31, 2013

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: There have been no changes in study investigators or personnel during years 1-3. As detailed in the year 2 report, the timeline and study protocol were modified to better overlap with the aims of projects 2-3. During years 1-2 (Project 1; study #1), the project was modified to perform human coarse CAP exposures at a rural site (Dexter MI) and an urban site (Dearborn MI).

From the time period ending at 6/30/13, 32 subjects completed the protocol at Dexter. Thirty subjects received exposures to both filtered air (FA) and rural coarse CAP at Dexter in a randomized blinded crossover fashion. Two subjects elected to not complete a second exposure due to personal issues unrelated to the study. Thus, we completed a total of 62 exposures at Dexter by the end of study year 2. During year 3 (6/30/12 to 7/31/13) we transported the coarse CAP

exposure facility (AirCARE-2) to Dearborn MI to begin urban coarse PM exposures. The facility was established at the site (e.g., electrical and power couplings) and the concentrator system was tested and validated as properly functioning prior to human studies. We began urban coarse CAP exposures after the end of winter season in April 2013. Exposures continued until the end of May 2013. Exposures were put on hold from June until Sept 2013 (planned date) as personnel were required to complete animal exposures for study 2 during this time. We completed exposures for 11 subjects at Dearborn during this time period.

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, 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. (In press)

Brook R, Bard R, Morishita M, Dvonch J.T, Wang L, Yang H, Spino C, Mukherjee B, Kaplan M, Yalavarthi S, Oral E, Ajluni N, Sun Q, Brook J, Harkema J, Rajagopalan S. (2013) The Hemodynamic and Vascular Effects of Acute Exposure to Coarse Particulate Matter Air Pollution in a Rural Location. January 25, 2013 MS ID#: HYPE201301122. (In Review)

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.

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. (In Review)

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. (2013) Cardiovascular dysregulation caused by inhalation exposures to PM2.5 and ozone is augmented in rats fed a high fructose diet. Environ Perspect. MS ID#: 13-07085-ART. (In Review)

Future Activities for year 4: We will recommence human exposures to urban coarse CAP at Dearborn in September 2013. To accord with the rural exposure study results, we will complete enrollment of a minimum of 32 subjects (receiving both urban CAP and filtered air exposures) during the period of Sept 2013 to early spring 2014. We will complete analyses of our study outcomes during this time period as well. We then begin study 2 in the summer of 2014 (start of year 5). In addition to the manuscripts listed above, we have 2 papers currently in review, and 2 additional papers in preparation as of July 2013. We will complete these papers and subsequently analyze and finish abstracts and manuscripts pertaining to urban coarse CAP during year 4.

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 3 Progress Report)

Date of Report: 7/31/13
EPA Agreement Number: RD83479701
Center Name: 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, 2012 - July 31, 2013

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 the study investigators or personnel in year 3 of this Project. Our objectives in Project 2 have also remained the same in Year 3. Specifically, 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 3 addressed all four of these hypotheses, and focused on field exposure studies during summer months, and was followed by mechanism-driven experiments during the winter months. 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 and 2 were 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 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 3 several research milestones were achieved that led directly to a better understanding of the health effects of multipollutant exposures in the face of experimentally induced facets of the cardiometabolic syndrome (CMS). Specifically, we 1) finished the statistical analyses of the data from a large inhalation 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, 2) used murine models of diet- and genetically induced CMS to investigate the effects of O3 exposure on the development of insulin resistance and other health facets of the CMS, and 3) started a new inhalation toxicology study in Dexter, MI to study the toxicity of PM2.5, O3 and PM2.5/O3 exposures in this rural/regional airshed. In addition, we started an intraCLARC collaborative animal toxicology study with Harvard University in the Fall of 2012 employing our rat model of diet-induced CMS and their inhalation exposure system to traffic emissions from the Boston Tunnel. A brief summary of our Project 3 accomplishments in the last year and future studies in Year 4 are briefly described below.

1) Results from our Rat Inhalation Toxicology Study in Dearborn MI. Sprague-Dawley rats fed a diet with 60% fructose-derived calories induced multiple facets of the human CMS. These rats were insulin-resistant, hypertensive and dyslipidemic, which are three major facets of the CMS (Figure 1A, B, C). In addition, high fructose (HFr) fed rats developed hepatic steatosis and elevated heart rate. As such, HFr-fed rat model demonstrated cardiovascular and metabolic abnormalities that are consistent with human CMS, and provided a reproducible rodent model to further elucidate the adverse health effects of multi-pollutant exposures in a potentially susceptible population suffering from CMS.

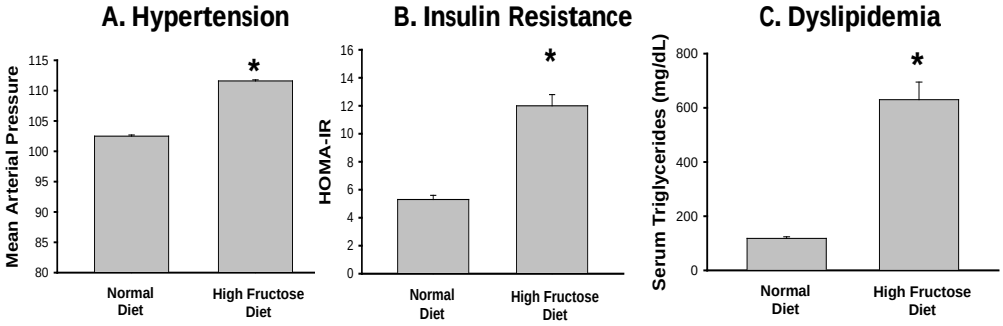


Figure 1. Changes in Blood Pressure (A), Insulin Resistance (B) and Serum Triglycedrides (C) in Sprague Dawely Rats after 10 weeks on a High Fructose Diet. * - indicates significant difference from rats fed a Normal Diet.

We conducted a series of acute inhalation exposures of rats, on normal or HFr-diets (HFrD), to O3 and/or concentrated PM2.5 (CAP), for 9 days, 8 h/day, in our mobile air research laboratory (AirCARE I) parked in an urban/industrial setting in Dearborn, MI. Two 9-day exposure regimens were conducted in order to acquire data from all experimental groups. Inhalation exposures were coordinated with Drs. Dvonch and Morishita in the Exposure Characterization Core at the University of Michigan. They characterized the physicochemical composition of both ambient and concentrated PM2.5 at this exposure site. Initiated by Drs. Harkema, Wagner and Fink (MSU-Project 2), the details of the study design and data and tissue collection protocols were finalized after consultation with Drs. Rajagopalan (Project 3) and Brook (Project 1), so as to ensure cohesion of endpoints in acute and chronic rodent and acute human studies. Acute cardiovascular responses (e.g., blood pressure, heart rate, heart rate variability) were measured every 5 minutes by radiotelemetry during and after daily exposures. Twenty-four hours after the last exposure, tissue samples were taken at necropsy from a wide-range of targeted organs, along with blood and bronchoalveolar lavage fluid samples, and analyzed for biochemical, molecular, and morphometric alterations related to the CMS.

This past year, 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 found that inhalation of either concentrated PM2.5 or O3 alone caused significant drops in mean arterial pressure (10-15 mmHg) in HFr-fed rats that were maintained for most days of the exposures (Figure 2A, B). These decreases persisted during post-exposure periods on the weekends (7:30AM-3:30PM) and during evening hours of weekdays (12:00-5:00AM) when rats were removed from the inhalation chambers. In comparison, vascular responses of rats fed a normal diet were minimal and less frequent, with episodic elevations in blood pressure on certain days. Interestingly, co-exposure to both pollutants caused marked decreases in blood pressure in HFr-fed rats on the first two

days of exposure, yet this vascular response did not persist with repeated exposures, as was observed with single pollutant exposures (Figure 2C).

