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ORIGINAL ARTICLE

Acute cardiovascular autonomic responses to inhaled particulates

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Abstract
Purpose Harmful effects of inhaled particulates have been established in epidemiologic studies of ambient air pollution. In particular, heart rate variability responses to high levels of environmental tobacco smoke (ETS), similar to responses observed during direct smoking, have been reported. We sought to determine whether such responses could be observed at lower particulate concentrations.
Methods We monitored cardiovascular responses of non-smoking 21 women and 19 men to work-place-relevant levels of: ETS, cooking oil fumes (Coil), wood smoke (WS), and water vapor as sham control. Responses, tested on three consecutive days (random order of aerosol presentation), were averaged for each subject.
Results Low frequency spectral powers of heart rate and blood pressure rose during recovery from exposure to particulate, but not to sham exposures. At breathing frequencies, spectral power of men's systolic pressure doubled, and baroreflex effectiveness increased, following ETS exposure. An index of sympathetic control of heart rate was more pronounced in men than women, in response to ETS and Coil, compared to WS and sham.
Conclusions When measured under controlled conditions, autonomic activities in non-smoking men and women exposed to low level, short term, particulate concentrations were similar to those observed during longer term, higher level exposures to ETS and to direct smoking. These increased indexes of sympathetic control of heart rate and peripheral vasomotion followed introduction of particulates by about 15 min. Finally, coupling of heart rate and systolic pressure indicated an increase in baroreflex activity in the response to breathing ETS that was less effective in men than women.

Keywords Autonomic control · Baroreflex · Blood pressure variability · Cooking oil · ETS · Gender · Heart rate variability · Wood smoke

Abbreviations

ANOVA	Analysis of variance
BEI	Baroreflex Effectiveness index
BRS	Baroreflex sensitivity
BP	Blood pressure
CO	Carbon monoxide
CO_2	Carbon dioxide
Coil	Cooking oil fumes
DBP	Diastolic blood pressure
ECG	Electrocardiogram
ETS	Environmental tobacco smoke
h	Hours
HR	Heart rate
IRB	Institutional review board
NO	Nitric oxide
RSP	Respirable suspended particulate matter
SBP	Systolic blood pressure
SNA	Sympathetic nerve activity
WS	Wood smoke

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Introduction

Epidemiologic studies continue to link ambient air pollution to cardiovascular morbidity (Link and Dockery 2010; Brook et al. 2010; Bartell et al. 2013). Increased cardiovascular risks from the most prevalent form of air pollution, passive inhalation of cigarette smoke, have also been established (Glantz and Parmley 1996; Barnoya and Glantz 2005; Steenland et al. 1996; Office on Smoking and Health (US) 2006). Morbidity effects, thought to be outcomes of long term exposure to nicotine and other byproducts of smoking, include accelerated atherosclerosis, endothelial dysfunction, increased coronary vasoconstriction and platelet aggregation (Office on Smoking and Health (US) 2006; Narkiewicz et al. 1998; Celermajer et al. 1993; Moliterno et al. 1994; Winniford et al. 1986).

Direct pathways linking inhaled particulates to long term cardiovascular morbidities have not been made but changes in autonomic balance controlling cardiac function have been documented in response to direct cigarette smoking (Narkiewicz et al. 1998; Andrikopoulos et al. 1999; Nabors-Oberg et al. 2002): parasympathetic withdrawal in control of heart rate is a hallmark of cigarette smoking (Andrikopoulos et al. 1999; Nabors-Oberg et al. 2002). In addition, one study established clear evidence for increased sympathetic outflow to muscle and skin, after baroreflex buffering of blood pressure had been removed (Narkiewicz et al. 1998). This smoking-induced shift in autonomic balance toward sympathetic dominance may also occur in response to inhaling environmental tobacco smoke (ETS).

Such evidence, linking inhaled ETS to shifts in autonomic control of the heart, has been documented in a real world study that used heart rate variability to index autonomic activity (Pope et al. 2001). With portable monitoring, Pope et al. (2001) recorded ECG in volunteers in two environments: smoking and nonsmoking rooms at the Salt Lake City airport. Their results clearly demonstrated that the heart rate spectral power index of sympathetic activity was elevated during the 2 h periods their subjects spent in rooms where smoking was permitted. However, because this was a field study, some variables, like subject activity, humidity and temperature of the rooms could not be controlled and gender differences were not reported. Finally, measurements reported by those authors indicate that the ETS level present inside the smoking areas was quite high, in at least one experimental session, mean nicotine concentration exceeded that of respirable suspended particulate (RSP) matter.

