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Acute Peat Smoke Inhalation Sensitizes Rats to the Postprandial Cardiometabolic Effects of a High Fat Oral Load

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Abstract

Wildland fire emissions cause adverse cardiopulmonary outcomes, yet controlled exposure studies to characterize health impacts of specific biomass sources have been complicated by the often latent effects of air pollution. The aim of this study was to determine if postprandial responses after a high fat challenge, long used clinically to predict cardiovascular risk, would unmask latent cardiometabolic responses in rats exposed to peat smoke, a key wildland fire air pollution source. Male Wistar Kyoto rats were exposed once (1 hr) to filtered air (FA), or low (0.36 mg/m³ particulate matter) or high concentrations (3.30 mg/m³) of peat smoke, generated by burning peat from an Irish bog. Rats were then fasted overnight, and then administered an oral gavage of a HF suspension (60 kcal% from fat), mimicking a HF meal, 24 hr post-exposure. In one cohort, cardiac and superior mesenteric artery function were assessed using high frequency ultrasound 2 hr post gavage. In a second cohort, circulating lipids and hormones, pulmonary and systemic inflammatory markers, and circulating monocyte phenotype using flow cytometry were assessed before or 2 or 6 hr after gavage. HF gavage alone elicited increases in circulating lipids characteristic of postprandial responses to a HF meal. Few effects were evident after peat exposure in un-gavaged rats. By contrast, exposure to low or high peat caused several changes relative to FA-exposed rats 2 and 6 hr post HF gavage including increased heart isovolumic relaxation time, decreased serum glucose and insulin, increased CD11 b/c-expressing blood monocytes, increased serum total cholesterol, alpha-1 acid glycoprotein, and alpha-2 macroglobulin ($p = 0.063$), decreased serum corticosterone, and increased lung gamma-glutamyl transferase. In summary,

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Brandi Martin performed all of the work related to this study at the U.S. EPA location listed above.

these findings demonstrate that HF challenge reveals effects of air pollution that may otherwise be imperceptible, particularly at low exposure levels, and suggest exposure may sensitize the body to mild inflammatory triggers.

Keywords

Air Pollution; Exposure; Cardiovascular; Metabolic; Risk; Health

1.0: Introduction

Wildland fires represent a major source of air pollution, and are increasingly linked to adverse health impacts related to poor air quality (Haikerwal et al., 2015). Moreover, nearly 40 % of total suspended black carbon (a key component of particulate matter) in the United States is linked to biomass burning (Agency, 2013), increasing the need for understanding the associated risks from exposure to various biomass combustion emissions. The cardiopulmonary effects of air pollution exposure, however, are often latent (Agency, 2009), posing a significant challenge to controlled exposure studies that rely on measures of spontaneous effects. Interestingly, air pollution exposure has been found to modify responses to stressors (e.g. exercise). For instance, Volpino et al. (2004) demonstrated that traffic policemen exposed to ambient air pollution and then subjected to exercise had exaggerated changes in cardiopulmonary function only after exercise (Volpino et al., 2004). Analogous responses to air pollution were evident in our previous findings in rodents using treadmill exercise (Carll et al., 2013) or sympathomimetic infusion (Hazari et al., 2012).

Day-to-day activities beyond exercise also stress the cardiovascular system, and when modeled experimentally, may have similar utility in demonstrating latent effects of air pollution exposure. For example, consumption of a high fat (HF) meal causes multiple transient effects including endothelial and microvascular dysfunction (Vogel et al., 1997), increases in LDL cholesterol and triglycerides (Langsted et al., 2008), oxidative stress and inflammation (de Vries et al., 2014), and altered insulin sensitivity (Robertson et al., 2002) and pulmonary function (Rosenkranz et al., 2010). Moreover, the severity of such postprandial responses varies depending upon a host of intrinsic (e.g. sex, disease, age) and extrinsic (e.g. diet, exercise) factors and as such has long been used as a tool much like exercise tests to define elevated or diminished risk. For example, postprandial responses after a HF meal revealed greater endothelial dysfunction in patients with elevated blood triglycerides (Giannattasio et al., 2005), hypotension in type 2 diabetics (Smits et al., 2014), and greater oxidative stress in men compared to women (Bloomer and Fisher-Wellman, 2010). Conversely, co-ingestion of dietary anti-oxidants improved vascular responses (Plotnick et al., 2003), while exercise diminished inflammatory responses (Fuller et al., 2017). Postprandial responses, previously unexplored in air pollution health effects studies, may prove similarly useful in indicating risk from exposure.

Peat, a fuel source comprised of decaying vegetation found in wetlands, emits more fine particulate matter (PM) when burned than any other type of wildland fire (Rein, 2013). Moreover, short-term exposure to smoldering peat air pollution in eastern North Carolina

during separate burns in 2008 and 2011 led to increased cardiopulmonary emergency room visits (Rappold et al., 2011; Tinling et al., 2016). The purpose of this study was to assess the effects of a one-time inhalation exposure to smoldering peat smoke on postprandial cardiopulmonary responses after a HF oral load in rats. To mimic a single HF meal challenge, an approach using oral gavage of a HF suspension was developed. To coincide with reported peak postprandial functional consequences, one cohort of rats was exposed to low or high concentrations of smoldering peat smoke, administered a HF gavage suspension one day later, and then examined for cardiac and superior mesenteric artery function using high frequency ultrasound 2 hr post gavage. A second cohort of similarly exposed rats was assessed 24 hr after exposure for time-dependent changes in systemic lipids and hormones, and pulmonary and systemic inflammatory markers immediately before, or 2 hr or 6 hr after HF gavage.

2.0: Methods

2.1: Animals

12 week-old male Wistar Kyoto (WKY) rats (Charles River Laboratories Inc., Raleigh, NC) (n = 6-8, depending on the experiment) were housed 2/cage in polycarbonate cages, maintained on a 12 hr light/dark cycle at approximately 22°C and 50% relative humidity in our Association for Assessment and Accreditation of Laboratory Animal Care-approved facility, and held for a minimum of one week before exposure. All animals received standard (5001) Purina pellet rat chow (Brentwood, MO) and water ad libitum unless otherwise stated. The Institutional Animal Care and Use Committee of the U.S. Environmental Protection Agency (U.S. EPA) approved all protocols.

2.2: Experimental Design

2.2.1: Study Layout and Group Size Determinations—Three experiments were performed within this study to examine the impacts of air pollution exposure on postprandial responses (Fig. 1). Experiment A describes the development of a HF challenge using oral gavage administration of a HF emulsion. Experiment B describes the use of the HF oral gavage challenge to assess the priming effects of exposure on cardiovascular functional responses to a HF oral load. Experiment C describes the impacts of air pollution exposure on the time-dependent postprandial changes in metabolic, systemic and pulmonary factors after HF gavage challenge.

For Experiment A, a group size of n = 6 was selected since this was a pilot study and was done to match group size from a previous study with analogous species and endpoints (Farraj et al., 2016). To determine group size for Experiments B and C, sample size analysis was done based on Experiment A lipid data. Sample size analysis was performed using R Studio software with the 'pwr' package and 'pwr.anova.test' command. Original sample size analyses based on changes in triglyceride levels between the HF group and vehicle group in Experiment A were too robust to yield a meaningful sample size value (yielded n = 2). Instead, the analysis was based on blood LDL measurements. Sample size analysis was based on the (k) number of experimental groups (k = 3 groups: filtered air, low peat and high peat), a significance level = 0.05, a power = 0.8, and the effect size index (f), which is

derived by multiplying the expected effect size (d) by the standard deviation (SD). We calculated d (effect range/SD), using the widest LDL SD from Experiment A, in this case the vehicle group SD. The widest effect range (i.e. range of means) was between the low fat and the HF groups ($8.6 - 6.8 = 1.8$). These calculations yielded a $d = 1.5$ and an $f = 0.75$. Using these values, n was calculated at 6.81, or 7 rats per group. In order to offset potential oversights in analysis parameters we selected an $n = 8$ for Experiments B and C.

