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Abstract

Background: High ambient levels of ozone (O₃) and fine particulate matter (PM_{2.5}) are associated with cardiovascular morbidity and mortality, especially in people with pre-existing cardiopulmonary diseases. Enhanced susceptibility to the toxicity of air pollutants may include those with the metabolic syndrome (MetS).

Objective: We tested the hypothesis that cardiovascular responses to O₃ and PM_{2.5} will be enhanced in rats with diet-induced MetS.

Methods: Male Sprague-Dawley rats were fed a high fructose diet (HFrD) to induce MetS and then exposed to O₃, concentrated ambient PM_{2.5}, or the combination O₃+PM_{2.5} for 9 days. Data related to heart rate (HR), HR variability (HRV) and blood pressure (BP) were collected.

Results: Consistent with MetS, HFrD rats were hypertensive, insulin resistant and had elevated fasting levels of blood glucose and triglycerides. All exposures caused decreases in HR and BP that were greater and more persistent in HFrD rats compared to rats fed a normal diet (ND). Coexposure to O₃+PM_{2.5} induced acute drops in HR and BP in all rats, but only ND rats adapted after two days. HFrD rats had little exposure-related changes in HRV, while ND rats had increased HRV during O₃ exposure, modest decreases with PM_{2.5}, and dramatic decreases during O₃+PM_{2.5} coexposures.

Conclusions: Cardiovascular depression in response to exposures to O₃ and PM_{2.5} was enhanced and prolonged in rats with HFrD-induced MetS. These results in rodents suggest that people with MetS may be prone to similar exaggerated BP and HR responses to inhaled air pollutants.

Introduction

Metabolic syndrome (MetS) is a group of risk factors for developing type II diabetes mellitus and cardiovascular disease, that includes at least three of the following conditions concurrently: hypertension, central obesity, elevated fasting glucose, high serum triglycerides and low circulating high density lipoprotein (NCEP 2001). MetS affects approximately 32% of the U.S. population and is expected to grow to 34% by 2020 (Ford et al. 2010). Among dietary and other lifestyle factors, high intake of fructose has been proposed to contribute to the the development of MetS, although a casual relationship is controversial (Stanhope 2012; Feinman and Fine, 2013). Fructose-sweetened beverages can increase visceral adiposity and induce insulin resistance and dyslipidemia after only 10 weeks of consumption (Stanhope et al. 2009). Fructose-induced MetS has been effectively modeled in rats, where hypertension, hypertriglyceridemia and insulin resistance are induced after an 8-10 week period of fructose supplementation (Patel et al. 2009; Tran et al. 2009).

Recent epidemiological studies have indicated that elevated ambient concentrations of fine particulate matter (aerodynamic diameter $\leq 2.5 \mu\text{m}$; $\text{PM}_{2.5}$) or carbon monoxide are linked to greater decreases in heart rate variability, an index of autonomic balance, in MetS compared to healthy subjects (Min et al. 2009; Park et al. 2010). In addition, we have recently reported that small increases in urban ambient $\text{PM}_{2.5}$ can decrease insulin sensitivity in healthy subjects (Brook et al. 2013), suggesting that $\text{PM}_{2.5}$ may contribute to MetS etiology or to the progression from MetS to diabetes. Given the high prevalence of MetS, the cardiovascular and metabolic health risk of exposure to ambient pollutants may be substantial.

By comparison to MetS, the link between morbidity and mortality due to diabetes and exposure to ambient air pollution are more documented (Ostro et al. 2006; Zanobetti and Schwartz 2011). For example exposure to PM_{2.5} is associated with enhanced vascular reactivity (O'Neill et al. 2005) and cardiac function abnormalities (Baja et al. 2010) in diabetics. Acute cardiovascular responses to ozone (O₃) exposure are also exaggerated, with increased heart rate (Hampel et al. 2012) and decreased blood pressure (Hoffmann et al. 2012). In addition, obesity and hypertension, common comorbidities of both MetS and diabetes, are themselves susceptibility factors for adverse responses to PM_{2.5} (Dubowsky et al. 2006).

Health complications from air pollutant exposures have been modeled in rodents with hypertension and various other chronic cardiovascular diseases, but comparable studies in experimental metabolic disorders are lacking. In the present study we used a novel model of high fructose feeding to induce the MetS phenotype and then exposed these metabolically challenged rodents to different air pollutant atmospheres to test the hypothesis that adverse cardiovascular effects of air pollution would be exacerbated by the MetS. Our exposure regimens consisted of O₃, PM_{2.5}, and O₃+ PM_{2.5}, an approach which is consistent with calls for more research on the health risks of multipollutant atmospheres (Johns et al. 2012). We analyzed changes in heart rate (HR), heart rate variability (HRV) and blood pressure (BP) to assess the effects of exposure on vascular, cardiac, and autonomic function in the face of diet-induced metabolic dysregulation.