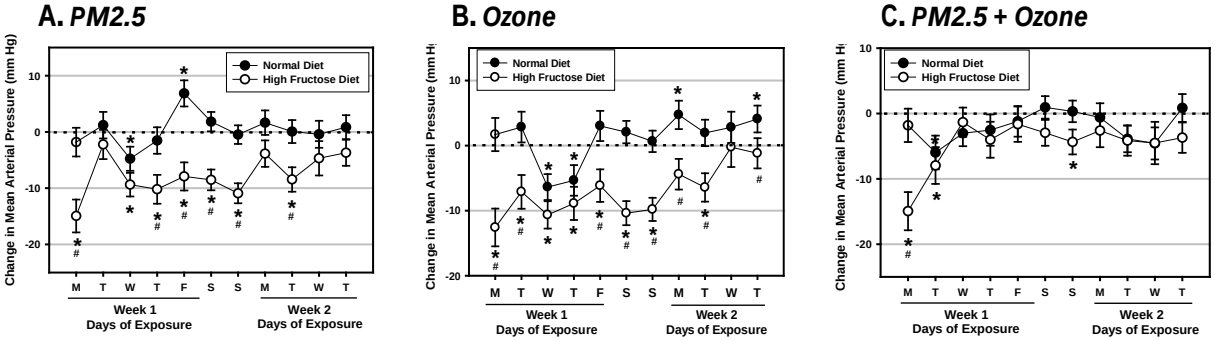


Figure 2. Daily Effect of PM2.5 (A), Ozone (B) and Ozone + PM2.5 Coexposure (C) on Blood Pressure In Rats Fed Normal (black circles) or High Fructose (white circles) Diets. Data is plotted as the effect estimate on blood pressure (mm Hg) of the indicated exposure compared to filtered air exposed rats (indicated by dotted line, zero axis). * - indicates significant difference from Air-exposed rats given the same diet; # - indicates significant difference from rats fed a Normal Diet.

Both PM2.5 and O3 exposure alone caused drops in heart rate that were consistently greater and persistent in HFr-fed rats (Figure 3A, B). Decreases of up to 25 beats per minute (bpm) in normal chow-fed rats and up to 60 bpm in HFr-fed rats were observed. During co-exposures to both O3 and PM2.5, drops in heart rate were remarkable during the first two days (decreases of 40-70 bpm), but were less dramatic with repeated exposures (0-20 bpm decreases; Figure 3C). Thus decreases in both blood pressure and heart rate were greater and more sustained with single pollutant exposures, whereas cardiovascular responses during co-exposures appeared to attenuate more quickly. The reason for this difference in the persistence of these cardiovascular responses to repeated single or multiple pollutant exposures is unknown. We speculate that the rapid adaptation of co-pollutant-induced blood pressure and heart rate responses in HFr-fed rats may have been the result of a greater adaptive defense mechanism(s) due to a greater total pollutant dose with the co-pollutant exposures. The biological mechanisms underlying these physiologic responses to exposure will be the focus of future studies.

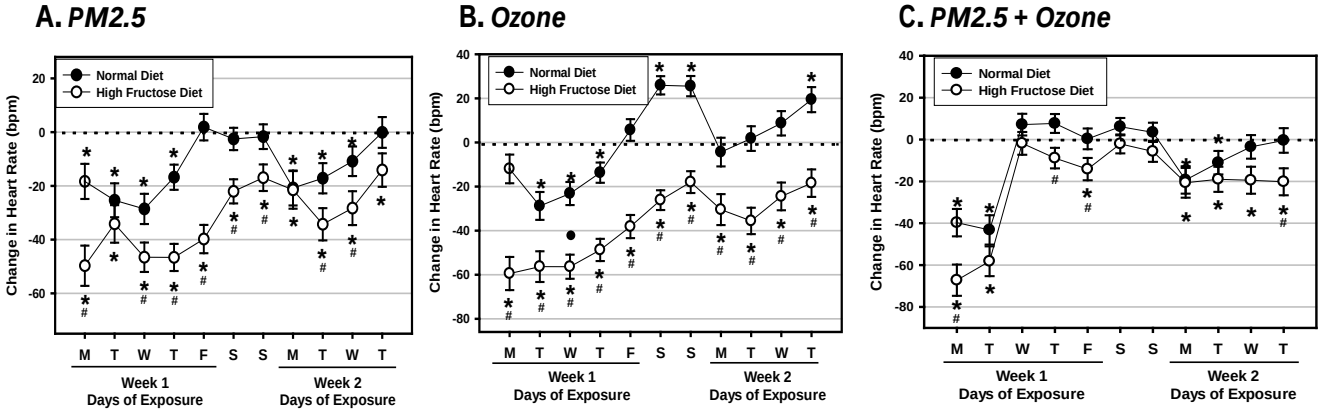


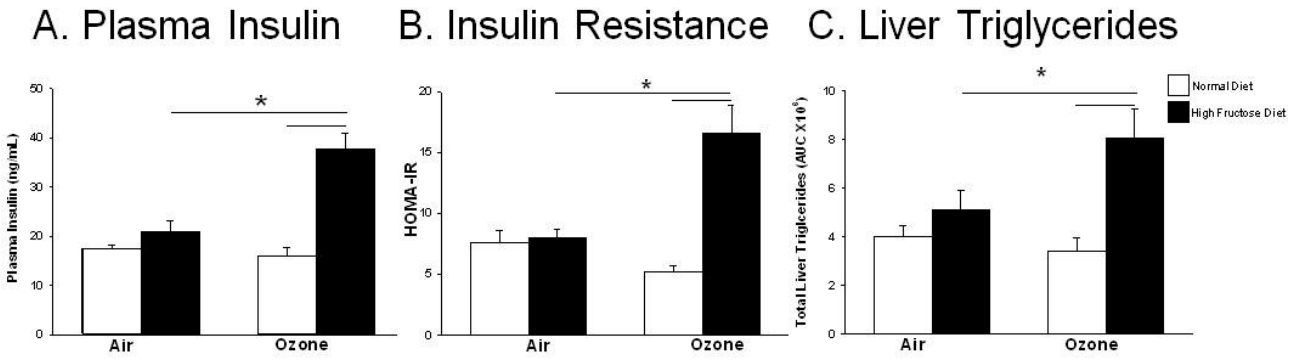
Figure 3. Daily Effect of PM2.5 (A), Ozone (B) and Ozone + PM2.5 Coexposure (C) on Heart Rate In Rats Fed Normal (black circles) or High Fructose (white circles) Diets. Data is plotted as the effect estimate on heart rate (bpm) of the indicated exposure compared to filtered air exposed rats (indicated by dotted line, zero axis). * - indicates significant difference from Air-exposed rats given the same diet; # - indicates significant difference from rats fed a Normal Diet.

From these results, we conclude that cardiovascular depression in response to exposures to O3 and PM2.5 was enhanced and prolonged in rats with HFrD-induced CMS. This suggests that people with CMS may be prone to similar exaggerated BP and HR responses to inhaled air pollutants. We have submitted a manuscript describing this study to *Environmental Health Perspectives* (EHP) for peer review publication. Initial reviews were encouraging and a revised paper will be submitted to EHP in July or early August 2013.