2.2.2: Experiment A: Development of a High Fat Challenge—The goal of Experiment A was the development and characterization of an oral gavage regimen that allowed for bolus administration of a HF emulsion that mimics the systemic effects of a single HF meal for the purpose of later assessing postprandial responses after air pollution exposure. To that end, rats ($n = 6$ per group) received a single 10 mL/kg (3 mL for a 300-gram rat) oral gavage of one of the following three suspensions immediately after an overnight fast (8 hours: 12 AM to 8 AM; rats were given access to water ad libitum during this period): 1) a 1 kcal/mL HF emulsion that derived 60% calories from fat (i.e. 60 kcal%; water-suspendable rodent diet (Product # D12492L, Research Diets Inc., New Brunswick, NJ)), 2) an isocaloric 1 kcal/mL emulsion that derived 10% of calories from fat (i.e. 10 kcal %; most derived from carbohydrates, water suspendable rodent diet (Product # D12450JL, Research Diets Inc., New Brunswick, NJ)), or 3) water vehicle. The HF diet suspension contained 60 kcal% fat (lard and soybean oil), 20 kcal% protein (casein) and 20 kcal% carbohydrate (corn starch, maltodextrin 10, and sucrose). A 1 kcal/mL HF suspension was prepared by mixing 191 g of the dry HF suspendable diet (contained 200 g casein, 3 g L-cystine, 125 g maltodextrin 10, 68.8 g sucrose, 40 g cellulose, 10 g Xanthan Gum, 25 g soybean oil, 245 g lard, as well as vitamins and minerals per 773.85 g of diet) with 809 mL water. The low fat diet suspension contained 10 kcal% fat (lard and soybean oil), 20 kcal% protein (casein), and 70 kcal% carbohydrate (cornstarch, maltodextrin 10, and sucrose). A 1 kcal/mL low fat suspension was prepared by mixing 260 g of the dry low fat suspendable diet (contained 200 g casein, 3 g L-cystine, 506.2 g corn starch, 125 g maltodextrin 10, 68.8 g sucrose, 40 g cellulose, 10 g xanthan gum, 25 g soybean oil, 20 g lard, as well as vitamins and minerals per 1055 g of diet) with 740 mL water. For comparison, the standard rat chow (Laboratory Rodent Diet 5001; Barnes Supply Co., Durham, NC) used at this facility contains 13.5% fat, 28.5 % protein, and 58 % carbohydrate, made with predominantly ground corn, dehulled soybean meal, dried beet pulp, fish meal and ground oats. The percent fat of the HF emulsion was selected to approximate the fat content of high fat fast food meals (Paeratakul et al., 2003). In addition, administration of 10 mL/kg of a 1 kcal/mL suspension in rats is analogous to a 700 kcal meal in a 70 kg person, which is in the calorie range of typical fast food meals (Schoffman et al., 2016). Echocardiography (i.e. noninvasive ultrasound assessment of cardiac structure and mechanical function) and whole body plethysmography (an approach used to assess breathing/ventilation) were used to assess cardiopulmonary function 2-4 hr after gavage. Furthermore, systemic lipid and inflammatory responses in blood, lung, heart, and liver tissue were also assessed. Sensitivity to aconitine-induced arrhythmia was also assessed after gavage (aconitine is a cardiotoxic substance widely used to produce cardiac arrhythmia).

2.2.3: Experiment B: Cardiovascular Functional Responses after High Fat

Challenge—The goal of Experiment B was to assess the impacts of a single exposure to low and high concentrations of smoldering peat combustion emissions on cardiovascular function measured using cardiovascular ultrasound, shortly after a HF oral load (i.e. 60 kcal %, 1 kcal/mL). As such, all animals (n = 8 per group) exposed once for 1 hr to either filtered air, low peat, or high peat underwent an 8 hr fast from food beginning 16 hr after exposure. All animals were then administered a HF oral gavage approximately 24 hr after exposure to smoldering peat and underwent cardiovascular ultrasound beginning 2 hr after gavage.

2.2.4: Experiment C: Time-dependent Postprandial changes in Pulmonary and Systemic Endpoints

—The goal of Experiment C was to assess the impacts of a single exposure to smoldering peat combustion emissions on time-dependent changes in postprandial lipids, hormones, and pulmonary and systemic indicators of inflammation and injury. The time course of responses post gavage (0, 2 and 6 hr) for each exposure group (FA, low peat, and high peat) were examined using separate cohorts of rats (n = 8 in each exposure group and each time point). Time = 0 hr rats were exposed to smoldering peat or air, fasted overnight, and euthanized at ~ 8 am. Time = 2 hr rats were exposed to smoldering peat or air, fasted overnight, and received a HF gavage at 8 am followed by euthanasia 2 hr later. Time = 6 hr rats were exposed to smoldering peat or air, fasted overnight, and received a HF gavage at 8 am followed by euthanasia 6 hr later.

2.3: Peat Smoke Inhalation Exposure

2.3.1: Acclimation to Exposure Chambers and Peat Smoke Concentrations

—All animals were acclimated twice in thirty minute increments to a full-body inhalation chamber at room temperature over a 2-day period leading up to exposure. We used Irish peat briquettes from County Clare, Ireland for our biomass fuel source (Glynn Bros., Boston, MA). Animals were exposed once for a duration of 1 hr to smoke generated using an automated control tube furnace system wherein PM was diluted to low (target concentration = 0.4 mg/m³) or high (target concentration = 4.0 mg/m³) concentrations (actual concentrations are listed in Table 2). The low concentration target was chosen based on ambient particulate concentrations typical in highly polluted cities (i.e. Delhi, India) and in order to establish a dose response, we selected the high concentration to be one order of magnitude higher (Delhi, 2018). Importantly, this high concentration is on par with respirable particulate matter exposure levels experienced by firefighters while combating wildland fires (Swiston et al., 2008).

2.3.2: Tube Furnace Exposure System and PM and Gas Monitoring

—We generated smoldering peat smoke using an automated quartz-tube furnace system. An automated mass flow controller (Mass-Flo, MKS Instrument, Inc., Andover, MA) based on a proportional-integral-derivative (PID) feedback loop was incorporated into the system to precisely control smoke concentration. Biomass smoke (2 L/min) generated from the tube furnace system was diluted with air (~3 and ~60 L/min for 1st and 2nd dilution air, respectively) and then delivered to a whole body inhalation chamber (0.3 m³ Hinners style stainless steel and glass exposure chamber) (Hinners et al., 1968). The smoke concentration was continuously monitored and adjusted by the PID feedback control loop linked to a

continuous PM monitor in the chamber to an exhaust flow control valve in a smoke inlet line. The adjustment was made immediately as soon as a change in PM concentration in the chamber was detected to maintain the PM concentration close to its set point (<10% of the target set point). The peat smoke in the chamber was maintained at a temperature of ~72 °F, and humidity of ~40 %RH, controlled by a humidifier. A pressure gauge (Magnehelic, Dwyer Instruments Inc., Michigan City, IN) was placed in the chamber to ensure constant pressure throughout the inhalation exposure. We monitored carbon dioxide (CO₂) and carbon monoxide (CO) levels using a non-dispersive infrared analyzer (Model: 602 CO/CO₂; CAI Inc., Orange, CA) and nitrogen oxides (NO, NO₂, and NO_x) using a chemiluminescent analyzer (Model: 42i NO/NO₂/NO_x; Thermo Scientific, Franklin, MA). We also collected PM on a glass-fiber filter installed in an exhaust line of the inhalation chamber to determine mean PM concentrations gravimetrically by weighing the filter before and after inhalation exposure. The real-time measurements of biomass smoke properties and engineering parameters (e.g., temperature, relative humidity, static pressure, and flow rate) were continuously monitored, recorded, and displayed using data acquisition software (Dasylab version 13.0, National Instruments, Austin, TX).

2.3.3: PM Sampling during Exposure: We conducted PM sampling through ports on the inhalation chamber during combustion for chemical speciation. PM in the biomass smoke and FA (control) were collected on a pre-baked quartz filter for analysis of carbon species. The flow rate of PM sampling controlled by a vacuum controller (Model: XC-40; Apex Instruments Inc., Fuquay-Varina, NC) was ~ 1 L/min.

2.4: Oral Gavage Administration of High Fat Emulsion

To ensure that the rats avoided consumption of their own fecal matter during the fasting period, all animals were transferred to cages with wire platforms with only access to water overnight for a duration of 8 hr. For those animals exposed to peat smoke (Experiments B and C), this occurred 16 hr post exposure. After the conclusion of the fasting period (Time = 24 hr post-exposure), animals were manually restrained (unanesthetized) and administered a 10 mL/kg body weight of a 1 kcal HF emulsion in water via oral gavage using an 18G gavage feeding needle, 3 inches in length with a 2.25 mm ball diameter. Because the HF diet derives 60% of its calories from fat and will separate quickly, the suspension was freshly made just before gavage by blending the diet with distilled deionized water in a standard food blender.