Methods

Animals

Eight-week-old male Sprague Dawley rats, weighing 250-275 grams (Charles River Laboratories, Portage, MI) were fed either a normal diet (ND; 8640 Teklad 22/5 Rodent Diet,

Harlan Laboratories, Madison, WI, or a high-fructose diet (HFrd; 60% fructose by mass; TD.89247; Harlan) to induce the MetS phenotype. After 8 weeks on ND or HFrd rats were transported to AirCARE 1, a mobile laboratory parked in Dearborn, MI, and randomly assigned to one of four exposure groups, filtered air (FA), O₃, PM_{2.5}, or O₃+PM_{2.5}, for a total of 8 experimental groups (n = 7-8 per group for metabolic outcomes; n = 4 for telemetry endpoints). Rats were housed individually in polycarbonate shoebox-type cages with corn-cob bedding where they had access to food and autoclaved and filtered water. Inhalation exposures were conducted 8 h/day (7:30AM-3:30PM) for nine consecutive weekdays (Week1:Mon-Fri; Week2:Mon-Thrs) to capture hourly and daily variation of ambient PM. All rats were sacrificed 24h after the last exposure (Week2:Fri). Food and water were removed during exposures. Study protocols were approved by the Institutional Animal Care and Use Committee of MSU to ensure human treatment of animals; MSU is an AAALAC accredited institution.

Exposure to O₃ and PM_{2.5}

We conducted inhalation exposures in AirCARE 1, a mobile air research laboratory parked at Salinas Elementary School in Dearborn, MI during the summer of 2011. This urban industrial location is also a stationary air pollution monitoring site operated by the Michigan Department of Environmental Quality and experiences some of the highest annual PM_{2.5} concentrations in Michigan (MDEQ 2012). The site is located within 5km of iron/steel production facilities, a coke oven, oil refinery, sewage sludge waste incinerator, a coal-fired power plant and major highways.

Concentrated PM_{2.5} was generated from ambient PM_{2.5} using a Harvard-type fine particle concentrator and Hinnert whole body animal exposures chambers as previously described in

detail (Harkema et al. 2004). O₃ was generated using an OREC O₃ generator (Model V1; *uv* method), and O₃ concentration was targeted at 0.5 ppm. Due to exposure chamber configurations, exposures to O₃+PM_{2.5} and FA groups were conducted on different weeks (July 25-Aug 4, 2011) than exposures to O₃ or PM_{2.5} alone (Aug 15-25, 2011).

Exposure Characterization

We conducted air quality monitoring on both concentrated and ambient PM_{2.5} samples using previously described methods (Morishita et al. 2006). Briefly, integrated and concentrated PM_{2.5} mass samples were determined during each 8-hour exposure period using Teflon (PTFE) filters. Ambient and concentrated PM_{2.5} mass was also measured continuously (5 minute interval). Particle compositions of acidity, sulfate, nitrate and ammonium ion, elemental carbon (EC) and organic carbon (OC) were determined. Trace elements concentrations were assessed using high-resolution inductively coupled plasma-mass spectrometry (ICP-MS) (ELEMENT2, Thermo Finnigan, San Jose, CA). Further details of the exposure assessment can be found in Supplemental Materials.

Cardiovascular Telemetry

Ten weeks before exposures, we surgically implanted PhysioTel Multiplus transmitters (# C50-PXT; Data Sciences International, St. Paul, MN) that emit radio signals of electrocardiograms (ECG) and blood pressure (BP) in rats. Transmitters were placed with ECG leads terminating in a Lead II configuration to sample cardiac parameters and the pressure catheter placed in the aorta via the femoral artery. Telemetry receivers (RLA3000, DSI) were modified and affixed inside individual cages in exposure chambers. Due to space limitations, four rats from each dietary group were used for collection of pressure and ECG waveforms. We collected data streams of 30

second duration every 5 minutes during exposures (7:30AM-3:30PM), and during nonexposure times in evenings (12:00AM-5:00AM) and weekends (7:30AM-3:30PM). Automated ECG analysis (DSI Ponemah) allowed for R-wave detection on a beat-to-beat basis. We use R-R intervals for all normal beats to calculate HR and time-domain measures of HRV: standard deviation of the normal-to-normal beats (SDNN), an indicator of overall autonomic tone, and the square root of the mean squared differences of successive normal-to-normal intervals (RMSSD), an estimate of parasympathetic tone.

Metabolic Endpoints

We collected serum from fasted animals for determination of glucose, insulin and triglycerides levels. Briefly, blood glucose was measured using a Bayer Contour glucometer. Serum insulin levels were measured using the ultrasensitive rat insulin ELISA kit (Crystal Chem, Downers Grove, IL) , and triglycerides were determined using the L-Type Triglyceride M Assay (Wako Diagnostics, Richmond, VA). Insulin resistance was determined by the homeostatic model assessment of insulin resistance (HOMA-IR), which assesses the ratio of fasting blood glucose and insulin and is routinely used for human clinic assessment (Brook et al. 2013) as described further in Supplemental Material.