2) Effects of Ozone Exposure on Facets of the Cardiometabolic Syndrome in Mice. In year 3, we conducted two mouse inhalation toxicology studies. In the first study, we exposed C57BL/6 male mice to 0 or 0.5 ppm O3, 4h/day, for 24

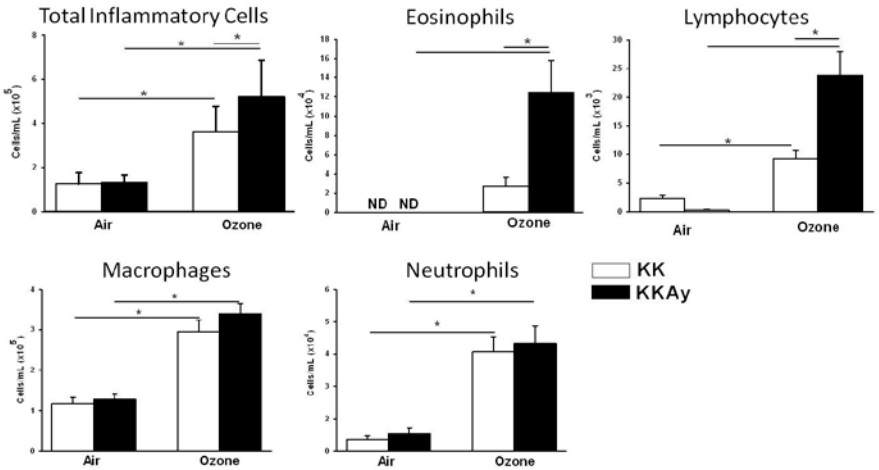
consecutive weekdays. Half of the mice were fed a high fructose diet (HFrD) and the other half a normal rodent chow diet (ND). We found that O3 induced upper and lower respiratory tract inflammation that was more marked in ND-fed mice. However, O3 exposure induced insulin resistance and increased blood insulin levels (facets of the CMS and diabetes) only in mice fed the HFrD. Insulin resistance (HOMA-IR) was not found in the HFrD-fed mice exposed only to filtered air (0 ppm O3). In addition, O3 enhanced the severity of diet-induced fatty liver (hepatic steatosis) and levels of total liver triglycerides in the HFrD-fed mice. Figure 4 graphically summarizes selected O3-specific metabolic changes. These initial results suggest that metabolic and pre-diabetic facets of HFrD-induced CMS can be enhanced by subacute inhalation exposure to this common gaseous air pollutant. These health effect findings caused by O3 exposure are similar in nature to those previously reports in mice fed a high fat diet and chronically exposed to PM2.5.

Figure 4. Effects of 24-day exposure of ozone on plasma insulin levels, insulin resistance (HOMA-IR) and total liver triglycerides in C57BL/6 mice fed a normal or high fructose diet, white and black bars respectively. Bars represent the mean ± the standard error of the mean (SEM). * Statistical difference between groups, $p \leq 0.05$.



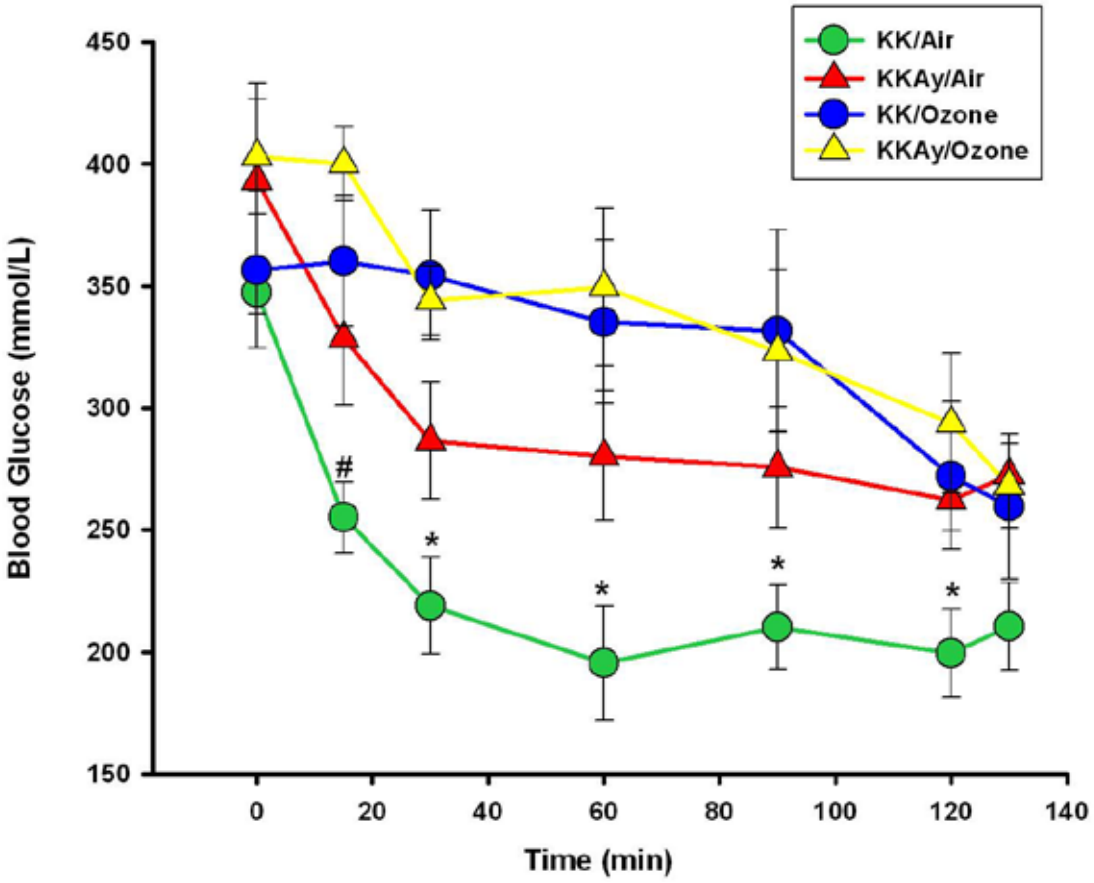
In collaboration with our colleagues in Project 3, we also conducted a second O3 inhalation toxicology study using murine strains, KK and KKAy, which are genetically prone to develop different severities of type two diabetes mellitus (T2D). KKAy develop obesity and have more severe facets of T2D compared to KK mice. Both strains of mice (8-10 weeks of age) were exposed to 0 or 0.5 ppm O3, 4h/day, for 13 consecutive weekdays. O3 caused pulmonary inflammation (as measured in the collected bronchoalveolar lavage fluid – BALF) in both strains of mice, but the severity was greatest in the KKAy mice due to striking increases in BALF eosinophils and lymphocytes that was not found in the lungs of O3-exposed KK mice (Figure 5).

Figure 5. Changes in the number of inflammatory cells in the bronchoalveolar lavage fluid (BALF) collected from the lungs of KK and KKAy mice after exposure to either 0 or 0.5 ppm O3 for 13 consecutive weekdays. Bars represent the group means ± SEM.
* statistical difference between groups, $p \leq 0.05$.



In terms of metabolic indices, both KK and KKAy had hyperglycemia that was not affected by the O3 exposure. Interestingly, KK mice exposed to filtered air (controls) had normal insulin tolerance tests (ITT) indicating no insulin resistance (Figure 6). In contrast KK mice exposed to O3 had abnormal ITT indicating insulin resistance (a major factor in the development of CMS and diabetes). KKAy mice exposed to filtered air or O3 (0 or 0.5 ppm O3, respectively) had insulin resistance (abnormal ITT). These results indicate that subacute exposure to O3 can induce insulin resistance in KK mice that is similar to the induction of insulin resistance that is genetically induced by the transfer of the yellow obesity gene A^Y in KK mice (Figure 6). We will continue this line of research next year by exploring the effects of subacute PM2.5 and O3/PM2.5 exposures of both the diet- and genetically induced murine models of the CMS and diabetes.

Figure 6. Results of insulin tolerance tests (ITT) in KK and KKAy mice after exposure to 0 or 0.5 ppm O3 for 13 consecutive weekdays. Mice were intraperitoneally injected with 0.5 IU/Kg body weight of insulin (Novolin) and blood glucose measurements were taken via the tail vein at 1, 30, 60, 90 and 120 minutes after insulin administration. KK mice exposed to filtered air (0 ppm O3) had normal blood glucose responses to insulin, while the other groups of mice had abnormal ITT indicating insulin resistance. * Statistically different from all groups. # Statistically different from O3-exposed groups, $p \leq 0.05$.



3) 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 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. Twenty-four hours after the last exposure, tissue samples were taken at necropsy from a wide-range of targeted organs, along with blood and bronchoalveolar lavage fluid samples, and are being analyzed for biochemical, molecular, and morphometric alterations related to CMS. Collected data is currently being analyzed and results are not yet available.