2.5: Ultrasound Echocardiography

Cardiac function of animals in Experiments A and B was determined 2 hr after oral gavage using a high frequency echocardiography ultrasound system (Vevo 2100, FujiFilm Visual Sonics Inc., Toronto, Canada). Animals were first placed in a sealed whole-body chamber and anesthetized with 2-5% isoflurane delivered in 100% O₂ at 0.8-1 L/min. Once anesthetized, animals were transferred to a heated ECG-monitoring table in dorsal recumbency where anesthesia levels were maintained with 1-3% Isoflurane (100% O₂ @ 0.8-1 L/min) via a nose cone. Eye lubricant was placed on the subject's eyes to avoid ocular drying, and each paw was grounded to ECG electrodes coated with Electrode Crème (Cat# 600-0001-01-S, Indus Instruments, Webster, TX) for physiological monitoring/recording of

electrocardiogram, and heart and respiratory rate. The surface of the Vevo[®] Rat Handling Table was set to 38 °C to support animal core temperature. The subject's chest and upper abdomen were shaved using an electric razor, and Nair gel was used to remove any remaining fur from the imaging location. The application area was wiped with clean gauze to remove any residual Nair from the skin. Pre-warmed ultrasound gel was applied to the chest prior to imaging. An MS-201 transducer was used to noninvasively record 3 video loops of the parasternal long axis views of the left ventricle in M-mode (15 MHz) for functional measurements and pulsed wave Doppler (12.5 MHz) of pulmonary artery and transmitral blood flow. The sonographer was blinded to exposure group identities.

2.6: Echocardiographic Analysis

Echocardiography data analyses were also performed while blinded to identities of exposure groups. Short-axis B-mode cine-loops were used to analyze wall deformation in the lateral and anterior free walls of the left ventricle via speckle tracking echocardiography (STE) using VevoStrain[™] software (FujiFilm VisualSonics Inc., Toronto, Canada). Peak circumferential strain, peak diastolic circumferential strain rate, and contractile synchrony between the lateral wall and the anterior free wall of left ventricular endocardium were evaluated. The time that elapsed between peak strain in the lateral and anterior free walls was reported as circumferential strain “delay.” For STE data, two beats from each of three cine-loops were analysed, yielding a total of six beats analyzed per animal at each time-point. For the remaining endpoints, two beats between breaths from each of the three cine-loops were collected. This yielded a total of six beats analyzed per animal at each time-point. We used Vevo[®] LAB software version 1.7.0 (FujiFilm VisualSonics Inc.) to analyse data collected via pulsed wave Doppler of transmitral flow in order to determine isovolumic contraction time (IVCT), aortic ejection time (AET), and isovolumic relaxation time (IVRT) (see Fig. 2d–f). The Tei index of myocardial performance was calculated with the following equation: $(IVCT + IVRT)/AET$. Short-axis M-mode loops were used to determine stroke volume (SV), cardiac output (CO), ejection fraction (EF), fractional shortening, end diastolic volume (EDV), and end systolic volume (ESV) using Vevo[®] LAB software (FujiFilm VisualSonics Inc.).

2.7: Whole Body Plethysmography

Ventilatory parameters of Experiment A rats only were monitored using the unanesthetized, unrestrained Buxco System (model PLY3223, Buxco Electronics, Wilmington, NC) as previously described (Kodavanti et al., 2011). Rats were placed in whole-body plethysmographs (WBP) and monitored for breathing frequency, tidal volume, minute ventilation and enhanced pause (Penh), 2–4 hr after oral gavage (Experiment A only). Rats were acclimated to the WBP chambers for 2 days prior to exposure. Chamber temperature, humidity, and air flow were continuously monitored throughout the WBP sessions. Food and water were restricted during the WBP sessions.

2.8: Aconitine Challenge Cardiac Arrhythmia Sensitivity Test

Aconitine challenge was performed in rats 2–4 hr after gavage (Experiment A only). Rats were anesthetized with urethane (1.5 g/kg, i.p.) and surgically catheterized into the left jugular vein with saline-filled PE50 tubing for administration of drugs. The experiments

were performed immediately following implantation of the i.v. catheter. Body temperature was supported during and after surgery. Ten mg/mL aconitine (in saline) was infused into the jugular vein at a speed of 0.2 mL/min (Li et al., 2007) using a ISMATECH IPC infusion pump while electrocardiogram (ECG) was continuously monitored and timed (using an external telemeter attached to the skin). Susceptibility was measured as the threshold dose of aconitine required to produce ventricular premature beats, ventricular tachycardia, and ventricular fibrillation and calculated using the following formula:

$$\text{Threshold dose (ug/kg) for arrhythmia} = 10\text{ug/ml} \times 0.2\text{ml/min} \times \text{time required for inducing arrhythmia (min)} \\ \text{/body weight (kg)} = 1\text{ug/min} \times \text{time (min)/body weight (kg)}$$

2.9: Necropsy and Tissue/Blood Collection

2.9.1: Necropsy and Blood Collection: Prior to necropsy, blood glucose levels were measured by pricking through the tip of the tail using a sterile 25-gauge needle (Experiment C rats only). Approximately 1 µl of blood was brought into contact with the glucometer strip attached to a Bayer Contour glucometer. Glucose levels were measured within 5 secs and recorded. Animals were euthanized with I.P. injection of 1 mL/kg pentobarbital (Fatal Plus, Dearborn, MI) diluted (1:1) approximating to ~200 mg/mL. When animals were completely non-responsive to hind paw pinch, blood was collected through the abdominal aorta in three blood collection tubes: one 3.0 mL draw in EDTA tube, one 2.7 mL draw in citrate tube and one 4.0 mL draw in serum separator tube (no coagulant). EDTA and citrate tubes were gently inverted and set on ice. From the EDTA sample was first used for Complete Blood Cell counts (CBC). Red blood cells, white blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, lymphocytes and lymphocytes % (of white blood cells) were measured using a Beckman-Coulter AcT blood analyzer (Beckman-Coulter Inc., Fullerton, CA). EDTA, citrate and serum blood tubes were then centrifuged at 3500 rpm, 4°C for 10 min. The buffy coat from EDTA tubes was collected for flow cytometry (see below). Plasma and serum samples were stored at – 80 °C until further analysis.

2.9.2: Bronchoalveolar Lavage Fluid Collection: The trachea was cannulated and then a suture was used to tie the left lung. The right lung was lavaged using $\text{Ca}^{2+}/\text{Mg}^{2+}$ free phosphate buffered saline (pH 7.4) equal to 28 mL/kg body weight (total lung capacity) × 0.6 (right lung is ~ 60% of total lung w8). Three in-and-out washes were performed using the same aliquot of buffer, and bronchoalveolar lavage fluid (BALF) was transferred to ice until further processing. The left lung lobe was clipped off and frozen at –80°C.

2.9.3: Heart Tissue Collection: The whole heart was weighed and then the left ventricle was collected by excising the heart, cleaning the heart of excess blood then by removing atria and right ventricle. Heart tissue was snap-frozen and stored in a –80° C freezer.

2.10: Measures of Blood Lipids, Hormones, and Mediators of Inflammation

Several serum factors were measured using the Konelab Arena 30 Clinical Chemistry Analyzer (Thermo Clinical LabSystems, Espoo, Finland including: total cholesterol (TC),

triglycerides (TG), creatine kinase (CK), alkaline phosphatase (AP), and glucose (TECO Diagnostics, Anaheim, CA), high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol (Sekisui Diagnostics, PE, Canada). TG, TC, LDL, and HDL in the plasma were also measured using the same kits as their serum counterparts using the Konelab Analyzer since postprandial studies have measured them in both. Serum levels of lpha-1 acid glycoprotein and alpha-2-macroglobulin (Meso Scale Diagnostics, Rockville, MD), insulin (Millipore, Billerica, MA) and corticosterone (Arbor Assays, Ann Arbor, MI) were measured according to manufacturer's protocols. The following endpoints were measured in plasma (EDTA or citrate tubes): free fatty acids (FFA) (Cell Biolabs, Inc., San Diego, CA), fibrinogen, D-dimer, C-reactive protein (CRP), C3 and C4 (Kamiya Biomedical, Seattle, WA), alanine amino-transferase (ALT) and angiotensin-converting enzyme (ACE) (Thermo Scientific, Middletown, VA), and epinephrine (Rocky Mountain Diagnostics, Colorado Springs, CO). A Homeostatic Model Assessment (HOMA) for both insulin resistance (IR) and beta-cell (β) function were calculated using the following formulas:

$$\text{HOMA-IR: } (\text{glucose} \times \text{insulin})/405 \text{ and}$$

$$\text{HOMA-}\beta\text{: } ((360 \times \text{insulin})/(\text{glucose}-63)) \% \text{ (Matthews et al., 1985).}$$