Statistical Analyses

We used a two-way factorial design with repeated measures over time, consisting of two diet groups (HFrd and ND) and four exposure groups (O₃, PM_{2.5}, O₃+ PM_{2.5}, and FA). To determine differences between experimental groups, repeated measures analyses using a linear mixed model with nested random effects of time within date were implemented in estimating the effects of exposure, diet, and their interaction on each outcome (Table 1) for the entire time series

observed over a nine-day study period. Autoregressive model of order 1 correlation structure was considered in the analysis due to the fact that the correlation between observations decreases if they are further apart in time. To reduce the skewness of the HRV measures, variables SDNN and RMSSD were transformed with the natural logarithm after adding 1.

Additionally, the same analytical approach of using linear mixed models for the effects of exposure and diet was performed for each of the nine days in the study period to obtain daily results, where the random effect is time only (Figures 2, 3, 4). Statistical analysis was performed using both R2.12.1 and SAS 9.2. Criteria for significance were set at $p \leq 0.05$ for all parameters.

Results

Exposure Characterization

The average daily chamber concentrations of PM_{2.5} were $356 \pm 87 \mu\text{g}/\text{m}^3$ (mean \pm SD) for the group exposed to PM_{2.5} alone, and $441 \pm 65 \mu\text{g}/\text{m}^3$ during the O₃+PM_{2.5} coexposures. Average O₃ concentrations were 0.485 ± 0.042 ppm for the group exposed to O₃ alone, and 0.497 ± 0.030 ppm during the O₃+PM_{2.5} coexposures. Data on the major components of PM_{2.5} (sulfates, nitrates, carbon, ammonium, organic matter), and of trace elements can be found in Supplemental Materials, Tables S1 and S2, respectively. Relative contributions from major components and trace elements in PM_{2.5} were typical for urban industrial southwest Michigan in summer months that we have documented in previous field exposures (Harkema et al. 2004; Rohr et al. 2011).

Metabolic Endpoints

Ten weeks of HFrD induced hyperglycemia, dyslipidemia with elevated serum triglycerides, and insulin resistance (Figure 1). Body weights were not different between dietary groups.

Blood Pressure

Baseline mean arterial pressure (MAP) prior to inhalation exposures was significantly greater in HFrD rats compared to ND rats (116.3 ± 8.9 vs. 103.4 ± 11.4 mm Hg, respectively), which is consistent with previous findings in HFrD rats (Patel et al. 2009). MAP was unaffected by FA-exposure (102.5 ± 9.5). In ND rats, only the coexposure to $O_3+PM_{2.5}$ affected MAP, with a modest but significant decrease of 3.2 mmHg that was similar to the response in HFr rats (Table 1). In comparison, HFrD rats were much more sensitive to exposure-related change in MAP, with reductions in blood pressures of 6.9 and 7.6 for O_3 and $PM_{2.5}$, respectively (Table 1). With the exception of a modest increase in diastolic pressure in ozone-exposed HFr rats (2.4 mmHg), diet- and exposure-related changes in systolic and diastolic pressures were similar to those observed with MAP (Table 1).

Acute decreases in MAP of 10-15 mmHg were evident in HFrD-rats during the first day of exposure to O_3 or $PM_{2.5}$ and were sustained during the first week of exposures (Fig 2 A, B). Interestingly, depressed MAP was sustained over the weekend when rats were not exposed. By the last day of exposure, MAP of pollutant-exposed HFrD rats was not different from that measured in rats exposed to FA, suggesting that adaptive responses to repeated exposures had occurred in these animals. Modest increases in MAP in ND rats were sporadically observed during exposure to O_3 (Fig 2a, second week) and to $PM_{2.5}$ (Fig 2b, first week). During post-exposure evenings when animals were resting in polycarbonate boxes, exposure-related changes

were sustained in both ND and HFrD rats (Figure 2D,E). In HFrD rats, MAP decreased dramatically during coexposure to O₃+PM_{2.5} during the first two days (Fig 2C), but then had minor changes with remaining exposures. In contrast the coexposures had no effect in ND rats.

Heart Rate

Baseline HR prior to the start of the inhalation exposures was significantly greater in HFrD rats compared to ND rats (329 ± 27 vs. 300 ± 35 bpm, respectively). Elevated HR is a consistent finding in human MetS (Grassi et al. 2009; Guize et al. 2008), though it has not been previously reported in HFrD rats. HR was unaffected during FA-exposures (299.9 ± 34.9). Decreases in HR were induced by exposures to O₃, PM_{2.5}, or O₃+PM_{2.5} regardless of diet (Table 1). Greater declines, however, occurred in HFrD rats compared to ND rats for all exposures, with reductions in HR of up to 12.5% (~ 40 bpm) during O₃ exposure.

Daily responses to O₃ or PM_{2.5} (Fig 3A, B) caused acute drops in HR during the first few days of exposure in all rats, but by the end of the first week HR returned to control levels in ND, but not in HFrD rats. During the weekend when animals were not being exposed to air pollutants, ND and HFrD had divergent carryover responses from exposures. Decreased HR during O₃ or PM_{2.5} exposure was sustained in HFrD rats (~ 20 bpm), despite the rats breathing FA during this time. By comparison, HR increased up to 25 bpm over the weekend in ND rats that had been exposed to O₃ (Fig 3A). During the second week of exposures, significant depression in HR was again induced by O₃ or PM_{2.5} exposures in HFrD rats, and by PM_{2.5} exposure to ND rats. In contrast, O₃ did not induce HR decreases in ND rats, rather increased HR occurred in the final day of exposure (Fig3A).