4) 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: In this coming year we will complete the data analyses. 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, we will assess the following outcomes: 1) Cardiovascular parameters, including blood pressure, heart rate, heart rate variability, and body temperature 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. From the non-telemetered rats, we will assess differences in *in vivo* chemiluminescence of the heart and lungs after one day of exposure in 6 animals/TRUAP

or filtered air group. In another group of non-telemetered animals, we will also assess bronchoalveolar lavage fluid (BALF) parameters after 4 days of exposure including total cells and cell types as well as total protein and ß-N-Acetyl-Glucuronidase determinations in the lavage fluid using 6 animals/ TRUAP or fitered air group. From the rats on which BAL is done, we will also collect heart blood from which complete blood count and differential as well as platelet counts will be done, and blood chemistry (chem 17) including blood glucose, electrolytes, insulin, triglycerides, and hepatic function assessment.

The third group of non-telemetered 6 animals per TRUAP or Filtered air group will be exposed for a length of time to be determined for specific outcomes to be assessed by collaboratoring CLARC investigators at Havard University CLARC, GLACIER, and the University of Washington Center for Clean Air Research.

Publications/Presentations:

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. PMCID:PMC3402685.

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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. (In press)

Liu C, Zheing Y, Harkema J, Sun Q, Rajagopalan S. (2013) Epidemiological and Experimental Links Between Air Pollution and Type II Diabetes. Toxicol Pathol. 41(2):361-373. PMID:23104765.

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*. (In review)

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. (2013) Cardiovascular dysregulation caused by inhalation exposures to PM2.5 and ozone is augmented in rats fed a high fructose diet. *Environ Perspect*. MS ID#: 13-07085-ART. (In review)

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. (In Press)

Oral Presentation. Allen KM, Brooks P, Dereski M, Lewandowski RP, Hotchkiss I, Jackson-Humbles D, Brandenberger C, Bramble LA, Wagner JG, and JR Harkema. (2013) Inhaled Ozone Induces Metabolic Abnormalities in Mice Fed a High-Fructose Diet. *The Toxicologist* 132(1): A62. Annual Meeting of the Society of Toxicology, March 2013.

Oral Presentation. Wagner JG, Yang H, Allen KM, Morishita M, Nan B, Mukherjee B, Fink GD and JR Harkema (2013) Suppressed Responses in Heart Rate Variability during Inhalation Exposure to Ozone and Ambient Fine Particles in Rats on a High-Fructose Diet. *The Toxicologist* 132(1): A63.

Poster Presentation. Wagner JG, Allen K, Nan B, Lewandowski R, Fink G, and JR Harkema (2013) Role of Transient Receptor Potential (TRP) Channels in Ozone-Induced Decreases in Blood Pressure and Heart Rate in Rats on a High Fructose Diet. A5093. *Proceedings of the American Thoracic Society.* Annual International Meeting of the American Thoracic Society. May, 2013.

Oral Presentation. Wagner JG. (2013) Cardiovascular Toxicity of Acute Exposures to Multipollutant Atmospheres: Results from Field Studies Using a Rodent Model of the Cardiometabolic Syndrome. EPA Work-In-Progress Webinar for the Clean Air Research Centers, January 2013.

Oral Presentation. Harkema JR, Interface of Health Effects caused by the CardioMetabolic Syndrome and Exposures to Air Pollutant Mixtures. Society of Toxicology Webinar sponsored by the Risk Assessment and Mixtures Specialty Sections, April 2013.

Poster Presentation: Ong Chee B *et al*. Development of Ozone-Induced Eosinophilic Rhinitis in Mice. Annual Meeting of

the Society of Toxicologic Pathology, Portland, OR, June 2013.

Oral Presentation: Harkema JR and Brook RD (2013). GLACIER Center Update. EPA CLARC Annual Meeting, Seattle, WA, July 2013.

Future Activities in the Remaining Months of Year 3 and in Year 4:

Ongoing Analyses in Year 3

1. **Cardiotelemetry Data:** Data analysis of heart rate and blood pressure responses in rats exposed to the O3/PM2.5 mixture, 5 days/wk for four weeks (Dexter inhalation exposure study) should be finished by the end of the year. In addition, data analysis of heart rate variability is being calculated for all cardiotelemetry studies and ECG waveform analyses are being planned.
2. **Associations of Exposure/Health Outcomes:** With the help of the Biostatistics and Data Management Core, we will use data of cardiovascular responses (e.g., heart rate, mean arterial pressure, heart rate variability) and PM2.5 metrics (e.g., trace elements, gases, sources) collected over the same 30-minute timeframes to provide a high resolution correlation of specific PM2.5 components to the cardiovascular health effects. We will conduct these correlative analyses using data from both our Dearborn and Dexter studies.
3. **Brain Neurochemistry:** With the help of collaborators and neuroscientists Drs. P.S. and Sheba MohanKumar at MSU, exposure- and diet-related responses in specific brain regions that control autonomic functions are being examined. For example, neurochemical and molecular analyses in the paraventricular nucleus of the hypothalamus are being conducted for neurotransmitters and inflammatory cytokines after inhalation exposures in both normal and high fructose-fed rats.
4. **Effects of Subchronic Ozone Exposure on the Development of Cardiometabolic Sydrome in C57Bl/6, KK and KKAy mice.** With the help of the Biostatistics and Data Management Core, we will be completing the statistical analyses of data collected from our initial O3 studies in which we used three mouse strains to more fully understand the impact of genetics and O3 exposure on facets of CMS and diabetes.
5. **Initial studies on Sympathetic Neural Recordings in Rats exposed to Ozone.** Dr. Fink and colleagues have been working on perfecting the surgical implantation of radiotelemetric transmitters in the renal sympathetic nerves of Sprague-Dawley rats. In late summer or early Fall 2013, we will conduct pilot studies in telemeterized rats to record changes in nerve activity during acute O3 exposure. Once successful recordings are achieved, we will move to similar studies but using acute inhalation exposures to PM2.5 and PM2.5/O3.

New Studies in Year 4:

6. **Effects of Subacute PM2.5 and PM2.5/O3 Exposures on the Development of Cardiometabolic Sydrome in C57Bl/6 mice.** We will test the effects of subacute PM2.5 and PM2.5/O3 inhalation exposure (4h/day, 4d/wk, for 24 consecutive weekdays) on the development of HFr-diet-induced cardiometabolic syndrome in mice. Animals will be fed a normal chow or an HFr diet during the inhalation exposures.
7. **Effects of Subacute PM2.5 and PM2.5/O3 Exposures on the Development of Cardiometabolic Sydrome in KK and KKAy mice.** Based on the encouraging findings with our initial subacute O3 exposures, we will extend the inhalation toxicology studies with KK and KKAy mice by exposing these pre-diabetic strains of mice for 24 consecutive weekdays to PM2.5 and PM2.5/O3 at either our Dexter or MSU East Lansing exposure site, depending on available resources.
8. **Sympathetic Neural Recordings in Rodents Exposed to Ozone.** We will continue to work on refining the technique of radiotelemetric microneurography in order to apply this measurement in future inhalation studies of PM2.5 and O3.
9. **Inhalation Toxicology Studies in Dexter, MI.** In the spring and summer of 2014, we will resume inhalation toxicology studies in our rural Dexter, MI (predominantly regionally transported air pollution) to determine the cardiopulmonary, airway and metabolic effects of acute PM2.5±O3 exposures on normal- and HFr-fed rats.

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

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

GLACIER PROJECT 3 (Year 3 Progress Report)

Date of Report: 7/31/13
EPA Agreement Number: RD83479701 (subproject: R834797C003)
Center Name: 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, 2012 - July 31, 2013

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:

Conduct of Simultaneous Exposures in OASIS-1 and OASIS-2

During the last year we have accomplished significant progress towards beginning exposures in our near road-way site (OASIS-2) and comparing this site with OASIS-1 (regional site). We are now analyzing the data and are on our way towards preparation of a paper that will provide comparative information on the characterization of these two sites in Columbus. We are also in the process of determining if source apportionment can be performed to understand the differences in sources at these two sites. Our preliminary data indicates higher BC concentration at the OASIS-2 site in keeping with its proximity to the roadway.