2.11: Cell Differentials and Indicators of Inflammation and Injury in BALF

Aliquots of BALF were used to determine total cell counts with a Z1 Beckman-Coulter Counter (Beckman-Coulter Inc., Miami, FL). A second aliquot was centrifuged (Shandon 3 Cytospin, Shandon, Pittsburgh, PA) to prepare cell differential slides. Slides were dried at room temperature and stained with Leukostat (Fisher Scientific, Pittsburgh, PA). Macrophages, neutrophils, lymphocytes, and eosinophils were counted using light microscopy with at least 200 cells counted on each slide. The remaining BALF was centrifuged at $1500 \times g$ to remove cells, and the supernatant fluid was analyzed for markers of lung injury using the Konelab Analyzer. Separate kits were used to measure total protein (Coomassie Plus Protein Assay Kit; Pierce Biotechnology, Inc., Rockford, IL), albumin (Sekisui Diagnostics, PE, Canada), lactate dehydrogenase (LDH) activity and γ -glutamyl transferase (GGT) activity (Thermo Fisher Diagnostics, Middletown, VA), n-acetylglucosaminidase (NAG) activity (Sigma-Aldrich Diagnostics St. Louis, MO), and total superoxide dismutase (SOD), MnSOD and CuZnSOD activities (Randox Laboratories Ltd. Crumlin County, Antrim, UK). Glutathione peroxidase (GPX) and glutathione reductase (GTR) were measured by adapting a procedure previously described (Jaskot et al., 1983). Total reduced glutathione (GSH) was measured following an adaptation of a procedure previously described (Anderson, 1985). Catalase was measured by following an adaptation of a procedure previously described (Wheeler et al., 1990).

2.12: Flow Cytometry for Blood Monocytes

After centrifugation of blood samples collected in EDTA tubes (3500 rpm, 4°C, 10 min), the buffy coats (200 μ L) were collected and resuspended in staining buffer comprised of

Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+} (Thermo Fisher, Waltham, MA) containing 1 % bovine serum albumin (BSA) and 0.1 % sodium azide (Sigma, St. Louis, MO). Following centrifugation, cells were resuspended in RBC Lysis Buffer (Affymetrix, Santa Clara, CA) for 10 min to lyse red blood cells. Cells were then washed and resuspended in staining buffer. 100 μL aliquot of cell suspensions were incubated for 20 min at room temperature with anti-Rat CD 32 (BD Pharmingen, San Jose, CA) to block FC receptor-mediated nonspecific antibody binding. Cells were labeled for 30 min after incubation with the following monoclonal antibodies: FITC-CD36 (Santa Cruz Biotechnology Inc., Dallas, TX), PE-CD172a, PE/Cy7-CD11b/c, Alexa Fluor 647-CD43, APC/Cy7-CD45 (Biolegend, San Diego, CA). Cells labeled with fluorochrome-conjugated isotype control antibodies (FITC Normal Mouse IgG_{2a}, κ (Santa Cruz Biotechnology Inc., Dallas, TX), PE Mouse IgG_{2a}, κ PE/Cy7 Mouse IgG_{2a}, κ , Alexa Fluor 647 Mouse IgG1 κ , and APC/Cy7 Mouse IgG1 (Biolegend, San Diego, CA)) were used as negative controls. Additional cell samples, including unstained cells and fluorescence minus one (FMO) controls, were utilized to aid in identifying and ensuring accurate gating of negative and positive cell populations. After staining, cells were washed twice with serum-free and azide-free DPBS and incubated for 30 min at room temperature with LIVE/DEAD fixable violet stain (Thermo Fisher Scientific, Waltham, MA) to determine viable cells. Cells were washed 3 times with DPBS staining buffer, fixed with 0.05 % formaldehyde in PBS and kept in the dark at 4°C (no longer than 1 day) until FACS analysis. All samples were collected on a LSR II flow cytometer (BD Biosciences, San Jose, CA) using FACSDiva software (BD Biosciences). Data analysis was performed by using FlowJo software (TreeStar, Inc., Ashland, OR). A minimum of 50,000 events were collected per analysis.

2.13: Statistics

Data are reported as boxplots with all data points shown. Box edges mark the interquartile range, the middle line marks the median, the "+" marks the mean, and the whiskers mark the minimum and maximum data values. GraphPad Prism (GraphPad Software version 7.02, San Diego, CA) was used for all statistical analyses. A 1-Way ANOVA with Tukey's post-test and multiplicity-adjusted p values were conducted for all data presented in the following: Table 1, Table 3 and Fig.2. For all other data a 2-Way ANOVA non-repeated measure with Tukey's post-test and multiplicity adjusted p values were conducted since t = 0, t = 2 and t = 6 hr groups were separate cohorts of rats and no serial blood draws were made. A p value < 0.05 was considered statistically significant.

3.0: Results

3.1: Circulating Lipids After High Fat, Low Fat or Water Gavage in Unexposed Rats (Experiment A)

For all data, statistically significant changes ($p < 0.05$) are mentioned, as well as changes that approach significance ($0.05 < p < 0.1$). Table 1 shows the changes in serum markers at 2 hr post gavage. HF gavage increased triglycerides compared to water ($p < 0.05$) and free fatty acids (FFA) compared to low fat gavage ($p < 0.05$). The FFA increase with HF gavage did not reach the threshold for significance compared to water ($p = 0.07$). HF gavage also increased LDL cholesterol relative to low fat ($p < 0.01$), but this increase did not reach the

threshold for significance compared to water (up 10%, $p = 0.09$). Low fat gavage increased adrenaline levels relative to HF gavage ($p < 0.05$) and water ($p < 0.05$). There were no significant cardiovascular or pulmonary functional changes (data not shown).

3.2: Peat Smoke Exposure Concentrations and Characteristics (for Experiments B and C)

Table 2 shows the composition of the peat smoke. Actual PM concentrations for the high and low peat, i.e. 3.3 mg/m^3 and 0.36 mg/m^3 were slightly lower than target concentrations of 4.0 mg/m^3 and 0.4 mg/m^3 . High peat had, on average, greater particle number and greater particle size. Low peat, in contrast, had greater had a greater percentage of organic carbon. High peat had approximately 5-fold the amount of CO and 2-fold the level of NO_x, although the levels of each gas were very low in each exposure group. High peat also had slightly higher CO₂ levels.

3.3: Cardiac Function 2 hr After HF Gavage in Peat Smoke-Exposed Rats (Experiment B)

Fig. 2 shows changes in cardiac function 2 hr after high fat gavage. Low peat increased isovolumic relaxation time (IVRT) compared to both FA ($p < 0.05$) and high peat ($p < 0.05$), but there were no significant effects of exposure on isovolumic contraction time (IVCT) and aortic ejection time (AET). There was no effect of exposure at either concentration on HR relative to FA, although high peat was significantly greater than low peat (Table 3). High peat increased ejection fraction (EF) and fractional shortening (FS) compared to low peat, although it did not reach the threshold for significance ($p = 0.1$). High peat decreased end systolic volume (ESV) compared to low peat ($p < 0.05$), but not FA ($p = 0.20$), and decreased end diastolic volume (EDV) compared to low peat, although this change did not reach the threshold for significance ($p < 0.1$).

3.4: Metabolic Endpoints in Peat Smoke-exposed Rats with or without HF Gavage (Experiment C)