Co-exposure to O₃+PM_{2.5} produced dramatic ~ 73 bpm decreases in HR in all rats during the first two days of exposure (Fig 3C), but HR rapidly returned to control levels by the third day. No exposure-related effects were detected during the weekend. Depressed HR was again induced during the second week of exposures; however it remained attenuated compared to exposures to O₃ or PM_{2.5} alone.

Changes in HR induced by all exposure scenarios were sustained during post-exposure evenings (Figures 3D,E,F). Reduced HR was evident after a single 8h exposure (i.e., evening of Day 1), and reductions with repeated exposures were of similar magnitude as changes measured during exposures.

Heart Rate Variability (HRV)

Baseline SDNN and rMSSD prior to exposures were significantly greater in ND rats (14.2 ± 18.5 and 12.1 ± 14.4 , respectively) compared to HFrD rats (10.7 ± 15.1 , 8.9 ± 16.9). These results are consistent with the prevalence of low HRV in human MetS (Liao et al. 1998) and in HFrD-induced MetS in rats (Moraes-Silva et al. 2013). In ND-fed rats, O₃ exposures caused increases in SDNN and RMSSD (33 and 46%, respectively), whereas PM_{2.5} caused decreases (21 and 18%) (Table 1). In comparison the exposure to O₃+PM_{2.5} resulted in a 60% decrease in HRV. In contrast to ND rats, HRV responses in HFrD rats were less pronounced, with no changes elicited in SDNN, and modest increases in RMSSD during exposure to O₃ or PM_{2.5} (Table 1). In ND rats, O₃'s effects were predominately during the first week of exposure, whereas PM_{2.5}-elicited effects occurred during the second week (Figure 4). During O₃+PM_{2.5} exposures by comparison, daily decreases in both SDNN and RMSDD were similar each day. In HFrD rats, O₃ caused increased

HRV on two days, while PM_{2.5} caused elevated RMSSD on a single day of the nine days of exposure.

Discussion

Results of this study clearly demonstrate an interaction between diet and pollutant exposure where rats with HFrD-induced MetS had enhanced depression of HR and BP in response to inhalation exposures to O₃ and PM_{2.5}, compared to similarly exposed ND rats. Exposure-induced decreases of BP and HR occurred during the first day of exposure in HFrD rats, persisted with repeated exposures, and remained depressed during non-exposure periods of evenings and weekends. By comparison responses in healthy rats fed a normal diet were less robust and showed adaptation with repeated exposures. In contrast to the enhanced sensitivity for BP and HR responses, HFrD rats had muted HRV responses compared to ND rats. These findings are the first to describe perturbations of normal cardiovascular responses to air pollutant exposures in a rodent model of MetS.

Inhalation exposure-induced depression of HR in rodents has been documented for O₃ (Farraj et al. 2012; Uchiyama and Yokoyama 1989), diesel exhaust (Lamb et al. 2012), and ambient PM_{2.5} (Kamal et al. 2011). Furthermore acute exposure of laboratory rodents to O₃ (Uchiyama and Yokoyama 1989) or ambient PM_{2.5} (Cheng et al. 2003) also triggers a drop in BP, suggesting that inhalation of a variety of airborne toxicants can lead to cardiovascular depression in lab animals. Activation of sensory irritant receptors has long been proposed to mediate both the pulmonary and cardiovascular responses to a range of substances, including ozone and components of PM (Alarie 1973). In the present study we found that these responses are exaggerated and prolonged in rats with MetS. The enhanced cardiovascular depression in rats exposed to O₃ is consistent

with recent reports of ambient O₃-associated bradycardia in infants (Peel et al. 2011), and depressed systolic pressure in diabetics (Hoffmann et al. 2012). Both decreased and increased BP have been reported in diabetics in response to ambient PM_{2.5}, with these differences due to the time of the response relative to the start of exposure (acute versus lag responses, respectively; Hoffmann et al. 2012; Schneider et al. 2010).

Unlike rats fed the normal diet, rats with MetS experienced delayed adaption or failed to adapt to single-pollutant exposures. After acute responses of bradycardia and respiratory depression induced by O₃ or PM inhalation, rodents normally become refractory to repeated exposures (Hamade and Tankersley 2009; Watkinson et al. 2001). Not only did O₃-exposed ND rats adapt by four days, they also displayed increased, rather than decreased, BP and HR after several days. The rapid adaptation of HR during O₃+PM_{2.5} coexposures was matched with significant drops in rMSSD in ND rats, which suggests a diminution of parasympathetic dominance with repeated coexposures. By contrast HFrD rats, which also displayed rapid adaptation during coexposures, were refractory to HRV changes, and had dampened responses in SDNN and RMSSD to all of the pollutant exposures. This pattern of response in HFrD rats is consistent with cardiovascular autonomic neuropathy described in diabetics that display derangements in autonomic control (Pop-Busui 2012).