The present study was designed to address cardiovascular impacts of lower levels of ETS and other, work place relevant, levels of commonly inhaled particulates. In order to accomplish this goal, exposures were delivered under controlled environmental conditions to subjects whose activity levels were also controlled. Also, since the impact of inhaled particulates on autonomic control of peripheral vasomotion is rarely reported (Bartell et al. 2013), we monitored blood pressure variability, in addition to heart rate variability, so that comprehensive, autonomic effects of exposure to work place relevant levels of particulates on cardiovascular regulation could be established. The study had two hypotheses: (1) Under carefully controlled conditions, healthy, nonsmokers will respond to low levels of inhaled particulates with cardiovascular responses similar to those observed in subjects smoking cigarettes or inhaling high levels of ETS. (2) Since autonomic balance differs between men and women (Evans et al. 2001b), the inhaled particulate-induced shift in autonomic balance toward sympathetic dominance will be different between men and women.

Objectives

Our study builds upon and extends the understanding from the Pope study (Pope et al. 2001) by using a controlled environment, by recording ambient environment conditions, by recording cardiac, respiratory and peripheral vascular parameters, by including three work place relevant, particulate exposures and by including gender differences. Specifically, our study takes the next step in characterizing the autonomic balance of cardiac and vascular control during exposures to three different particulates (plus a water vapor sham) administered to men and women seated in an environmental chamber under controlled conditions.

Methods

Subjects

Nineteen men and 21 women were recruited, screened, underwent a physical exam, and were briefed for this study by Amick Research of Knoxville TN. Subjects signed a consent form, approved by both the University of Kentucky and Oak Ridge, Site Wide, Institutional Review Boards. Exclusion criteria included the presence of diabetes, systemic hypertension, ECG abnormalities compatible with the diagnosis of myocardial infarction/ischemia, ventricular enlargement, prolonged QT interval; 2nd or 3rd degree AV block; resting heart rate <50 or >100 beats/min; atrial flutter/fibrillation; frequent atrial and/or ventricular premature beats; broncho-pulmonary abnormalities in functional respiratory tests, or treatment with drugs known to alter directly or indirectly the autonomic drive to the heart and blood vessels. Subjects were then instructed to eat a light, low fat meal in the half hour before arriving at the laboratory.

Instrumentation of subjects

On the day of study, and after familiarization with experimental procedures, subjects were instrumented for continuous cardiorespiratory monitoring throughout the exposure protocol. Respiratory volume and frequency were determined from Respitrace bands placed around the thorax and abdomen (Inductotrace, Ambulatory Monitoring, Inc). Respiratory volume changes were calibrated from four inhalation/exhalation cycles using 800 cc Spirobags (Inductotrace, Ambulatory Monitoring, Inc). Non-invasive blood pressure, BP, was acquired from a plethysmographic cuff (Portapres, FMS) placed on the finger and subject activity was monitored using a three axis accelerometer (Crossbow, Jameco) placed on the subject's torso. Although the Nyquist requirement for BP and respiratory measurements were lower, all signals were digitized at 1,000 samples per second in order to maintain time alignment.

Pairs of subjects were studied per session. Each of the sessions (including instrument placement and removal and short breaks between each aerosol administration) lasted a little less than 3 h. Data for each exposure for each subject were averaged over the three exposure days and subject-averaged data were used for statistical testing.

Controlled experimental atmosphere chamber

A 30 m^3 volume, 10 × 12 × 8.5 feet high (internal dimensions) stainless steel interior chamber was used for these studies. The chamber (Bruischat Environmental Mfg, now SciTech), was operated under both active and static conditions. During periods of pre-exposure, flush out, and background determinations (see below) the chamber was operated in an active, fresh air provision mode with temperature and relative humidity adjusted automatically.

During the short periods of subject exposure, the chamber was operated in a static mode (no air recirculation). A small fan, running at 200 rpm, circulated air in the chamber. Air conditioning was activated as needed.