3.4.1: Time-dependent changes in lipid levels—Peat smoke impacts on lipid levels measured in un-gavaged rats ($T = 0 \text{ hr}$) or 2 ($T = 2 \text{ hr}$) or 6 hr ($T = 6 \text{ hr}$) after HF gavage are shown in Table 4. For the metabolic endpoints, markers of inflammation and monocyte data, effects are first described within each time point and then across time points. There were no effects of peat exposure on serum or plasma TG levels at $T = 0$ and $T = 2 \text{ hr}$. High peat at $T = 6 \text{ hr}$, however, had lower TG levels compared to FA ($p < 0.05$). Serum and plasma TG levels in the FA group at $T = 2 \text{ hr}$ were higher than levels in the corresponding group at $T = 0 \text{ hr}$, although this change did not reach the threshold for significance ($p = 0.053$ and 0.12 , respectively). In addition, TG levels in the high peat group at $T = 6 \text{ hr}$ were significantly lower than levels in the corresponding group at $T = 0 \text{ hr}$ ($p < 0.05$). There were no effects of peat exposure on serum or plasma LDL levels within each of the measured time points. Serum LDL levels in the FA and low peat groups at $T = 2 \text{ hr}$, however, were higher than levels in the corresponding groups at $T = 0 \text{ hr}$ ($p < 0.05$). Serum LDL levels in the high peat group at $T = 2 \text{ hr}$ were also higher than levels in the corresponding group at $T = 0 \text{ hr}$, although this change did not reach the threshold for significance ($p = 0.25$). High peat caused a decrease in serum HDL, but not plasma HDL, relative to FA at $T = 0 \text{ hr}$, although this change did not reach the threshold for significance ($p = 0.098$). HDL levels in the low

peat group at T = 2 hr and T = 6 hr were greater than levels in the corresponding groups at T = 0 hr ($p < 0.05$). Serum HDL levels in the high peat group at T = 2 hr were also greater than levels in the corresponding group at T = 0 hr ($p < 0.05$). There were no effects of peat exposure on serum total cholesterol at T = 0 hr. In contrast, high peat had higher total serum cholesterol levels relative to both FA and low peat at T = 2 hr ($p < 0.05$) and at T = 6 hr, although the comparison with FA at T = 6 hr was below the threshold for significance ($p = 0.081$). Total cholesterol levels in both the FA and low peat groups at T = 2 hr and T = 6 hr were significantly lower than levels in the corresponding groups at T = 0 hr ($p < 0.05$), whereas the levels in high peat did not differ across time. There were no effects of peat exposure on plasma FFA levels within each time point. FFA levels in both the low and high peat groups at T = 6 hr, however, were significantly lower than levels in the corresponding groups at T = 0 hr ($p < 0.05$).

3.4.2: Time-dependent changes in glucose and hormones levels—Peat smoke impacts on glucose, insulin and corticosterone levels measured in un-gavaged rats (T = 0 hr), 2 hr (T = 2 hr) or 6 hr (T = 6 hr) after HF gavage are shown in Fig. 3. There were no effects of peat exposure on blood glucose or serum insulin levels at T = 0 hr or T = 6 hr. In contrast, low peat decreased glucose, insulin and HOMA-IR levels relative to FA at T = 2 hr ($p < 0.05$). Insulin levels at T = 6 hr in the high peat group and HOMA-IR levels at T = 6 hr in the FA group were significantly greater than levels in the corresponding group at T = 0 hr ($p < 0.05$). There were no significant changes in HOMA- β data and is therefore not shown. There were no effects of peat exposure on corticosterone levels at T = 0 and T = 2 hr. High peat exposure, however, decreased corticosterone levels relative to FA at T = 6 hr, although this change did not reach the threshold for significance ($p = 0.085$). Corticosterone levels in the FA group were less at T = 2 hr ($p < 0.05$) and conversely greater at T = 6 hr ($p < 0.05$) compared to levels in the corresponding group at T = 0 hr.

3.5: Markers of Inflammation in Peat Smoke-Exposed Rats with or without HF Gavage (Experiment C)

3.5.1: Time-dependent changes in Indicators of Pulmonary Injury and Inflammation—Peat smoke impacts on pulmonary and systemic markers of inflammation and injury measured in ungavaged rats at T = 0 hr or after HF gavage are shown in Fig. 4. There were no effects of peat exposure on lung MIA levels within each of the measured time points. MIA levels in the high peat group at T = 2 hr, however, were higher than levels in the corresponding group at T = 0 hr (Fig. 4a; $p < 0.05$). There were no effects of peat exposure on lung GGT levels at T = 0 hr. In contrast, both low and high peat increased lung GGT levels relative to FA at T = 2 hr (Fig. 4b; $p < 0.05$). Lung GGT levels in the FA group at T = 2 hrs were less than the levels in the corresponding group at T = 0 hr, whereas GGT levels in the low peat group at T = 2 hr were greater than levels in the corresponding group at T = 0 hr ($p < 0.05$). There were little to no effects of exposure and/or gavage on other indicators of pulmonary injury and inflammation (data not shown).

3.5.2: Time-dependent changes in Systemic Indicators of Inflammation—There were no effects of peat exposure on serum A2M or AGP levels at T = 0 hr or T = 6 hr (Fig. 4). In contrast, high peat increased A2M and AGP levels relative to low peat and FA

($p < 0.05$), although the difference with FA for A2M did not reach the threshold for significance ($p = 0.063$). A2M and AGP levels in the FA group at $T = 6$ hrs were greater than the levels in both endpoints in the corresponding group at $T = 0$ hr, although the difference with $T = 0$ hr for A2M did not reach the threshold for significance ($p = 0.088$). There were little to no effects of exposure and/or gavage on complete blood count endpoints (Table 5) and other indicators of systemic injury and inflammation (data not shown).

3.6: Blood Monocytes in Peat Smoke-exposed Rats with or without HF Gavage (Experiment C)

Peat smoke impacts on blood monocytes measured in un-gavaged rats ($T = 0$ hr) or 6 hr ($T = 6$ hr) after HF gavage are shown in Fig. 5. Fig. 5a illustrates the selections made for quantification of white blood cells, CD45+ viable cells, and for granulocytes and monocytes. There were no effects of peat exposure on the percentage of WBCs that were monocytes within each time point (Fig. 5b). However, the percentage of WBCs that were monocytes in all exposure groups at $T = 6$ hr were greater than levels in corresponding groups at $T = 0$ hr ($p < 0.05$). Low peat increased the percentage of monocytes that were classical monocytes relative to FA at $T = 0$ hr and at $T = 6$ hr (Fig. 5c; $p < 0.05$). Finally, there were no effects of peat exposure on the percentage of monocytes that were CD11 b/c monocytes at $T = 0$ hr. Low peat at $T = 6$ hr, however, had elevated levels of CD11 b/c monocytes compared to FA and high peat at $T = 6$ hr and relative to the corresponding group at $T = 0$ hr.

4.0: Discussion

The present findings illustrate that acute exposure to a wildland fire-related biomass combustion emission modifies responses to a HF oral load (summarized in Table 6). Peat smoke was generated using a glass tube furnace system that allowed for automated combustion and has been previously used to compare health effects of different biomass fuel sources including peat (Kim et al., 2018). A simple approach using oral gavage of a HF suspension was developed and used to mimic a HF meal to examine the impacts of air pollution exposure on postprandial responses. Exposure to smoldering fumes from peat biomass combustion followed by a HF gavage caused several cardiopulmonary, systemic, and metabolic effects distinct from similarly challenge rats exposed to filtered air.

Oral gavage of a HF suspension in naïve unexposed rats caused a significant increase in TG levels and LDL cholesterol, characteristic of human postprandial responses after consumption of a HF meal (Teeman et al., 2016). By contrast, only the low fat suspension caused a significant increase in adrenaline in naïve rats, consistent with previous reports (Heseltine et al., 1990), and an effect likely driven by a comparatively high carbohydrate load from a starch-rich (i.e. maltose) suspension. There were few measurable functional changes after oral gavage of any suspension.

HF gavage after exposure to low peat smoke caused an increase in isovolumic relaxation time, an effect not observed with exposure to high peat. This effect usually indicates poor myocardial relaxation and impaired diastolic function in humans (Gibson and Francis, 2003). Furthermore, these data are consistent with reported effects of air pollutant exposure in humans and experimental models. For example, long-term exposure to ambient air

pollution in a cohort of aging women was associated with diastolic dysfunction (Ohlwein et al., 2016). Similarly, passive smoking was linked to impaired left ventricular diastolic function in healthy volunteers (Dogan et al., 2011) and tobacco smoke exposure over the course of 5 weeks increased IVRT in rats (Gu et al., 2008). There were few other cardiac effects of exposure and although there was also no measurable change in superior mesenteric artery flow after exposure, use of techniques that focus on endothelial or microvascular function in future studies may more readily uncover vascular effects of exposure. Likewise, more pronounced cardiac and vascular functional changes may present with repeated/chronic exposure to air pollution.