In a previous study, acute depression of HR during inhalation of 0.8 ppm O₃ in rats was accompanied by ECG profiles with prolonged PR interval and ST depression, alterations which are consistent with delayed atrioventricular conduction (Farraj et al. 2012). Similar ECG with bradycardia have been reported during inhalation of diesel exhaust or metal-rich PM (Farraj et al. 2011; Lamb et al. 2012). Though we did not assess electrical conduction in the present study, O₃, PM_{2.5} and O₃+PM_{2.5} exposures may initiate similar ECG alterations that were further modified in

HFrD rats to decrease heart rate. Cardiac conduction abnormalities, including atrioventricular block are common in the diabetic heart (Movahed 2007), but cardiac electrical conduction in HFrD models has not been extensively studied. Fructose can slow excitation-contraction coupling and prolong relaxation in cardiomyocytes (Ren et al. 1997) and HFrD rats have been reported to develop cardiac inflammation, myocardium remodeling and ventricular dilation (Patel et al. 2009). In separate analysis of the epicardial adipose tissue from our rats, we found exposure-related infiltration of macrophages that was associated with iNOS, TNF α and leptin expressions (Sun et al., 2013). Further studies are needed to describe the nature of exposure-related ECG changes in rats with cardiomyopathies.

Susceptibility of HFrD rats to exaggerated autonomic responses to inhaled pollutants may be centrally mediated at cardiovascular regulatory sites in the brain. Stimulation of α_2 -adrenoceptors in the anterior hypothalamus leads to decreased BP and HR that is greater in HFrD rats compared to control rats (Mayer et al. 2007). Supersensitivity of hypothalamic adrenoceptors in response to hyperglycemia has been hypothesized to underlie this effect. We have previously shown elevated production of norepinephrine, an α_2 -receptor agonist, in the paraventricular nucleus of the anterior hypothalamus after a single exposure of rats to PM_{2.5} (Sirivelu et al. 2006). Repeated exposures to PM_{2.5} resulted in sustained increases in hypothalamus norepinephrine in insulin resistant, obese JCR rats, but not in healthy lean control rats (Balasubramanian et al. 2012). This relationship is consistent with our current observation of dramatic drops in HR and BP during the first few days of exposure that are sustained in HFrD but not ND rats. Thus, the enhanced and sustained stimulation of sympathoinhibitory α_2 -adrenoceptors by chronic release of norepinephrine in the hypothalamus could explain the robust and relatively more persistent cardiovascular depression in exposed HFrD rats.

It is notable that exposure to either O₃ or PM_{2.5} alone both resulted in cardiovascular depression of similar magnitude and time-course, yet by comparison the effects of the multipollutant exposure to O₃+PM_{2.5} were blunted. We hypothesize that the combination of particulate- and oxidant-induced toxicity may stimulate defense and adaptive responses more quickly and strongly than those elicited by a single pollutant exposure. However because our PM exposures with and without ozone were conducted at different times, a direct comparison of these groups to determine the interaction between O₃ and PM_{2.5} was not possible in this study.

Translation of our results from this HFrD model may be limited to MetS subjects who have a high intake of dietary fructose. MetS associated with high fat or high caloric diets, or genetic predisposition may have different responses to air pollutant exposure. A second limitation is the use of rodents in inhalation studies to model human responses. While several research groups also report cardiovascular depression in animals from O₃ and PM exposure, many human studies report hypertensive and increased heart rate responses to ambient pollutant exposure. We have mentioned some important exceptions in diabetics and infants above (Hoffmann et al. 2012; Peel et al. 2011; Schneider et al. 2010), where elevations in ambient O₃ are associated with decreased HR and BP, similar to what we describe in HFrD rats. Lastly, our results should be interpreted cautiously due to the limited number of animals per group, even though statistical power was sufficient for our analysis.

Conclusion

This is the first report of dysregulation of normal cardiac, vascular and autonomic responses to inhalation exposure to O₃ and PM_{2.5} in rats with HFrD-induced MetS. Exaggerated depression and delayed adaptation of BP and HR to air pollutants in HFrD rats was accompanied by the lack

of adjustment in autonomic control as measured by HRV. This suggests that underlying cardiovascular and autonomic neuropathies caused by MetS or metabolic disorders such as diabetes may promote inappropriate cardioregulatory responses to repeated exposure to ambient air pollutants. Specific alterations in central versus local neurotransmission, cardiac tissue remodeling and production of soluble mediators in HFrd rats during inhalation exposure remain to be identified in future studies. With one-third of the U.S. population compromised by MetS, the health impact of oxidant and particulate air pollutants in this sensitive population is likely to be significant. Future research using this model of HFrd-induced MetS will contribute to the development of prevention and intervention strategies to protect this susceptible population from the adverse cardiovascular effects of multipollutant atmospheres.

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Table 1. Change in Cardiovascular and Autonomic Parameters during 9-Day Exposure to O₃, PM_{2.5}, or O₃+PM_{2.5}.