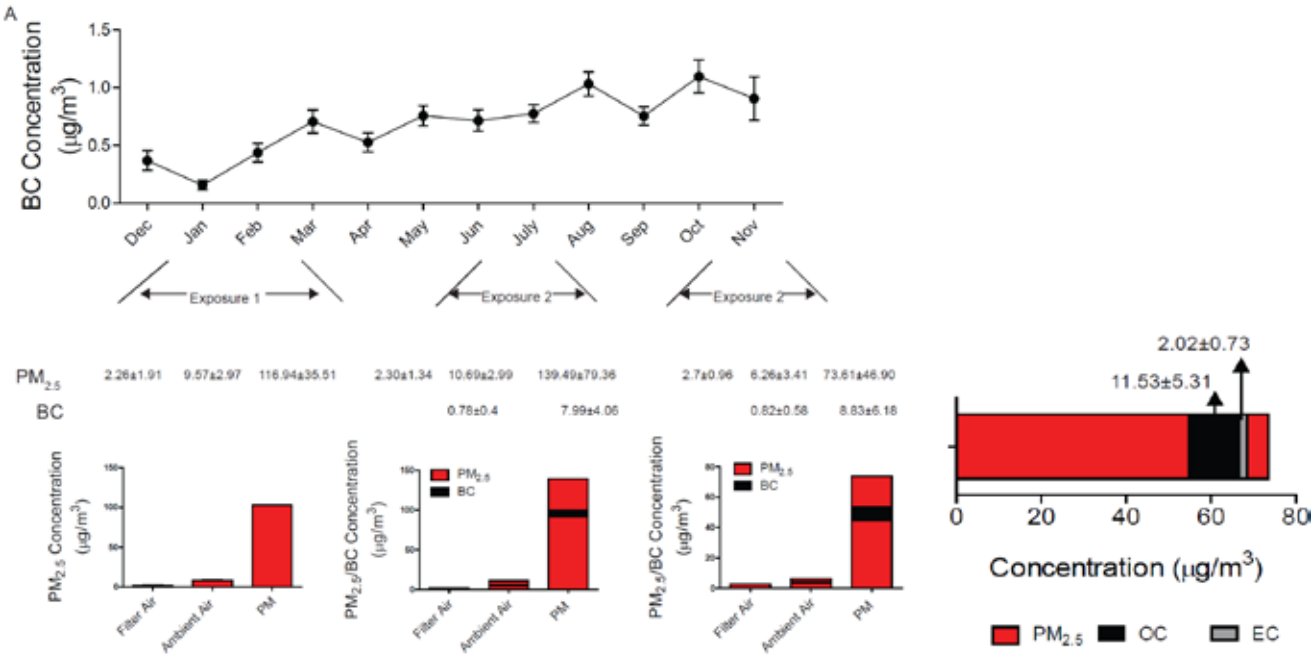
Elemental Characterization of Exposures in OASIS-2 October 2012 to November 2012 by ICP-MS.

Elements	Ambient PM2.5		Filtered Air		Concentrated PM2.5	
	Mean	SD	Mean	SD	Mean	SD
S	641.40	412.66	39.8	10.93	4812.33	3568.81
Ca	104.30	27.44	97.69	21.68	825.43	276.99
Na	47.62	17.67	72.8	23.18	295.14	118.79
Fe	62.06	26.94	5.92	0.91	488.22	208.13
K	56.63	22.39	31.39	10.74	377.02	159.94
Zn	19.20	9.90	4.36	2.38	144.56	77.18
Mg	17.81	6.02	6.7	1.15	159.94	54.84
Al	16.99	7.26	20.21	5.71	144.31	27.47
P	5.72	2.18	2.15	0.51	46.28	17.96
Pb	3.36	1.48	2.39	0.48	22.14	11.15
Cu	4.19	1.64	3.22	3.99	35.76	12.19
Ba	4.45	1.82	0.61	0.06	38.94	16.61
Mn	2.49	1.04	0.25	0.07	19.53	7.89
Cr	3.84	0.48	8.18	0.89	15.81	1.92
Se	0.76	0.46	BID	0.02	6.76	3.79
Ti	1.19	0.53	0.08	0.04	9.75	4.14
Sb	0.82	0.42	0.05	0.01	6.54	3.46
Sr	0.72	0.28	0.13	0.02	5.72	1.80
As	0.74	0.52	0.03	0.01	5.36	4.11
Mo	0.50	0.29	0.23	0.06	3.44	2.15
Ni	0.43	0.31	0.22	0.06	2.79	1.28
V	0.18	0.18	0.02	0.00	1.40	1.47
Cd	0.23	0.08	0.36	0.08	1.39	0.53
Rb	0.08	0.04	0.05	0.01	0.62	0.34
Ce	0.04	0.02	0.01	0.00	0.33	0.17
La	0.03	0.01	BID	0.00	0.22	0.09
Co	0.04	0.02	0.05	0.01	0.33	0.05

Concentrations in ng/m^3
BID: below instrument detection limit

Progress in Aim 1

We have completed Specific Aim 1 and as part of these experiments report on data on the effects of concentrated ambient $PM_{2.5}$ when used alone in potentiation of glucose intolerance and IR in a genetic model of Type II DM. KKay mice, which are susceptible to Type II DM, were assigned to either concentrated ambient $PM_{2.5}$ or filtered air (FA) for 4-8 weeks. The mice were exposed for 6 h/d, 5 d/wk, 5 -8 weeks in a mobile trailer exposure system ("Ohio Air Pollution Exposure System for Interrogation of Systemic Effects 1," located at the near-roadway site located in Polaris (OASIS-2). The figure outlines the BC and PM2.5 concentrations at the site during 3 separate exposures and EC/OC concentrations for one of the exposures (Exposure 3).



$PM_{2.5}$ and BC concentration to which mice were exposed at the study site. A, BC concentration in the ambient air in Columbus at OASIS-2 from Dec.2011-Nov.2012. Exposure 1-Exposure 3 labeled under the X-axis with exact date information. B, $PM_{2.5}$ concentration in the filter air, ambient air and concentrated ambient air for Exposure 1. C, $PM_{2.5}$ concentration in the filter air, ambient air and concentrated ambient air, and BC concentration in the ambient air and concentrated air for Exposure 2. D, $PM_{2.5}$ concentration in the filter air, ambient air and concentrated ambient air, and BC concentration in the ambient air and concentrated air for Exposure 3. The values of $PM_{2.5}$/BC concentration were shown above the according bar graphs in B-D. E, OC & EC concentration in the $PM_{2.5}$ from Exposure 3. BC denotes black carbon; OC denotes organic carbon; EC denotes element carbon.

Demonstration of Time Course of Development of IR and Inflammation in KKay with $PM_{2.5}$

As part of our original proposal we had proposed investigating the time course of development of the IR response to air-pollution using a genetically pre-disposed model. We found that there were no changes in body weight or food intake during exposure. Circulating adiponectin was decreased at 5 weeks of exposure, accompanied by an increase in leptin levels. There were no further changes in adiponectin or leptin after 8 weeks exposure. An increase in fasting blood glucose was seen within 1-week of exposure to $PM_{2.5}$, while insulin levels trended upwards, with the highest values after 6 weeks of exposure and significant differences between FA/ $PM_{2.5}$ at the 3- and 8-week time-points. Corresponding HOMA-IR levels were significantly higher with concentrated ambient $PM_{2.5}$ at the 1, 3- and 8-week time-points. Responses to intra-peritoneal glucose challenges were abnormal after 5 weeks of $PM_{2.5}$ exposure, characterized by marked worsening of glycemic response to glucose challenge. This change in glucose tolerance persisted at 8 weeks (Figure 1H). Insulin tolerance was also impaired at 5 and 8 weeks.