Peat smoke exposure caused metabolic dysregulation after HF gavage. Exposure to high peat smoke caused a significant increase in serum total cholesterol at T = 2 hr and T = 6 hr post gavage, an effect not evident in similarly challenged rats exposed to low peat or smoke-exposed T = 0 rats that did not receive an oral HF load. Postprandial lipemia, a sustained increase in circulating lipids following a meal, has been linked to promotion of thrombosis and atherosclerotic processes including foam cell formation and is a known cardiovascular risk factor (Badimon et al., 2012). Interestingly, however, there were no effects of HF gavage in smoke-exposed rats on LDL or HDL cholesterol or TG, making the significance of the total cholesterol findings unclear. Moreover, low, but not high, peat smoke caused decreases in circulating levels of glucose, insulin and HOMA-IR at T = 2 hr. HF meals alone typically cause postprandial increases in glucose and insulin (Shin et al., 2009) and HOMA-IR (Wang et al., 2017), consistent with the findings with high fat gavage in filter air-exposed rats in the present study, and responses attributed to FFA-mediated inhibition of insulin-induced glucose uptake in skeletal muscle (Boden et al., 1994) and meal-induced activation of insulin-secreting beta cells, respectively (Sears and Perry, 2015). The mechanisms driving these peat effects are unclear, but may be related to altered activity of the autonomic nervous system, which many studies have shown is a prominent effect of air pollution exposure (Brook, 2008) (Kodavanti, 2016) (Miller et al., 2016b) (Miller et al., 2016a). Activation of parasympathetic fibers innervating the liver decreases circulating glucose levels by favoring glycogen synthesis, whereas sympathetic hepatic nerve activation has the opposite effect (Bruinstroop et al., 2013). Furthermore, vagus nerve stimulation has been linked to decreases in postprandial levels of insulin after ingestion of a mixed meal (Tang et al., 2017). Notably, chronic liver disease is often associated with impaired cardiac autonomic tone (Frith and Newton, 2011). Further research is required to fully characterize the impacts of air pollution on postprandial metabolic changes and any potential short- and long-term ramifications on cardiovascular function.

Peat smoke increased pulmonary and systemic inflammation after HF gavage. Exposure to either low or high peat smoke caused a significant increase in lung GGT levels at T = 2 hr post HF gavage that subsided by 6 hr post gavage. Importantly, exposure-induced changes in lung GGT were not evident in smoke-exposed T = 0 hr rats that did not receive an oral HF load. Given that HF meals on their own alter pulmonary function (Rosenkranz et al., 2010), these findings suggest that exposure to air pollution may prime the respiratory tract to heightened responses to pro-inflammatory triggers including HF meals. Although GGT is typically associated with the synthesis of glutathione in liver and kidneys (Goldberg, 1980), it plays an important role in regulating oxidative stress in the lungs (Jean et al., 2003), and

may indicate some level of lung injury and inflammation, since an increase in GGT has been shown to be associated with increases in proinflammatory cytokines. For example, mice that were exposed to unleaded gasoline exhaust showed elevated levels lung GGT in addition to elevated levels of tumor necrosis factor- α and interleukin-6, indicating Type II alveolar epithelial cell injury (Sureshkumar et al., 2005). Unsurprisingly, intra-pulmonary administration of organic extracts of PM collected from a peat wildfire in North Carolina also caused an increase in GGT in mice (Kim et al., 2014). Soluble mediators of inflammation that are released in the lung in response to air pollution exposure have been postulated to leak in to the circulation triggering systemic inflammatory responses (Brook et al., 2010). While the origin of the inflammatory insult is not known for this study, exposure to high peat smoke, which caused a greater lung GGT response than low peat, also caused a systemic inflammatory response as indicated by increases in serum A2M and AGP levels at T = 2 hr post high fat gavage that subsided by 6 hr post gavage. Increases in circulating A2M and AGP have been previously demonstrated after ozone exposure in rats (Bass et al., 2013). Because these acute phase proteins are likely originating from the liver (Blackburn, 1994), it is plausible that A2M and AGP release was in part mediated by autonomic stimulation.

Perhaps most compelling is our finding that smoke exposure caused changes in circulating monocyte phenotype after HF gavage. While HF gavage alone increased the proportion of white blood cells that were monocytes, confirming the potential for HF consumption to elicit a mild inflammatory response, only low peat caused an increase in CD11 b/c expressing monocytes at T = 6 hr post gavage. This effect was not evident in similarly exposed T = 0 hr un-gavaged rats. CD11 b/c monocytes are pro-inflammatory and pro-atherogenic and have been previously linked to accelerated vascular wall remodeling in a mouse model of vascular wall injury (Martinez et al., 2015). It is possible that classical monocytes converted to CD11 b/c expressing monocytes after gavage, since exposure to low peat alone caused an increase in classical monocytes in T = 0 hr un-gavaged rats. Classical monocytes are linked to inflammatory responses (Kratofil et al., 2017) as evidenced by increases in these cells after pulmonary exposure to lipopolysaccharide in rats (Barnett-Vanes et al., 2016). These findings collectively indicate that HF challenge after even short-term air pollution exposure causes a transient shift towards a pro-inflammatory phenotype that when extrapolated over a life time of exposure and consumption of a HF diet may set the stage for initiation and/or progression of cardiovascular diseases including atherosclerosis.

The biological responses to low and high peat smoke exposure point to an absence of a traditional concentration-response pattern, wherein the magnitude of responses increases with higher exposure concentration/dose. In fact, unique low dose effects have been previously reported including the elicitation of a lung cytokine expression pattern with repeated low dose diesel exhaust exposure that was absent at higher doses in a mouse model (Saito et al., 2002). We previously reported elicitation of electrocardiographic alterations (i.e. ST depression) with a single exposure to diesel exhaust gases that was not evident at the higher concentration (Lamb et al., 2012). Furthermore, we demonstrated that exposure to low concentrations of residual oil fly ash ((Hazari et al., 2009); (Farraj et al., 2011)) or diesel exhaust (Hazari et al., 2011) were as potent in increasing sensitivity to cardiac arrhythmogenic challenge as higher concentrations, while eliciting disparate electrocardiographic and autonomic effects. The precise phenomena driving these effects are

unclear, but reflect the complexity inherent in particle-gas mixtures, the health effects of which are likely determined by the interplay of physicochemical and biological mechanisms at each exposure dose. Although the low and high peat smoke had similar organic carbon content, pointing to potentially comparable chemical composition, low peat had smaller particle size, which may have influenced the response pattern observed. Compared to larger particles, smaller particles have a greater capacity for deep lung penetration and likewise elicitation of pulmonary and systemic inflammatory responses and injury (Brook et al., 2010). Furthermore, the results from the present and previous studies are congruent with findings from Pope et al. (2009) who in an assessment of data from a large American Cancer Society cohort found that the exposure-response relationship between fine particulate exposure and cardiovascular mortality was in fact nonlinear.

Many postprandial studies are designed to allow serial assessments of blood levels of lipids and other factors in the same subject over time after meals. The findings of the present study are limited because of the use of separate cohorts at different time intervals. This was largely done to enable assessment of other endpoints including pulmonary inflammation and injury at each interval after HF gavage. In addition, while the ultrasounds performed after gavage were originally planned to take place 2 hr after gavage, they actually took place 2 -5 hr after gavage largely because of the sequential nature of the ultrasound scans. Although the exposure groups were randomly scanned, this disparity in timing may have influenced registration of effects, particularly since peak postprandial cardiovascular responses after a HF meal are transient (Kearney et al., 1995). Notably, many effects that were described in the present study, while statistically significant, were small in magnitude, and other effects, did not reach the threshold for significance. These findings therefore limit the conclusions that can be made about the effects of exposure on postprandial responses. Nonetheless, the changes in aggregate point to exposure-induced enhanced sensitivity to the cardiometabolic effects of a high fat load. Finally, approximately 10% of the rats were excluded from Experiments B and C due to pre-existing spontaneous cardiomegaly that was unrelated to exposure or treatment.

5.0: Conclusions:

In conclusion, these findings demonstrate that non-specific stressors of the cardiovascular system like HF meal consumption may reveal effects of air pollution that would otherwise be imperceptible, particularly at low exposure levels, and when modeled experimentally, may limit the potential for mischaracterization and/or underestimation of the impacts of exposure. Importantly, peat smoke exposure sensitized the body to HF challenge resulting in exaggerated postprandial responses. Given the increased prevalence of air pollution episodes such as wildland fires and the pervasiveness of diets rich in fat, such effects may set the stage for development and/or progression of cardiovascular disease. Furthermore, the $PM_{2.5}$ concentrations used in this study, particularly with low peat, were comparable to ambient concentrations reported in peat bog fires (e.g. $PM_{2.5}$ exceeded $100 \mu g/m^3$ over a 24 hr period in North Carolina, USA) (Tinling et al., 2016) and on par with recent peak ambient concentrations in highly polluted cities (e.g. $PM_{2.5}$ levels in Anand Vihar, Delhi, India measured $549 \mu g/m^3$ on November 27, 2017) (Delhi, 2018). That a one-time, one-hour,

exposure has the potential to cause such metabolic imbalance and systemic and pulmonary injury and inflammation should only add to the level of concern.