Parameter	Diet	Experimental Exposure		
		O ₃	PM _{2.5}	O ₃ +PM _{2.5}
Mean Arterial Pressure (<i>mmHg</i>)	ND	0.45 + 0.81	-0.07 + 0.93	-3.20 + 0.82 *
	HFrD	-6.87 + 0.89 ***	-7.65 + 0.89 ***	-4.9 + 0.95 *
Systolic Pressure (<i>mmHg</i>)	ND	-2.09 + 1.09	-1.20 + 1.09	-2.93 + 1.10 *
	HFrD	-13.82 + 1.20 ***	-8.83 + 1.20 ***	-7.92 + 1.28 ***
Diastolic Pressure (<i>mmHg</i>)	ND	2.37 + 0.66 *	0.77 + 0.66	-3.01 + 0.67 *
	HFrD	-1.9 + 0.72 ***	-6.73 + 0.73 ***	-2.75 + 0.77 *
Heart Rate (<i>bpm</i>)	ND	-6.19 + 2.14 *	-15.24 + 2.16 *	-10.75 + 2.17 *
	HFrD	-40.94 + 2.34 ***	-34.68 + 2.34 ***	-24.67 + 2.35 ***
SDNN (<i>ms</i>)	ND	3.87 + 0.74 *	-2.51 + 0.75 *	-6.54 + 0.75 *
	HFrD	1.27 + 0.87 **	1.52 + 0.81**	-1.43 + 0.81 **
RMSSD (<i>ms</i>)	ND	6.23 + 1.01 *	-2.46 + 1.01 *	-8.7 + 1.01 *
	HFrD	2.35 + 1.1 ***	2.82 + 1.1 ***	-1.46 + 1.1 **

Data are the estimated changes in BP (mean, systolic and diastolic; mmHg), HR (bpm) and HRV (SDNN and rMSSD; msec) due to Diet and Experimental Exposures compared to filtered air (Mean ± SEM). Effect estimates were determined using linear mixed modeling as described in Methods. n = 4; ND - normal diet, HFrD- high fructose diet. * = Significantly different from respective group exposed to filtered air. ** = Significantly different from respective group fed a normal diet (ND).

Figure Legends

Figure 1. Metabolic Responses in Rats Fed Normal versus High Fructose Diets. Eight week-old Sprague Dawley rats were fed diets for 10 weeks, and then serum glucose (A), serum triglycerides (B), insulin resistance as measured by HOMA-IR (C) and body weight (D) were determined as described in Methods. Data are expressed as mean \pm SEM, analyzed by student's *t*-test, $n=8$; * significant difference from Normal Diet rats, $p < 0.05$.

Figure 2. Daily Effects of Exposures on Blood Pressure. Changes in mean arterial pressure (mmHg) were determined in rats fed a normal diet (black circles) or a high fructose diet (white circles) during exposures to O₃ (A,D), PM_{2.5} (B,E) or O₃+PM_{2.5} (C,F) on weekdays and during non-exposure hours on weekends (A,B,C), and non-exposure hours during evenings (D,E,F). Data are expressed as mean \pm SEM, analyzed by linear mixed models, $n=4$; * significant difference from rats exposed to Air (dotted line), ** significant difference from rats fed a Normal Diet, $p < 0.05$.

Figure 3. Daily Effects of Exposures on Heart Rate. Changes in heart rate (bpm) were determined in rats fed a normal diet (black circles) or a high fructose diet (white circles) during exposures to O₃ (A,D), PM_{2.5} (B,E) or O₃+PM_{2.5} (C,F) on weekdays and during non-exposure hours on weekends (A,B,C), and non-exposure hours during evenings (D,E,F). Data are expressed as mean \pm SEM, analyzed by linear mixed models, $n=4$; * significant difference from rats exposed to Air (dotted line), ** significant difference from rats fed a Normal Diet, $p < 0.05$.

Figure 4. Daily Effects of Exposures on Heart Rate Variability. Changes in SDNN (A,B,C) and RMSSD (D,E,F) were determined in rats fed a normal diet (black circles) or a high fructose diet

(white circles) during exposures to O₃ (A,D), PM_{2.5} (B,E) or O₃+PM_{2.5} (C,F). Data are expressed as mean ± SEM, analyzed by linear mixed models, n= 4; *significant difference from rats exposed to Air (dotted line), ** significant difference from rats fed a Normal Diet, p < 0.05.