Aerosol generation

Subjects were exposed to three different aerosols and a sham exposure. The aerosols consisted of environmental tobacco smoke (ETS), cooking oil fumes (Coil), and cedar wood smoke (WS). Composition of the sham aerosol consisted of condensed water vapor and nitrogen. Generation of the wood smoke, environmental tobacco smoke, and cooking oil aerosols has been described in detail previously (Jenkins et al. 2004). The water vapor/nitrogen sham aerosol was generated by pouring 350 mL of liquid nitrogen on to warmed copper fittings while drawing the vapor into the chamber. This resulted in a condensed water vapor aerosol, clearly visible to the subjects that evaporated quickly. All aerosols were generated in an external glove box and directed into the exposure chamber as required.

Targeted peak particle concentrations for the Coil, ETS, and WS aerosols were 1750, 600, and 350 μg/m^3, respectively. Typical average concentrations during the 20–25 min individual exposure periods were ~two-thirds of the targeted maximum concentrations.

Aerosol monitoring

Real time monitoring of particle concentrations in the chamber used a DustTrak (Model 8520) calibrated previously against gravimetric collection of aerosol respirable suspended particulate matter (Jenkins et al. 2004). Over-reporting by the DustTrak was about three times higher than the actual concentration (determined gravimetrically) of aerosols. In addition, a Personal Data Ram (pDR-1000, Thermo Electron Corporation) was used to monitor particle concentrations, and to provide time points for beginning and end of exposures synchronized to cardiovascular monitoring instrumentation (Fig. 1). Real time monitoring of temperature, relative humidity, carbon monoxide (CO) and carbon dioxide (CO_2) concentrations (Fig. 1) was conducted using a Q-Trak Plus (Model 8554, TSI).

Calculation of particle concentrations and exposures

The response of the DustTrak was measured every second, reported in 10 s intervals and corrected for over-reporting using appropriate factors for each aerosol. The estimated background contribution was not subtracted from the exposure, since it represented a trivial contribution to individual test aerosol exposures.

Subject exposure protocol

Aerosol exposures were conducted over a period of 51 weeks, from mid-January 2005 to early January, 2006, over the course of 33 morning, and 33 afternoon, sessions that included exposure to each of three test aerosols and a sham. As reviewed and approved by both IRB's, subjects were exposed via a protocol that permitted short duration exposures to relatively high particle concentrations, followed by non-exposure or background periods; typically exposure sessions lasted 2.5–3 h. Maximum concentrations never exceeded the 8-h time weighted average exposure limit for non-specific respirable particles of 5,000 μg/m^3. Aerosol exposures were sufficiently low to as to keep daily particulate exposures below the EPA's single day ambient (outdoor) standard at that time of 65 μg/m^3.

Exposure sequences

Subjects were exposed in pairs, in either a morning or an afternoon session, for three sequential days. When subjects arrived at the test facility, they were quickly re-screened and females were asked to take a urine-based pregnancy test. Inside the chamber, subjects selected one of eight available movies to watch and were outfitted with cardiac and respiratory monitors. Once subjects were seated and comfortable, exposures were initiated, typically with a 10 min background exposure to fresh, conditioned air. The order of actual exposure sequence to the three test aerosols and one sham was random. Following the 10 min background period, the chamber was shifted to static mode operation, and aerosol generation commenced. For the ETS exposure, about 10 min were required to smoke the two cigarettes according to Federal Trade Commission standards, so aerosol concentrations increased to a maximum over the course of 10 min. In the case of the other test aerosols, maximum concentrations were reached in 5 min. Then, with the chamber still in a static mode, exposures were continued for 10 min. At the end of the 10 min exposure period, the chamber was switched to active mode and the test aerosol was flushed out of the chamber. This flushing period was followed by a brief air-recirculation mode and then a background period of 10 min. At that point, subjects were asked to stand and move about, were allowed bathroom breaks and were then reseated before the introduction of another aerosol was initiated.

Cardio-respiratory data evaluation (offline)

Heart rates (HR) and RR intervals were computed by identifying R waves of the ECG in each data collection period. Artifacts in HR and BP signals, including premature beats and Portapres servo adjustments, were manually removed. Only data segments which did not include servo adjustments were used for computation of the spectral estimates. Systolic (SBP) blood pressures were determined by computing the maximum values of clean arterial BP for each heart beat. Mean values of RR interval, breathing frequency and SBP were computed for each 5 min time segment: pre exposure 1 (data not used here), pre exposure 2, exposure 1, exposure 2, recovery 1, and recovery 2. All subsequent data analyses were performed using MATLAB.