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Abbreviations

ACE	angiotensin-converting enzyme
AET	aortic ejection time
ALT	alanine amino-transferase
AP	alkaline phosphatase
BALF	bronchoalveolar lavage fluid
BSA	bovine serum albumin
CBC	complete blood count
CK	creatinine kinase
CO	cardiac output
CRP	C-reactive protein
DPBS	Dulbecco's phosphate-buffered saline
ECG	electrocardiogram
EDV	end diastolic volume
EF	ejection fraction
ESV	end systolic volume
FA	filtered air
FFA	free fatty acids
FMO	fluorescence minus one
GGT	γ -glutamyl transferase
GPX	glutathione peroxidase
GSH	total reduced glutathione
GTR	glutathione reductase

HDL	high density lipoprotein
HF	high fat
IVCT	isovolumic contraction time
IVRT	isovolumic relaxation time
LDH	lactate dehydrogenase
LDL	low density lipoprotein
NAG	n-acetylglucosaminidase
PM	particulate matter
RH	relative humidity
SD	standard deviation
SOD	superoxide dismutase
STE	speckle tracking echocardiography
SV	stroke volume
TC	total cholesterol
US EPA	United States Environmental Protection Agency
WKY	Wistar Kyoto

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Experiment A

Development of a high fat challenge

Experiment B

Cardiovascular responses after high fat challenge

Experiment C

Time-dependent postprandial changes in systemic and pulmonary endpoints

Fig. 1.

Experimental design for Experiments A, B, and C. In Experiment A, naïve un-exposed rats were administered an oral gavage of isocaloric HF or low fat suspensions or water vehicle after an 8 hr fast. In Experiment B, rats exposed for 1 hr to either low or high concentrations of smoldering peat biomass smoke, or filtered air were fasted overnight for 8 hr and then given an oral gavage of a HF emulsion 24 hr after exposure. Cardiovascular ultrasound was then performed 2 hr post gavage. Experiment C rats were exposed as in Experiment B and then underwent assessment of postprandial changes in pulmonary and systemic markers after no gavage (T = 0 hr), 2 hr (T = 2 hr) or 6 hr (T = 6 hr) after HF gavage.

Fig. 2.

Cardiovascular function in peat smoke-exposed rats measured 2 hr post gavage (Experiment B rats). Note: all rats in Experiment B received a HF gavage. A) Schematic of a rat heart oriented in the apical 4 chamber view, modified from [45], originally modified from [46]. The mitral and aortic valves are denoted because Tei index is derived using transmitral blood flow. B) Image of echocardiographic apical 4-chamber view with color Doppler imaging and pulsed wave Doppler placement. During data collection from the apical view, the pulsed wave Doppler beam was placed in the region of highest velocity flow (aliasing region) identified using color Doppler. C) Tracing of pulsed wave Doppler measurements of transmitral blood flow. Tei was calculated by dividing the sum of the isovolumic contraction time (IVCT) and isovolumic relaxation time (IVRT) by aortic ejection time (AET). D) IVCT E) IVRT F) AET. Data are reported using boxplots with significant ($p < 0.05$) changes indicated with *.

Fig. 3. Postprandial changes in metabolic endpoints in peat smoke-exposed rats measured after no gavage, 2 or 6 hr post HF gavage (Study C rats). **A)** serum glucose **B)** serum insulin **C)** homeostatic model assessment for insulin resistance (HOMA-IR) **D)** serum corticosterone. Data are reported using boxplots with significant ($p < 0.05$) changes in corresponding groups across time points indicated with * and significant changes ($p < 0.05$) compared to FA within a certain time point are indicated with #.

Fig. 4.

Postprandial changes in pulmonary and systemic indicators of inflammation and injury in peat smoke-exposed rats measured after no gavage, 2 or 6 hr post HF gavage (Experiment C rats). **A)** lung microalbumin **B)** lung gamma-glutamyl transferase (GGT) **C)** serum alpha-2 macroglobulin (A2M) **D)** serum alpha-1 acid glycoprotein (AGP). Data are reported using boxplots with significant ($p < 0.05$) changes in corresponding groups across time points indicated with * and significant changes ($p < 0.05$) compared to FA within a certain time point are indicated with #.

Fig. 5.

Postprandial changes in circulating monocyte phenotype determined using flow cytometry in peat smoke-exposed rats measured after no gavage or 6 hr post HF gavage (Experiment C rats). **A)** FlowJo-produced histograms of a single animal that show (left to right): the total white blood cell population, then only the CD45+ expressing viable cell population, then narrowed further into subpopulations showing granulocytes and monocytes, the latter from which data was analyzed for each animal. **B)** Monocytes of animals expressed as % of total white blood cells at the T = 0 hr and T = 6 hr post HF gavage. **C)** Classical monocytes

expressed as a percent of total monocytes. **D)** CD11 b/c expressing monocytes expressed as a percent of total monocytes. Data in panels B, C, and D are reported using boxplots with significant ($p < 0.05$) changes in corresponding groups across time points indicated with * and significant changes ($p < 0.05$) compared to FA within a certain time point are indicated with #.

Table 1.

Serum markers from Experiment A

	Water	Low Fat	High Fat
TG (mg/dL)	27.0 ± 2.5	30.6 ± 4.1	35.5 ± 5.9 ^a
FFA (μM)	214.7 ± 56.3	188.3 ± 61.3	299.2 ± 75.0 ^b
LDL (mg/dL)	7.8 ± 1.2	6.8 ± 0.3	8.6 ± 0.7 ^b
Adrenaline (pg/mL)	60.8 ± 12.1	83.4 ± 15.7 ^c	65.9 ± 7.2

Animals were assessed 2 hr after oral gavage of either a HF or low fat emulsion, or water. TG = triglycerides, FFA = free fatty acids, LDL = low-density lipoprotein.

^a denotes a significant ($p < 0.05$) change v. water and

^b denotes a significant change v. low fat and

^c denotes a change that is different from both groups.

Table 2.

Exposure concentrations for Experiments B and C

	FA [*]	Low Peat	High Peat
PM (mg/m ³)	BLD	0.36 ± 0.00	3.30 ± 0.04
size (µm)	---	0.71 ± 0.07	1.3 ± 0.2
number (#/cc)	---	3714 ± 739	4562 ± 1108
OC (% PM mass)	---	76.9 ± 15.3	67.6 ± 5.4
EC (% PM mass)	---	0.0 ± 0.0	0.4 ± 0.0
CO (ppm)	---	2.45 ± 0.06	11.55 ± 0.22
CO₂ (ppm)	---	659 ± 8.00	907 ± 4.00
NO_x (ppb)	---	3.9 ± 0.30	8.3 ± 0.40

PM = particulate matter, BLD = below level of detection, OC = organic carbon, EC = elemental carbon, CO = carbon monoxide, CO₂ = carbon dioxide and NO_x = nitrogen oxides.

* Particle sizes were characterized with the TSI Optical Particle Counter Model 3330. The mean size reported is in relation to mass. One set of calibrated instruments was available for the measurement of CO, CO₂ and NO_x for this project. They ran continuously during the exposures; therefore, FA measurements for these parameters are not available.

Table 3.

Cardiac and superior mesenteric function values 2 hr after HF gavage (Experiment B)

	units	<u>FA</u>	<u>Low Peat</u>	<u>High Peat</u>
HR	BPM	345.5 ± 20.45	320.1 ± 29.28	355.5 ± 18.64 ^b
ESV	μL	65.14 ± 23.97	81.74 ± 23.26	46.82 ± 13.34 ^b
EDV	μL	235.9 ± 41.72	249.9 ± 23.18	203.6 ± 36.19
SV	μL	170.7 ± 32.22	168.1 ± 22.45	156.8 ± 27.24
CO	mL/min	58.89 ± 10.58	54.17 ± 11.62	59.15 ± 9.920
EF	%	72.62 ± 8.389	67.48 ± 7.834	77.20 ± 4.336
FS	%	43.38 ± 7.441	39.00 ± 6.555	47.16 ± 4.406
MPI	n1	0.541 ± 0.058	0.570 ± 0.051	0.523 ± 0.029
Strain Delay	% CC	4.028 ± 2.096	4.964 ± 3.386	1.716 ± 0.872
Pk Strain	%	25.21 ± 3.344	26.90 ± 4.214	26.58 ± 3.907
TPk Strain	%CC	50.36 ± 7.248	45.75 ± 2.050	46.81 ± 4.511
SMA RI	n1	0.719 ± 0.026	0.756 ± 0.072	0.672 ± 0.097

HR = heart rate, ESV = end systolic volume, EDV = end diastolic volume, SV = stroke volume, CO= cardiac output, EF = ejection fraction, FS = fractional shortening, MPI = myocardial performance index, Pk = peak, TPk = time to peak, SMA = superior mesenteric artery, RI = resistance index, BPM = beats per minute and %CC = percent of cardiac cycle.