Effect of $PM_{2.5}$ exposure on energy homeostasis and BAT gene expression

We next investigated the effect of concentrated ambient $PM_{2.5}$ exposure on whole body energy homeostasis. $PM_{2.5}$ exposure reduced oxygen consumption, carbon dioxide production, respiratory exchange ratio and thermogenesis. In light of the significant change in thermogenesis, we investigated uncoupling protein 1(UCP1), a pivotal player in thermogenesis via uncoupling of oxidative phosphorylation. UCP1 protein but not mRNA expression was down-regulated in interscapular brown adipose tissue (BAT) of $PM_{2.5}$ exposed mice, consistent with decreased thermogenic activity. PPARγ-coactivator-1alpha (PGC1α) and PRD1-BF1-RIZ1 homologous domain-containing 16 (PRDM16) expression, transcriptional activators that play a key role in BAT development and acquisition of thermogenic phenotype were however no different. An increase in IL-6 and TNFα was also noted in BAT, indicating $PM_{2.5}$ induced inflammation in brown adipose tissues. Taken together, alteration of UCP1 via post-transcriptional mechanisms and inflammation in BAT may account for the decreased thermogenesis and oxygen consumption in response to $PM_{2.5}$ exposure.

Systemic Inflammation in Response to Concentrated Ambient $PM_{2.5}$ Exposure

Another essential question that we sought to address in our proposed investigations in Aim 1 was to elucidate the extent of systemic inflammatory response with concentrated ambient $PM_{2.5}$. We investigated a population of inflammatory monocytes that we and others have shown to be relevant in response to diverse triggers including high-fat feeding and high cholesterol chow. We found that $PM_{2.5}$ exposure increased $CD11b^{+}Ly6G^{low}7/4^{hi}$ cells in the peripheral circulation with a corresponding trend towards a reduction in this population in the bone marrow. Although there were no differences in splenic $CD11b^{+}Ly6G^{low}7/4^{hi}$ cells (Figure 3A), IFN production from splenocytes of $PM_{2.5}$ exposed mice was significantly higher compared to that of FA mice with a corresponding decrease in IL-4 release. These results suggest a redirection of Th1/Th2 balance towards a Th1 polarized state in response to $PM_{2.5}$ exposure. Type 2 DM in humans and animal models is associated with increased levels of recruitment and/or activation of innate immune cells in visceral adipose depots. It has been shown that $PM_{2.5}$ exposure results in an increase in adipose tissue macrophages with a shift to a pro-inflammatory phenotype characterized by an increase in F4/80 macrophages in the visceral adipose and a pro-inflammatory "M1 phenotype" typified by TNF-α, IL-6 and a decrease in IL-10, MgI1 gene expression. We observed an increase in F4/80+/CD11c+ cells in VAT in response to $PM_{2.5}$ exposure. Together with data demonstrating that $PM_{2.5}$-mediated monocyte infiltration into VAT is $CCR2^{-/-}$ dependent (unpublished data), these results suggest mechanisms similar to those involved in diet mediated aggravation of the VAT infiltration by monocytes via CCR2 dependent pathways.

Hypothalamic Inflammation in Response to Concentrated Ambient $PM_{2.5}$ in KKay Mice

This is a new observation that we have made as part of our investigations that may provide an integrated understanding of the diverse effects of PM2.5. CNS inflammation has been shown to be relevant to the pathogenesis of Type II DM and the development of peripheral inflammation in response to factors such as high-fat feeding in both animal models as well as studies in humans.

We examined mRNA encoding inflammatory mediators, including cytokines (IL-6, TNFα), Suppressor-of-cytokine signaling (Socs3), components of the NFκB pathway, and microglia/macrophage (MAC1) in hypothalamic centers. In mice exposed to $PM_{2.5}$, TNFα and IL-6 expression was elevated after 5-weeks of exposure to $PM_{2.5}$ as compared to FA. Longer duration (8 week) exposure to $PM_{2.5}$ elevated TNFα and IL6 expression, as well as significantly increased IKK expression. These results suggest that even short-term exposure of a few weeks is sufficient to induce increases in cytokine expression in the medial basal hypothalamus. SOCS-3 mRNA levels were unaltered in the hypothalamus. SOCS proteins were originally identified as anti-inflammatory, negative regulators of cytokine receptor signaling and attenuate leptin receptor signaling contributing to central leptin resistance. Mice deficient in SOCS-3 or with brain-specific SOCS-3 deletion are more sensitive to the anorectic effects of leptin and are resistant to DIO. The lack of an elevation in SOCS-3 expression with $PM_{2.5}$ in the face of hyperleptinemia could potentially drive CNS inflammation while allowing continued leptin signaling and may account for maintenance of body weight.

Metabolic Responses to intracerebroventricular Administration of TNF-a Blockade in Response to $PM_{2.5}$ Exposure

Based on the increased hypothalamic TNF expression in $PM_{2.5}$ mice and observations that increased hypothalamic inflammation appears to occur early following initiation of a high fat diet and may contribute to changes in peripheral inflammation including brown adipose tissue (BAT) dysfunction, we hypothesized that TNFα antagonism would restore peripheral glucose intolerance and altered thermogenesis following $PM_{2.5}$ exposure. Mice at age of 5 weeks old were continuously administered infliximab (0.2ug/day) or artificial CSF (aCSF) at a rate of 0.11uL/hr through a minipump connected to a cannula directed at the lateral ventricle (+0.2 posterior and -0.95 lateral to Bregma, extending 2.75 mm below the skull). ICV infliximab did not influence peripheral glycemia or insulin tolerance in response to $PM_{2.5}$ exposure; neither did body temperature differ between groups. However, infliximab treatment significantly impaired energy homeostasis, as evidenced by further decreases in O_2 consumption, CO_2 production, respiratory exchanging ratio, and heat generation in inflixmab treated mice compared to artificial CSF controls. This is contrary to reports in which the centrally TNFα administration reduced the expression of thermogenic proteins in brown adipose tissue and skeletal muscle, effects which were blunted in TNFα receptor knockout mice. It has been suggested that, depending on its local concentrations, TNFα can exert a dual functions in the hypothalamus, being catabolic at high concentrations and anabolic at low concentration. Consistent with these paradoxical effects on energy metabolism, TNFα levels in obese animals are higher than controls but significantly lower than tumor-bearing rats, accompanied by inhibition of feeding/anorexia in tumor-bearing and increase in feeding/obesity in obese animals respectively.

Central IKK2 Inhibition Prevents $PM_{2.5}$-Induced Disruption of Glucose/Energy Balance and Peripheral Inflammation

To determine whether increased hypothalamic IKK- NF-κB pathway contributes to $PM_{2.5}$'s effects, we administered a pharmacological inhibitor of IKK2 (IMD-0354) ICV and concomitantly exposed mice at age of 7 weeks old to $PM_{2.5}$ or FA for 4 weeks (3rd Exposure). IKK-2 inhibition had no effect on food intake and body weight in either FA or $PM_{2.5}$ mice. In contrast, $PM_{2.5}$-induced changes in glucose and insulin were normalized with IMD-0354 treatment. This effect was $PM_{2.5}$ dependent, as IMD-0354 did not further improve these parameters in the FA group. Taken together, these results suggest that central IKK2 inhibition prevents $PM_{2.5}$-induced abnormalities in glucose/insulin homeostasis. As expected, ICV IMD-0354 normalized energy metabolism, reflected by restored O_2 consumption, CO_2 production, respiratory exchanging ratio and heat generation, compared with $PM_{2.5}$-exposed mice administered vehicle. Moreover, inhibition of central inflammation improved energy homeostasis including oxygen consumption and heat generation, in the FA group as well. Interruption of IKK-2 signaling was sufficient to restore weight gain in these experiments. We did not see weight gain in response to $PM_{2.5}$ despite reduction in BAT thermogenesis and increased peripheral inflammation. While these changes may be specific to the model (KKay mice) and the short duration of exposure, they also suggest complex adaptations in satiation/adiposity signals that may warrant further study.