Spectral analysis: RR interval, SBP and respiratory time series were resampled at 4 Hz using a cubic spline method. Each segment was then linearly detrended. Power spectral densities of RR intervals, SBP, and respiratory activity were estimated using Welch's method of averaged periodograms. Spectral powers in low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.4 Hz) regions were obtained using trapezoidal integration over the specified frequency range. Low frequency spectral power of systolic arterial pressure was used to index neurally mediated vasomotion (Montano et al. 1998).

Baroreflex sequences: we adopted the sequence method (Bertinieri et al. 1985) to quantify the number of spontaneous blood pressure ramps and baroreflex sequences, as well as baroreflex sensitivity. The ratio between the number of baroreflex sequences and the total number of increasing or decreasing SBP ramps determined the baroreflex effectiveness index (BEI) (Di Rienzo et al. 2001). Because the numbers of SBP ramps and baroreflex sequences depend on the number of analyzed heartbeats, which varied among and within subjects, the numbers of SBP ramps and baroreflex sequences were normalized by the number of heartbeats in each time segment.

Statistical analysis

To account for first day and for adaptation effects, results from all 3 days of study were averaged for each subject for each aerosol, before group averaging or statistical analysis was performed. Three factor (gender, aerosol, stage of exposure), with repeated measures on the aerosol and stage of experiment factors, analysis of variance (ANOVA) was performed on all data. For most variables, each stage of the study was broken into 5 min segments. The first 5 min segment of the pre exposure control was considered an adaptation period and those results were excluded from further analysis. Data reported are from the following 5 min segments: end of control, early exposure, late exposure, early recovery and late recovery. When three factor interactions failed to achieve statistical significance, two factor ANOVA was conducted, followed by one factor ANOVA if two factor interactions were not significant. Post-hoc comparisons between particulate species, time and gender effects were made using least significant differences. Results are presented as the mean ± standard error of the mean (SEM).

Results

The median average temperature for all exposure sessions was 70.8 °F, and the median temperature range (maximum–minimum) was 3.2 °F.

Figure 1 provides a representative exposure session presenting concentration/time trace of aerosols with an overlay of carbon monoxide and carbon dioxide concentrations for that exposure session. Exposure durations averaged 24.3, 27.7 and 22.2 min for cooking oil, ETS, and wood smoke aerosols, respectively. Actual exposures to the test aerosols comprised ~75 min over a typical 2.5–3 h session.

Carbon monoxide (CO) and carbon dioxide (CO_2) concentrations fluctuated over the course of the exposure

Table 1 Summary of aerosol particulate exposures, in μg-h/m^3 $N = 66$

Parameter	Exposure matrix		
	Cooking oil fumes	Environmental tobacco smoke	Cedar wood smoke
Median	409.4	146.0	69.9
Mean	400.9	154.5	77.3
Std deviation	101.7	43.8	52.1
Relative std deviation, %	25.4	28.4	67.4
10th percentile	292.2	119.4	38.2
90th percentile	473.3	194.7	96.1

Table 2 Subject characteristics (mean ± standard error of the mean)

Gender	Height (in.)	Weight (lbs)	Exercise (h/week)	Age (years)
Females (21)	65.1 ± 0.7	163.6 ± 9.2	2.4 ± 1.8	34.6 ± 1.3
Males (19)	71.5 ± 0.6	231.8 ± 9.1	2.2 ± 0.6	34.8 ± 1.4

session, depending on which aerosol was being presented and the chamber status at any given time. Carbon monoxide levels increased above background only when combustion derived aerosols (wood smoke and ETS) were introduced into the chamber. While the introduction of combustion derived aerosols resulted in increased levels of CO_2 in the chamber, this figure demonstrates that the primary source of CO_2 was exhaled air from subjects.

Table 1 provides a summary of the particulate exposure levels delivered to the subjects. "Exposure level" is defined as the product of the magnitude and duration of the exposure and is given in units of μg-h/m$^{3.}$

Subject characteristics

Demographics of this study's subjects are given in Table 2. Other than typical height and weight differences between men and women, there were no gender differences in terms of age or exercise levels. One woman was Hispanic and one was Native American, otherwise all subjects were white Caucasians. None of the subjects smoked cigarettes but one man and one woman were regularly exposed to ETS at home or at work, while two women and one man were regularly exposed to workplace cooking oil fumes. Phase of the menstrual cycle was not controlled for the women of this study, five women were not ovulating, seven were in the follicular phase and nine were in the luteal phase of the menstrual cycle.