^adenotes a significant ($p < 0.05$) change v. FA and

^bdenotes a significant change v. low peat.

Table 4.

Lipid levels one day after exposure to peat smoke or filtered air (FA; Experiment C)

	<u>FA</u>		<u>Low Peat</u>		<u>High Peat</u>	
	<i>Serum</i>	<i>Plasma</i>	<i>Serum</i>	<i>Plasma</i>	<i>Serum</i>	<i>Plasma</i>
TG (mg/dL)						
T = 0	29.5 ± 3.40	24.6 ± 2.75	27.2 ± 3.55	26.4 ± 3.75	29.8 ± 3.48	27.1 ± 3.00
T = 2	31.2 ± 2.74	27.9 ± 1.52	29.0 ± 5.41	26.4 ± 4.00	28.5 ± 5.08	27.3 ± 4.22
T = 6	28.8 ± 4.80	26.6 ± 3.89	28.6 ± 3.00	24.8 ± 1.76	23.3 ± 1.17 ^{ab}	22.4 ± 0.93 ^{ab}
LDL Cholesterol (mg/dL)						
T = 0	7.43 ± 1.07	6.86 ± 0.84	7.36 ± 0.84	7.43 ± 0.87	6.97 ± 0.75	6.93 ± 0.75
T = 2	8.03 ± 1.05 ^b	7.25 ± 1.06	8.43 ± 0.49 ^b	7.39 ± 0.77	7.68 ± 0.95	7.47 ± 0.82
T = 6	6.02 ± 0.97	6.07 ± 0.96	6.91 ± 0.75	6.57 ± 0.94	5.90 ± 0.52	5.56 ± 0.33 ^b
HDL Cholesterol (mg/dL)						
T = 0	20.0 ± 1.25	18.2 ± 0.71	19.5 ± 1.16	17.6 ± 0.70	18.6 ± 1.02	18.3 ± 1.48
T = 2	20.5 ± 1.28	18.8 ± 1.03	21.2 ± 1.06 ^b	18.8 ± 0.93	21.4 ± 1.84 ^b	18.9 ± 1.06
T = 6	20.3 ± 0.71	18.7 ± 1.55	21.4 ± 1.83 ^b	18.8 ± 1.22	20.1 ± 1.17	18.0 ± 0.95
Total Cholesterol (mg/dL)						
T = 0	49.6 ± 2.31	44.7 ± 4.41	49.9 ± 3.17	42.9 ± 1.70	50.3 ± 2.26	42.2 ± 1.69
T = 2	45.4 ± 6.64 ^b	43.7 ± 2.96	45.6 ± 1.58 ^b	44.1 ± 1.84	52.4 ± 2.30 ^a	43.1 ± 1.83
T = 6	42.2 ± 3.92 ^b	39.1 ± 0.92 ^b	40.3 ± 1.54 ^b	39.6 ± 2.17 ^b	46.2 ± 2.85	39.7 ± 1.47 ^b
FFA (mg/dL)						
T = 0	---	241.5 ± 53.2	---	290.9 ± 70.7	---	266.7 ± 95.3
T = 2	---	310.8 ± 55.7	---	329.2 ± 33.4	---	247.6 ± 91.2
T = 6	---	217.2 ± 30.1	---	206.8 ± 25.7 ^b	---	176.7 ± 36.8 ^b

TG = triglycerides, LDL = low density lipoprotein, HDL = high density lipoprotein, FFA = free fatty acids. Note T = 0 rats were not gavaged.

^a denotes a significant (p < 0.05) change v. FA and^b denotes a significant change v. T = 0 (fasted) rats.

Table 5.

Complete Blood Counts 1 day after exposure to peat smoke for filtered air (FA).

	FA	Low Peat	High Peat
RBCs (10⁶/μL)			
T = 0 hr	7.52 ± 0.21	7.57 ± 0.17	7.44 ± 0.20
T = 2 hr	7.40 ± 0.23	7.41 ± 0.15	7.22 ± 0.12
T = 6 hr	7.57 ± 0.31	7.64 ± 0.17	7.48 ± 0.25
WBCs (10³/μL)			
T = 0 hr	2.83 ± 0.75	3.00 ± 0.44	2.83 ± 0.28
T = 2 hr	2.83 ± 0.38	3.10 ± 0.39	2.84 ± 0.54
T = 6 hr	2.57 ± 0.81	2.79 ± 0.33	2.46 ± 0.57
HGB (g/dL)			
T = 0 hr	14.1 ± 0.42	14.2 ± 0.30	13.9 ± 0.32
T = 2 hr	13.5 ± 0.54 ^b	13.7 ± 0.55	13.3 ± 0.35 ^b
T = 6 hr	14.5 ± 0.46	14.5 ± 0.29	14.1 ± 0.51
HCT (% RBCs)			
T = 0 hr	38.7 ± 1.0	38.9 ± 0.77	38.4 ± 1.0
T = 2 hr	37.9 ± 0.84	37.9 ± 0.34	37.5 ± 0.73
T = 6 hr	39.2 ± 1.7	39.5 ± 0.85	38.5 ± 1.2
MCV (fL)			
T = 0 hr	51.5 ± 0.56	51.4 ± 0.56	51.6 ± 0.70
T = 2 hr	51.2 ± 0.98	50.6 ± 0.55 ^b	51.9 ± 0.38
T = 6 hr	51.8 ± 0.39	51.7 ± 0.39	51.5 ± 0.64
MCH (pg)			
T = 0 hr	18.9 ± 0.14	18.7 ± 0.28	18.8 ± 0.13
T = 2 hr	18.3 ± 0.31 ^b	18.4 ± 0.48	18.5 ± 0.32
T = 6 hr	19.2 ± 0.45	18.9 ± 0.35	18.8 ± 0.25
MCHC (g/dL)			
T = 0 hr	36.6 ± 0.57	36.4 ± 0.42	36.3 ± 0.61
T = 2 hr	35.7 ± 0.86 ^b	36.5 ± 1.0	35.6 ± 0.62
T = 6 hr	37.0 ± 0.92	36.6 ± 0.53	36.6 ± 0.42
Platelets (10³/μL)			
T = 0 hr	676.3 ± 50.0	654.5 ± 25.1	712.3 ± 24.8
T = 2 hr	669.5 ± 45.7	657.6 ± 26.9	708.9 ± 21.6
T = 6 hr	655.3 ± 14.2	677.8 ± 44.7	686.8 ± 24.8
Ly # (10³/μL)			
T = 0 hr	2.06 ± 0.60	2.14 ± 0.37	2.14 ± 0.25

	<u>FA</u>	<u>Low Peat</u>	<u>High Peat</u>
T = 2 hr	2.05 ± 0.29	2.17 ± 0.23	2.18 ± 0.41
T = 6 hr	1.77 ± 0.64	1.91 ± 0.19	1.68 ± 0.32
<hr/>			
Ly % (% WBCs)			
T = 0 hr	73.2 ± 4.2	71.6 ± 5.6	76.9 ± 1.6
T = 2 hr	72.0 ± 5.5	70.4 ± 5.7	73.7 ± 3.7
T = 6 hr	66.7 ± 6.4 ^b	68.8 ± 4.5	70.5 ± 4.0 ^b

RBCs = red blood cells, WBCs = white blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, Ly # = lymphocyte number, Ly % = lymphocyte percentage (of WBCs).

^a denotes a significant ($p < 0.05$) change v. FA and

^b denotes a significant change v. T = 0 (fasted) rats.

Table 6.

Summary of statistically significant ($p < 0.05$) post gavage effects of peat smoke relative to FA.

	<u>Low Peat</u>	<u>High Peat</u>
Cardiac Function	$\uparrow$ Isovolumic relaxation time (IVRT)	---
Superior Mesenteric Artery Function	---	---
Blood Lipids	---	$\uparrow$ Total Cholesterol
Blood Glucose/Insulin	$\downarrow$ Glucose $\downarrow$ Insulin	---
Markers in Lung Lining Fluid	$\uparrow$ Gamma-glutamyl transferase (GGT)	$\uparrow$ Gamma-glutamyl transferase (GGT)
Systemic Markers of Inflammation	---	$\uparrow$ alpha-1 acid glycoprotein (AGP)
Circulating Monocytes	$\uparrow$ Classical Monocytes $\uparrow$ CD11 b/c Monocytes	---

$\uparrow$ denotes a significant increase and

$\downarrow$ denotes a significant ($p < 0.05$) decrease compared to FA.