Central IKK2 Inhibition Ameliorates $PM_{2.5}$-induced Peripheral Inflammation

We next investigated whether central IKK inhibition ameliorated peripheral inflammation induced by $PM_{2.5}$ exposure. There was a clear trend ($P = 0.0518$) for an increase in circulating $CD11b^{+}Ly6G^{low}7/4^{hi}$ cells with concentrated ambient $PM_{2.5}$ exposure that was nearly normalized by IMD-0354 treatment. $F4/80^{+}/CD11c^{+}$ cells in the epididymal adipose was markedly higher in response to concentrated ambient $PM_{2.5}$ exposure, which is consistent with the prior exposure period and this increase was attenuated by IMD-0354. These results indicate that peripheral inflammation in blood and adipose tissue in response to $PM_{2.5}$ is dependent to a considerable degree on CNS inflammation.

Central IKK2 Inhibition Suppresses $PM_{2.5}$-induced Hypothalamic Inflammation

IMD-0354 reduced IL-6 and IKK expression, both of which were upregulated by $PM_{2.5}$ exposure, but had no effect on TNFα and IκB expression. We next investigated the presence of reactive gliosis, characterized by the recruitment, activation, and proliferation of glial cells, such as astrocytes, NG2 cells, and microglia, in the in the arcuate nucleus of the hypothalamus. Using anti-Iba1 (ionized calcium binding adaptor molecule 1), a microglia-specific cytoplasmic marker, we found a 50% increase in microglial number in the arcuate nucleus of mice exposed to $PM_{2.5}$ compared to FA. Additionally, microglia from $PM_{2.5}$ mice were larger with a more activated morphology. Central IMD-0354 infusion restored both the numbers and size of microglia in response to $PM_{2.5}$. The effect of $PM_{2.5}$ on astrocytes was assessed with GFAP immunostaining. Astrocytes are abundant throughout the CNS and were apparent in the arcuate nucleus of all groups. The intensity of GFAP staining was elevated by exposure to $PM_{2.5}$ with the increase in GFAP staining among $PM_{2.5}$ mice prevented by treatment with the IKK inhibitor. While cell boundaries of astrocytes in the arcuate nucleus of the KKay mice exposed to FA was relatively preserved, the boundaries of astrocytes exposed to $PM_{2.5}$ coalesced into a dense network reminiscent of a syncytium (Figure 10), a finding pathognomonic of reactive gliosis.

Summary

During this project cycle we have generated the following new findings and contributed to further understanding of the link between $PM_{2.5}$ and inflammation

1. Began exposures in a near roadway site and performed characterization of the exposure milieu.
2. Concentrated ambient $PM_{2.5}$ exaggerated Type II DM development in a genetic model with effects evident within 5 weeks including decreased thermogenesis, increased peripheral inflammation.
3. Concentrated ambient $PM_{2.5}$ 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.

Publications/Presentations:

Papers

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. (In Press)

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. (In press)

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.

Liu C, Xu X, Bai Y, Wang T, Rao X, Wang A, Sun L, Ying Z, Gushchina L, Maiseyeu A, Sun Q, Harkema J, Rajagopalan. (2013) Air-Pollution Mediated Susceptibility to Inflammation and Insulin Resistance via CCR2 Dependent and Independent Effects. Arteriosclerosis, Thrombosis, and Vascular Biology. January 31, 2013 MS ID#:ATVB/2013/301272. (In Press)

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. (In Review)

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. (2013) Cardiovascular dysregulation caused by inhalation exposures to PM2.5 and ozone is augmented in rats fed a high fructose diet. Environ Perspect. MS ID#: 13-07085-ART. (In Review)

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. (In Press)

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 11, 2013.

Abstracts

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. (In Press)

Liu C, Xu X, Bai Y, Wang T, Rao X, Sun L, Ying Z, Maiseyeu A, Sun Q, and Rajagopalan S. (2013) Air-pollution mediated susceptibility to inflammation and insulin resistance via CCR2 dependent and independent effects. J Immunol (Suppl) P190.

Liu C, et al. (2013) Central IKKb Inhibition Prevents Particulate Matter Mediated Peripheral Inflammation and Exaggeration of Type II Diabetes. Submitted to the Annual CLARC meeting July 25-26, 2013 in Seattle, WA.

Sun L, et al. (2013) Ambient fine particulate pollution and ozone exposure induces inflammation in epicardial and perirenal adipose tissues in a rat model. Submitted to the Annual CLARC meeting July 25-26, 2013 in Seattle, WA.

Zhong J, et al. (2013) Ozone exposure promotes adipose inflammation in mice. Submitted to the Annual CLARC meeting July 25-26, 2013 in Seattle, WA.

Ying Z, et al. (2013) The Effects of Inhalation Exposure to Ozone on Systemic Inflammation and Vascular Functions. Submitted to the Annual CLARC meeting July 25-26, 2013 in Seattle, WA.

Future Activities: Before embarking on simultaneous exposures of PM2.5 + Ozone it was important for us to determine the extent of effects attributable to PM2.5 alone. We will perform the experiments in Aim 3, which 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.

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 3 Progress Report)

Date of Report: 7/31/13
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, 2012 - July 31, 2013

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. (In press)

Brook R, Bard R, Morishita M, Dvonch J.T, Wang L, Yang H, Spino C, Mukherjee B, Kaplan M, Yalavarthi S, Oral E, Ajluni N, Sun Q, Brook J, Harkema J, Rajagopalan S. (2013) The Hemodynamic and Vascular Effects of Acute Exposure to Coarse Particulate Matter Air Pollution in a Rural Location. January 25, 2013 MS ID#: HYPE201301122. (In Review)

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.

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.

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. (In Review)

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. (2013) Cardiovascular dysregulation caused by inhalation exposures to PM2.5 and ozone is augmented in rats fed a high fructose diet. Environ Perspect. MS ID#:13-07085-ART. (In Review)

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, 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 3 Progress Report)

Date of Report: 7/31/13
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, 2012 - July 31, 2013

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 has collaborated with investigators from Project 1 to QC and analyze the first human study. We have finished the analysis for the following three papers:
(1) 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 2013 (in press).
(2) The hemodynamic, autonomic, and vascular effects of exposure to coarse particulate matter in a rural location. Environ Health Perspect 2013 (in second review).
(3) No effect of coarse particulate matter air pollution on HDL function. (In preparation for submission).
In addition, Dr. Lu Wang has consulted on the design of the next study that will begin soon.

Dr. Bin Nan of the Biostatistics and Data Management Core has collaborated with Project 2 investigators on analyses of several experiments in rodent models. He has finished the analysis for the manuscript "Cardiovascular Dysregulation Caused by Inhalation Exposures to PM2.5 and Ozone in a Rodent Model of the Cardiometabolic Syndrome," which is under review for EHP. This paper summarizes the first experiment (study 815-816) investigating acute CAP and ozone exposures in rodent models of cardiometabolic syndrome. The Core has provided preliminary statistical analysis results for study 824 which sought to determine the role of TRP channel in ozone-induced changes in heart rate and blood pressure in high fructose-fed rats. Currently, Dr. Nan and the Core's graduate assistant are analyzing the exposure trace element and particulate matter composition data for the 815-816 study. This is the first GLACIER study for which such data are available. The Core statisticians are meeting with members of the Exposures Core 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. Dr. Nan has also consulted with Project 2 investigators on design issues.

Dr. Bhramar Mukherjee is returning from her sabbatical and will provide statistical support for the investigators on Project 3.

In addition to statistical activities, the Core has enhanced and maintained the public access website (http://greatlakesairresearchcenter.org/), collaborating with Dr. Jack Harkema and other project investigators. It provides information about GLACIER, its Projects and Cores, a directory of key personnel for each project, information on the Mobile Labs, and links to Clean Air Centers and other EPA links. There is a link to future publications that will arise from this research. The Core has also to built the GLACIER intranet, with user accounts created. Standard formatting of study information including study design, codebooks, descriptive statistics (both tabular and graphical) and analysis datasets were created and implemented for the studies from Projects 1 and 2 during this reporting period.