Mean values of cardiovascular variables

There were no gender or aerosol effects for mean values of systolic pressure, heart rate, or respiratory rate but mean

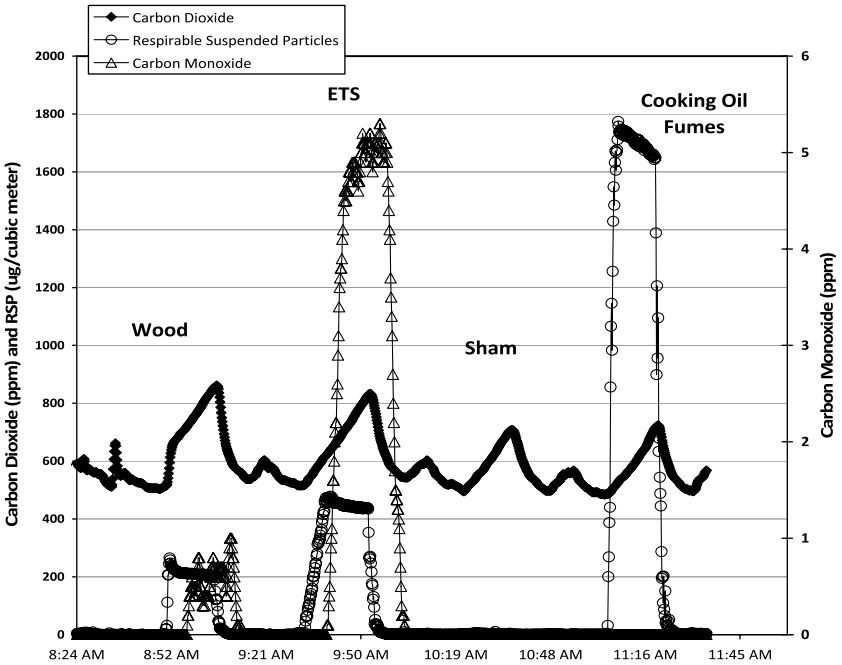

Fig. 1 Particle concentrations, as measured instrumentally, with carbon dioxide and carbon monoxide overlayed during a typical exposure sequence. Concentrations have been corrected for documented over-reporting by DustTrak

Table 3 Low and high frequency RR interval spectral powers (ms^2) of 19 men and 21 women at five stages of exposures to three particulates and a water vapor sham

	Male subjects					Female subjects				
	PRE	EXP1	EXP2	REC1	REC2	PRE	EXP1	EXP2	REC1	REC2
Low frequency (0.04-0.15 Hz) spectral power of RR intervals (ms^2)										
SHAM	463 ± 66	445 ± 55	490 ± 65	490 ± 65	547 ± 75	282 ± 40	262 ± 33	283 ± 37	278 ± 40	311 ± 51
ETS	398 ± 65	384 ± 47	416 ± 39	541 ± 61	476 ± 56	257 ± 40	334 ± 55	298 ± 49	367 ± 57	356 ± 62.5
CO	464 ± 71	438 ± 50	512 ± 74	627 ± 100	686 ± 104	263 ± 41	291 ± 43	316 ± 58	329 ± 51	321 ± 46
WS	490 ± 67	432 ± 56	505 ± 86	571 ± 77	560 ± 70	291 ± 45	337 ± 59	307 ± 53	400 ± 78	368 ± 78
High frequency (0.15–0.40 Hz) spectral power of RR intervals (ms^2)										
SHAM	90 ± 18	93 ± 12	104 ± 21	82 ± 13	87 ± 11	80 ± 15	81 ± 18	99 ± 23	79 ± 21	72 ± 16
ETS	77 ± 14	77 ± 17	87 ± 15	103 ± 22	98 ± 24	89 ± 10	98 ± 24	85 ± 19	90 ± 20	98 ± 23
CO	75 ± 15	87 ± 18	84 ± 18	92 ± 16	102 ± 23	85 ± 19	77 ± 17	73 ± 17	81 ± 18	76 ± 15
WS	103 ± 19	88 ± 14	92 ± 20	104 ± 18	120 ± 22	93 ± 19	99 ± 22	97 ± 23	87 ± 18	82 ± 16
Low-to-high frequency ratio of spectral power of RR intervals										
SHAM	6.7 ± 0.9	6.0 ± 0.9	7.7 ± 1.3	7.4 ± 1.0	7.3 ± 1.0	4.9 ± 0.7	4.7 ± 0.6	4.8 ± 0.7	6.0 ± 1.2	5.5 ± 1.2
ETS	7.2 ± 0.9	7.5 ± 1.1	7.0 ± 1.3	8.8 ± 1.7	7.6 ± 1.0	4.2 ± 0.7	4.9 ± 0.8	5.4 ± 0.8	6.1 ± 1.1	5.3 ± 0.9
CO	8.1 ± 1.0	8.7 ± 2.1	8.6 ± 1.1	9.0 ± 1.4	10.1 ± 1.5	4.6 ± 0.8	5.7 ± 0.8	5.9 ± 0.7	5.6 ± 0.8	5.7 ± 0.8
WS	5.9 ± 0.9	6.1 ± 0.9	6.8 ± 1.2	7.9 ± 1.4	6.3 ± 1.3	4.7 ± 0.9	5.1 ± 0.9	5.2 ± 0.8	6.0 ± 0.9	5.4 ± 0.7