Figure 1

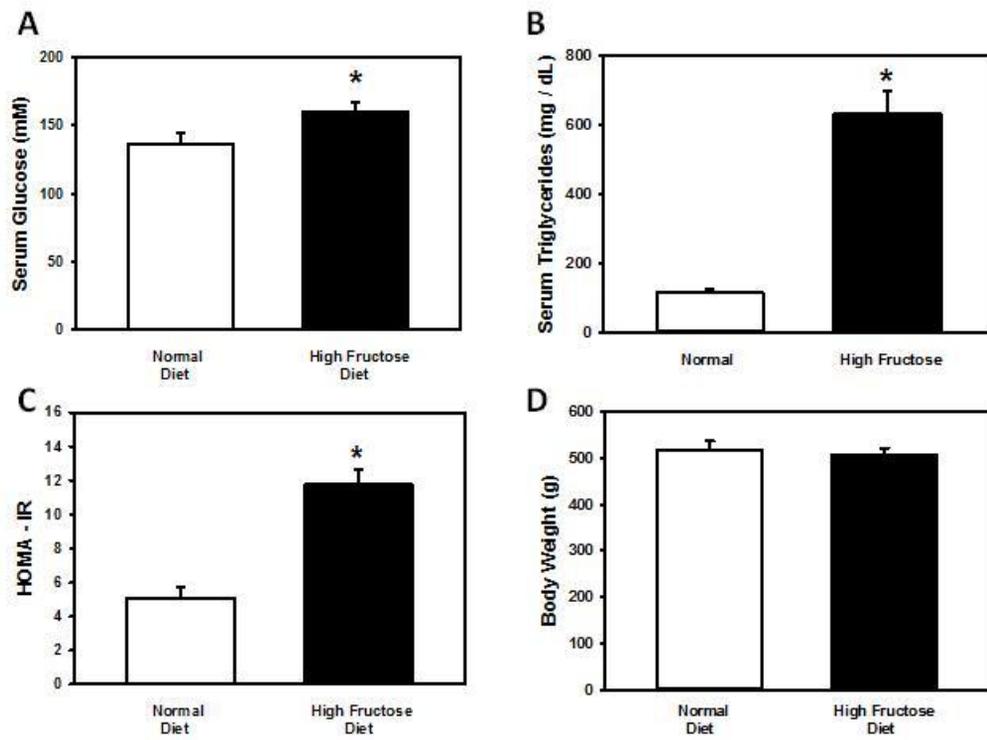
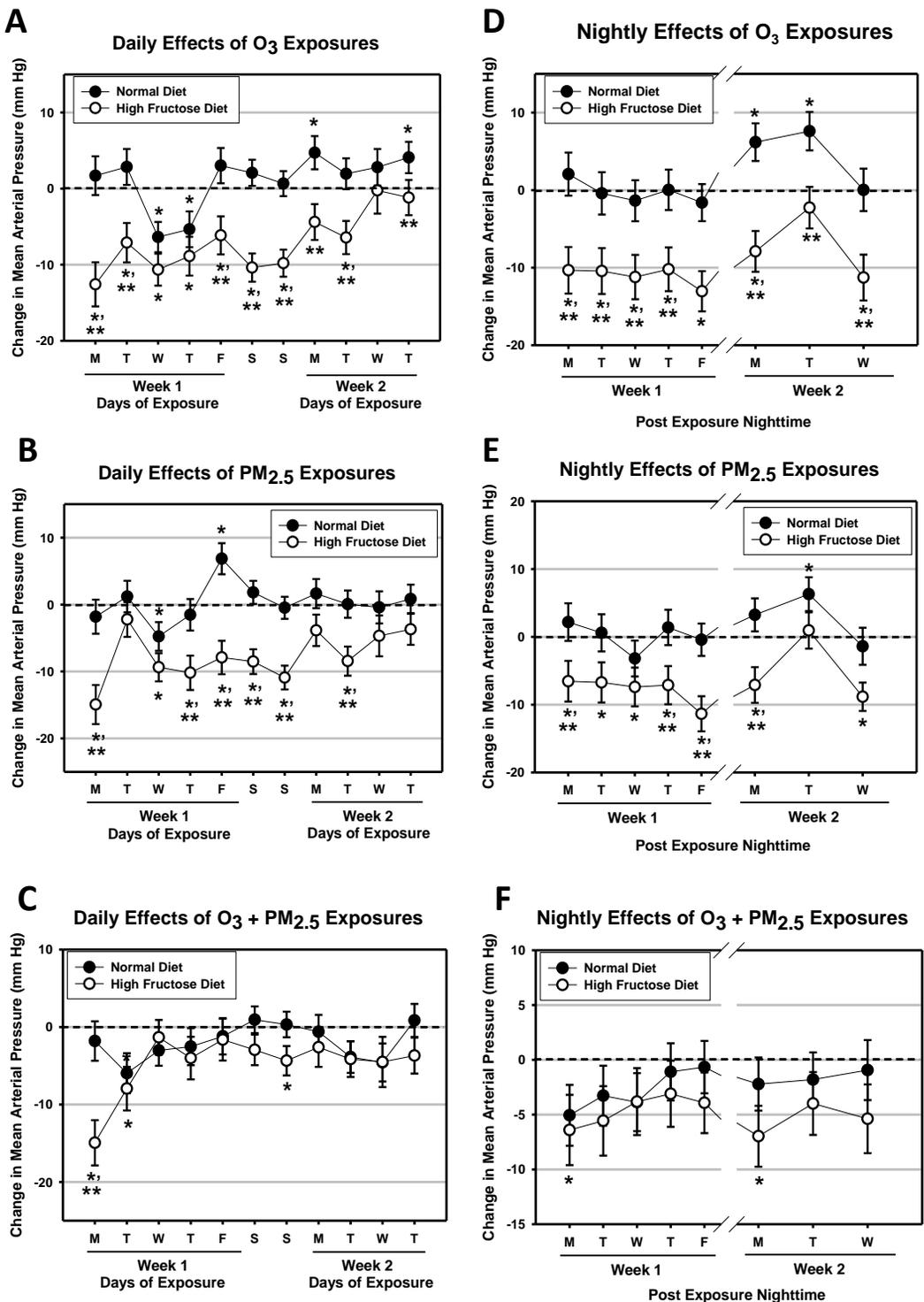