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. (In press)

Brook R, Bard R, Morishita M, Dvonch J.T, Wang L, Yang H, Spino C, Mukherjee B, Kaplan M, Yalavarthi S, Oral E, Ajluni N, Sun Q, Brook J, Harkema J, Rajagopalan S. (2013) The Hemodynamic and Vascular Effects of Acute Exposure to Coarse Particulate Matter Air Pollution in a Rural Location. January 25, 2013 MS ID#: HYPE201301122. (In Review)

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. (2013) Cardiovascular dysregulation caused by inhalation exposures to PM2.5 and ozone is augmented in rats fed a high fructose diet. Environ Perspect. MS ID#: 13-07085-ART. (In review)

Future Activities:

In the next reporting period, we are preparing data for the following two studies in Project 1: (1) To evaluate if there is any effect of coarse particulate matter air pollution on insulin sensitivity; and (2) To evaluate the associations between coarse particulate matter components and cardio-metabolic outcomes. Research methods development on high-resolution temporal data being generated in Project 1 using multi-scale models will remain a primary focus in Year 3. For Project 2, we will collaborate with investigators to prepare the manuscript for the analysis of study 824 (role of TRP) and to complete the analysis of the exposure trace element and particulate matter composition data for the 815-816 study. The development of methods to appropriate deal with these data for this study, as well as a common approach for all GLACIER studies, will be a key focus for this Project Year with close collaborations with the research investigators and the Exposure Core.

In addition, maintenance of the GLACIER public website and enhancement and further development of the GLACIER intranet will take continue. Project 3 data have been received for the study to evaluate the role of CC-chemokine receptor 2 (CCR2) in PM2.5-mediated inflammation and IR and will be transferred to the GLACIER intranet, as will Exposure Core data.

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

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

CENTER PUBLICATIONS:

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. 36:106-111. PMCID:PMC3402685.

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. PMID:22901427.

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

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 11, 2013.

Liu C, Ying Z, Harkema J, Sun Q, Rajagopalan S. (2012) Epidemiological and experimental links between air pollution and type 2 diabetes. Toxicol Pathol. 41(2):361-373. PMID:23104765.

CENTER PUBLICATIONS SUBMITTED AND IN REVIEW:

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. (In Press)

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. (In press)

Brook R, Bard R, Morishita M, Dvonch J.T, Wang L, Yang H, Spino C, Mukherjee B, Kaplan M, Yalavarthi S, Oral E, Ajluni N, Sun Q, Brook J, Harkema J, Rajagopalan S. (2013) The Hemodynamic and Vascular Effects of Acute Exposure to Coarse Particulate Matter Air Pollution in a Rural Location. January 25, 2013 MS ID#: HYPE201301122. (In Review)

Liu C, Xu X, Bai Y, Wang T, Rao X, Wang A, Sun L, Ying Z, Gushchina L, Maiseyeu A, Sun Q, Harkema J, Rajagopalan. (2013) Air-Pollution Mediated Susceptibility to Inflammation and Insulin Resistance via CCR2 Dependent and Independent Effects. Arteriosclerosis, Thrombosis, and Vascular Biology. January 31, 2013 MS ID#:ATVB/2013/301272. (In Review)

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. (In Review)

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. (2013) Cardiovascular dysregulation caused by inhalation exposures to PM2.5 and ozone is augmented in rats fed a high fructose diet. Environ Perspect. MS ID#: 13-07085-ART. (In Review)

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. (In Press)

RELATED PUBLICATIONS BY CENTER INVESTIGATORS:

Zheng Z, Xu X, Zhang X, Wang A, Zhang C, Hüttemann M, Grossman LI, Chen LC, Rajagopalan S, Sun Q, Zhang K. (2013) Exposure to Ambient Particulate Matter Induces a NASH-like Phenotype and Impairs Hepatic Glucose Metabolism in an Animal Model. J Hepatol. 58(1):148-154.

Brook RD, Rajagopalan S. (2012) Can what you breathe trigger a stroke within hours?: comment on "ambient air pollution and the risk of acute ischemic stroke". Arch Intern Med. 172(3):235-236. PMID:22332154.

Deiuliis JA, Kampfrath T, Zhong J, Oghumu S, Maiseyeu A, Chen LC, Sun Q, Satoskar AR, Rajagopalan S. (2012) Pulmonary T cell activation in response to chronic particulate air pollution. Am J Physiol Lung Cell Mol Physiol. 302(4):L399-409. PMCID:PMC3289266.

Wagner JG, Morishita M, Keeler GJ, Harkema JR. (2012) Divergent effects of urban particulate air pollution on allergic airway responses in experimental asthma: a comparison of field exposure studies. Environ Health. 11(1):45. PMCID:PMC3487754.

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Xu X, Liu C, Xu Z, Tzan K, Wang A, Rajagopalan S, Sun Q. (2012) Altered adipocyte progenitor population and adipose-related gene profile in adipose tissue by long-term high-fat diet in mice. Life Sci. 90(25-26):1001-1009. PMCID:PMC3390972.

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Kamal AS, Rohr AC, Mukherjee B, Morishita M, Keeler GJ, Harkema JR, Wagner JG. (2011) PM2.5-induced changes in cardiac function of hypertensive rats depend on wind direction and specific sources in Steubenville, Ohio. Inhal Toxicol. 23(7):417-430. PMID:21639710.

Maniar-Hew K, Postlethwait EM, Fanucchi MV, Ballinger CA, Evans MJ, Harkema JR, Carey SA, McDonald RJ, Bartolucci AA, Miller LA. (2011) Postnatal episodic ozone results in persistent attenuation of pulmonary and peripheral blood responses to LPS challenge. Am J Physiol Lung Cell Mol Physiol. 300(3):L462-471. PMCID:PMC3064293.

Morishita M, Keeler GJ, Kamal AS, Wagner JG, Harkema JR, Rohr AC. (2011) Identification of ambient $PM_{2.5}$ sources and analysis of pollution episodes in Detroit, Michigan using highly time-resolved measurements. Atmospheric Environment 45(8):1627-1637.

Morishita M, Keeler GJ, Kamal AS, Wagner JG, Harkema JR, Rohr AC. (2011) Source identification of ambient $PM_{2.5}$ for inhalation exposure studies in Steubenville, Ohio using highly time-resolved measurements. Atmospheric Environment 45:7688-7697.

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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.

Rohr AC, Kamal A, Morishita M, Mukherjee B, Keeler GJ, Harkema JR, Wagner JG. (2010) Altered heart rate variability in spontaneously hypertensive rats is associated with specific particulate matter components in Detroit, Michigan. Environ Health Perspect. 119(4):474-480. PMCID:PMC3080928.

ABSTRACTS/ORAL PRESENTATIONS:

Allen KM, Brooks P, Dereski M, Lewandowski RP, Hotchkiss I, Jackson-Humbles D, Brandenberger C, Bramble LA, Wagner JG, and JR Harkema. (2013) Inhaled Ozone Induces Metabolic Abnormalities in Mice Fed a High-Fructose Diet. Oral Presentation at: Annual Meeting of the Society of Toxicology - March 2013. The Toxicologist 132(1):A62.

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.

Harkema JR. (2013) Interface of Health Effects caused by the CardioMetabolic Syndrome and Exposures to Air Pollutant Mixtures. Oral Presentation at the Society of Toxicology Webinar sponsored by the Risk Assessment and Mixtures Specialty Sections - April 2013.

Harkema JR and Brook RD. (2013) GLACIER Center Update. Oral Presentation at the EPA CLARC Annual Meeting,

Seattle, WA - July 2013.

Liu C, et al. (2013) Central IKKb Inhibition Prevents Particulate Matter Mediated Peripheral Inflammation and Exaggeration of Type II Diabetes. Submitted to the EPA CLARC Annual Meeting, Seattle, WA - July 2013.

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