Statistical significance in LF: exposure stage × chemical species interaction, $p < 0.02$ and in LF/HF: gender, $p < 0.046$; exposure stage, $p < 0.007$, chemical species, $p < 0.009$, gender × chemical species, $p < 0.049$. Values are mean ± SE

SHAM water vapor sham, *ETS* environmental tobacco smoke, *CO* cooking oil, *WS* wood smoke, *PRE* last 5 min of control, *EXP1* first 5 min of full exposure, *EXP2* last 5 min of exposure, *REC 1* first 5 min of recovery from exposure, *REC2* second 5 min of recovery to exposure

systolic blood pressure rose by 4 mmHg ($p < 0.01$) across the course of the exposure sequence in all but the women's sham exposure. There was an accompanying minor (1 bpm, $p < 0.01$) rise in mean heart rate with no effect on mean values of respiratory frequency (not shown).

RR interval variability

In the very low frequency (VLF, 0.003–0.04 Hz) region, we found two main effects: men had overall higher power than women ($p < 0.011$), and a significant rise across exposure to particulate, but not sham, aerosols occurred (exposure stage × chemical species interaction, $p < 0.001$).

Low frequency spectral power of RR interval (Table 3) was similar to VLF, including a marginal main effect for gender ($p < 0.058$) and a significant (exposure stage × chemical species interaction, $p < 0.02$) rise in LF power across particulate, but not sham, exposures.

In the HF range, 0.15–0.40 Hz (Table 3), there were no significant effects of gender, exposure stage or aerosol species.

For the ratio LF/HF, a commonly used index of sympathetic control of heart rate, Table 3, women had lower ($p < 0.046$, main effect of gender) values than men. In addition, there was a significant rise in LF/HF across exposures ($p < 0.007$, main effect of exposure stage), reaching a peak during recovery. In addition, LF/HF was greater during cooking oil exposure than during exposure to either sham or wood smoke ($p < 0.009$, main effect of aerosol) and was marginally greater than during exposure to ETS ($p < 0.055$). Finally, the significant gender by chemical species interaction ($p < 0.049$) established that men's (but not women's) LF/HF values were greater during ETS ($p < 0.05$) and cooking oil ($p < 0.03$), as opposed to sham or WS, exposures.

For the HF/(LF + HF) index of parasympathetic control of heart rate (not shown), women had higher ($p < 0.023$) values than men and both men and women demonstrated a significant ($p < 0.001$) decline in power during particulate, but not sham, exposures, reaching a nadir during recovery.

Blood pressure variability

Spectral power of systolic blood pressure for one male subject before exposure, and during the build-up, steady state and wash-out of ETS is shown in Fig. 2. Note that high frequency (0.15–0.40 Hz) spectral power of SBP increased during exposure, but returned to near pre exposure levels by the end of the flush period. Note also, that low frequency (<0.15 Hz) spectral power of SBP increased during exposure and continued to be elevated above control values throughout the recovery period.