Figure 2



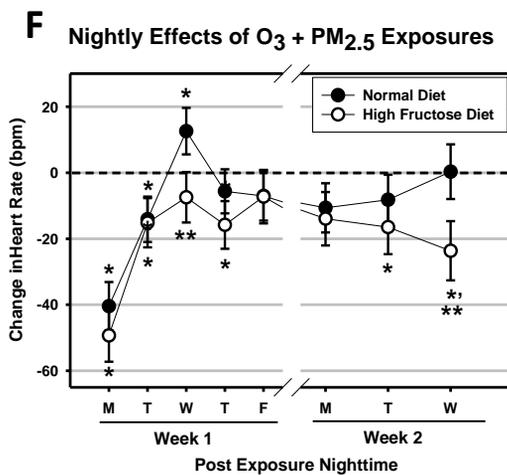
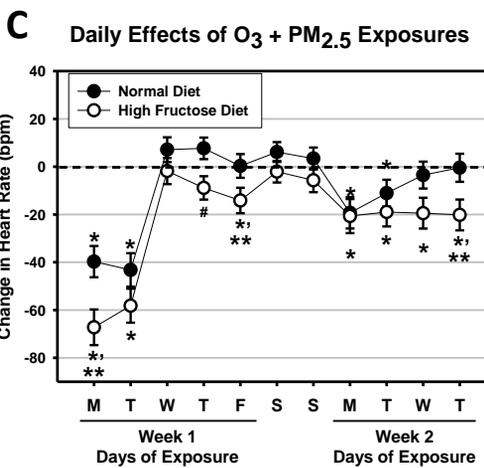
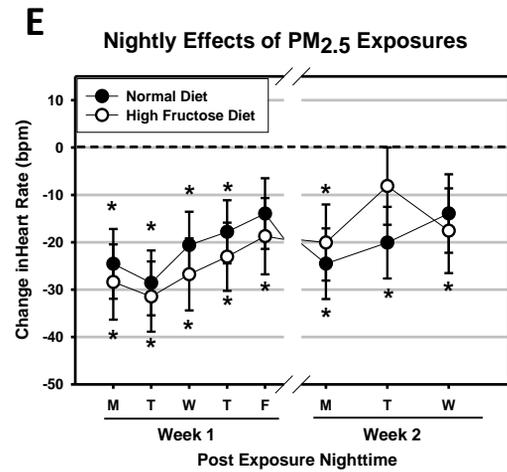
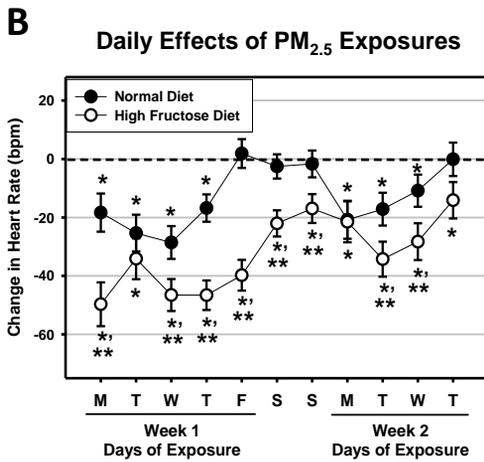
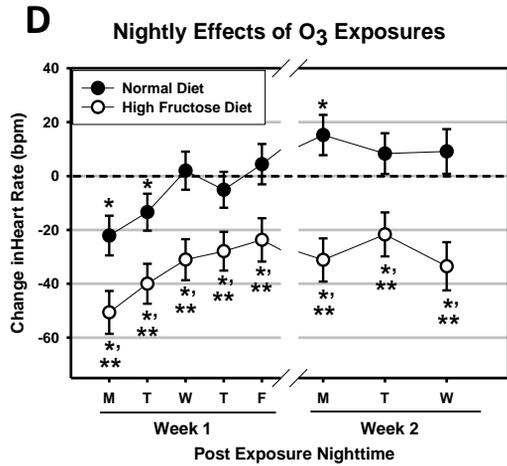
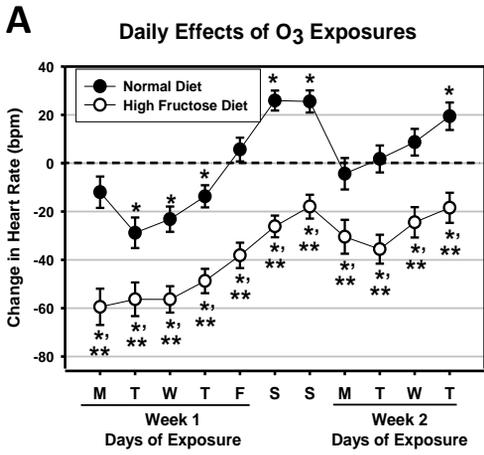


Figure 4