Group averaged (mean ± SEM), low frequency, spectral powers of systolic arterial pressure, are shown for men

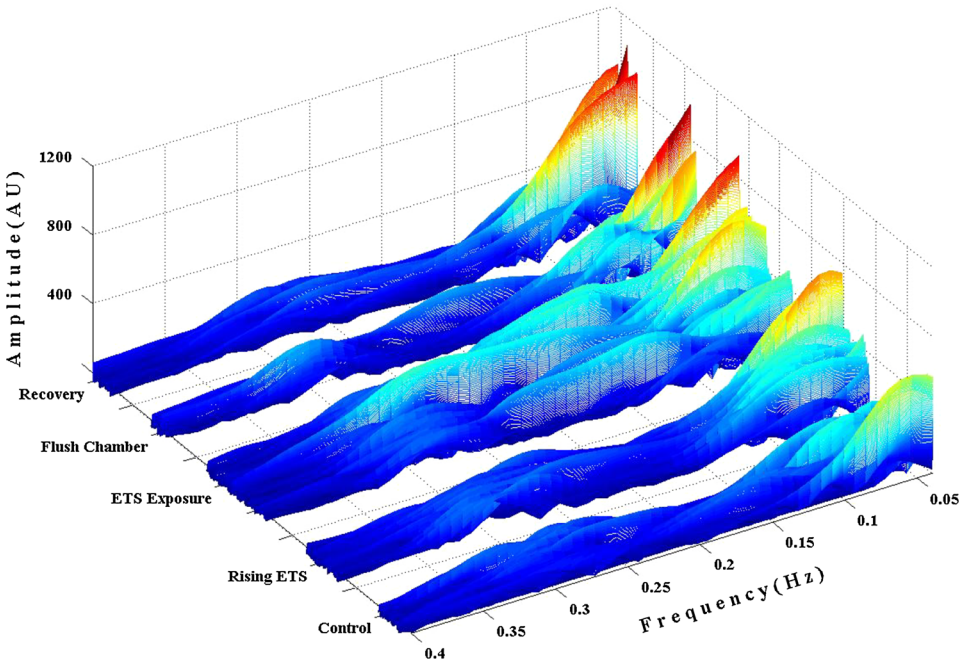

Fig. 2 Time frequency plot of one man's spectral power of blood pressure before exposure, during exposure to ETS, and clearing of the ETS aerosol from the chamber. Time is given on the *x* axis, magnitude of blood pressure excursions on the *z* axis and frequency on the *y* axis. Note increase in power in the breathing frequency range (0.15–0.35 Hz) during steady state exposure. This subject's peak, breathing frequency, power of blood pressure occurred during the exposure, while for most subjects, the peak occurred during the first 5 min of flushing the chamber. Note also increased low frequency power (<0.15 Hz) near end of exposure

and women in the top row of Fig. 3a–d for end of control, the first and last half of exposure to, and the first and last half of recovery from, ETS, cooking oil, wood smoke and to the water vapor aerosol. Increased low frequency spectral power of systolic pressure (exposure stage × chemical species, $p < 0.001$) was evident in all particulate (but not sham) exposures and peaked during recovery. Responses of men and women were not significantly different.

Group averaged, high frequency (0.15–0.40 Hz) spectral power of systolic blood pressure is shown in the bottom row of Fig. 3e–h for men and women in control, for the first and last half of exposure to, and the first and last half of recovery from, ETS, cooking oil fumes, wood smoke, and water vapor aerosols.

For this variable, there was a significant three factor interaction ($p < 0.003$): in response to ETS, men's SBP_{HF} doubled in the first recovery period ($p < 0.001$) compared to their responses to any other aerosol during the same period, and men's values were greater than women's during the same period. By the second recovery period, men's values were returning toward control. The increase in women's SBP_{HF} response to cooking oil was similar to men's response to ETS, but it was not significant. Both men's and women's responses to wood smoke exposure peaked during the second recovery period, but only the women's response was significantly ($p < 0.04$) greater than their response to sham. As with SBP_{LF}, the increase in SBP_{HF} was evident in all particulate (but not sham) exposures and peaked during recovery (exposure stage × chemical species interaction, $p < 0.011$).

Baroreflex sensitivity

Differences in blood pressure regulation (arterial baroreflex control of heart rate), most evident early in recovery from ETS exposure, are shown in Fig. 4. This figure shows the number of spontaneous baroreflex sequences (normalized by heart rate) in response to rising blood pressure ramps averaged over the first 5 min of recovery from exposure to each aerosol. There was a marginal gender by chemical species interaction ($p < 0.058$) in this variable indicating that, in men, the number of baroreflex sequences in response to ETS was greater than the number in response to sham or to the other particulates while the number of women's sequences in response to ETS exposure was significantly greater than their number in response to wood smoke.

The number of men's and women's rising blood pressure ramps during this period tended to be smaller for all particulate, compared to sham exposures, but this trend was not statistically significant. The baroreflex effectiveness index (number of baroreflex sequences/number of blood pressure ramps) therefore resembles the number of baroreflex sequences shown in Fig. 4, with a value greatest during recovery from ETS, compared to other exposures. These data reflect that the baroreflex was involved in buffering rises in blood pressure ~26 % of the time in response to sham, cooking oil and wood smoke, rising to ~33 % with ETS exposure ($p < 0.066$). Sensitivity of the baroreflex (slope of RR interval vs systolic arterial pressure) was not different for any exposure.

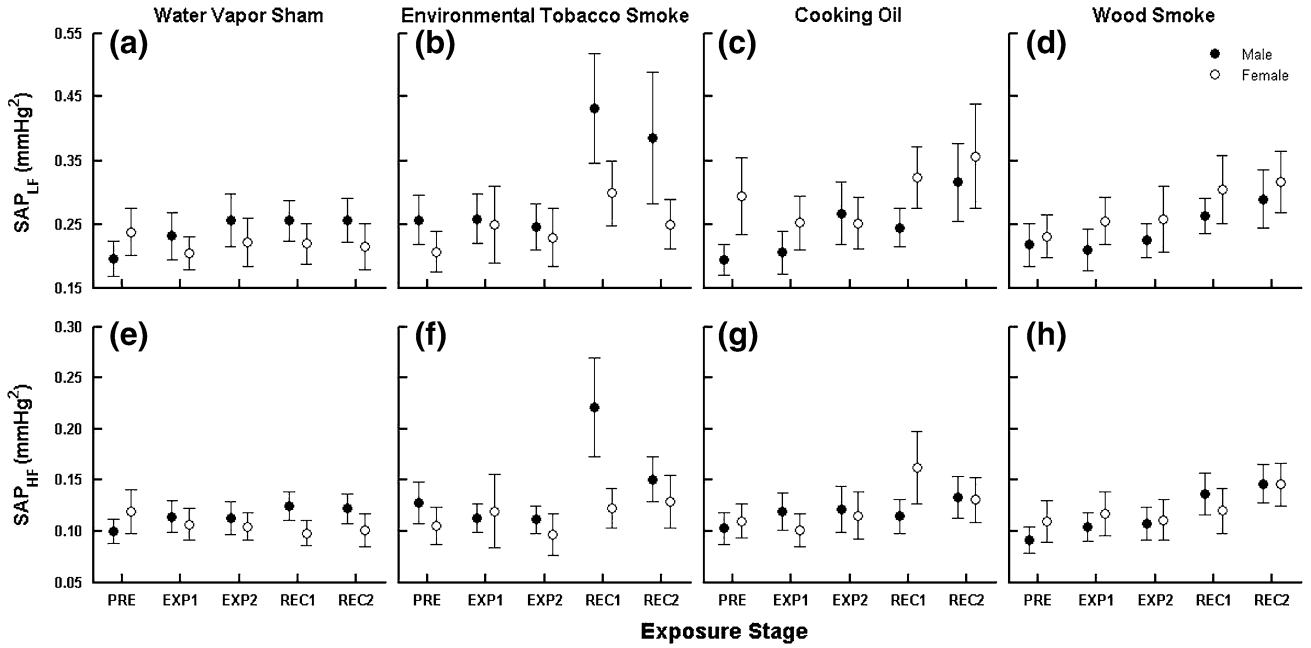

Fig. 3 Spectral power of systolic blood pressure, mean ± SEM. *Top row* Low (0.04–0.15 Hz) frequency spectral power of systolic pressure (SAP LF). *Bottom row* High (0.15–0.40 Hz) frequency spectral power of systolic blood pressure (SAP HF). Results are shown for men and women as a function of exposure stage: *PRE* last 5 min of control, *EXP1* first 5 min exposure, *EXP2* second 5 min exposure, *REC1* first 5 min recovery, *REC2* second 5 min recovery from exposure to water vapor sham, ETS, Coil, and WS. *SAP LF* exposure stage × chemical species interaction $p < 0.001$. *SAP HF* gender × chemical species × exposure stage interaction, $p < 0.003$

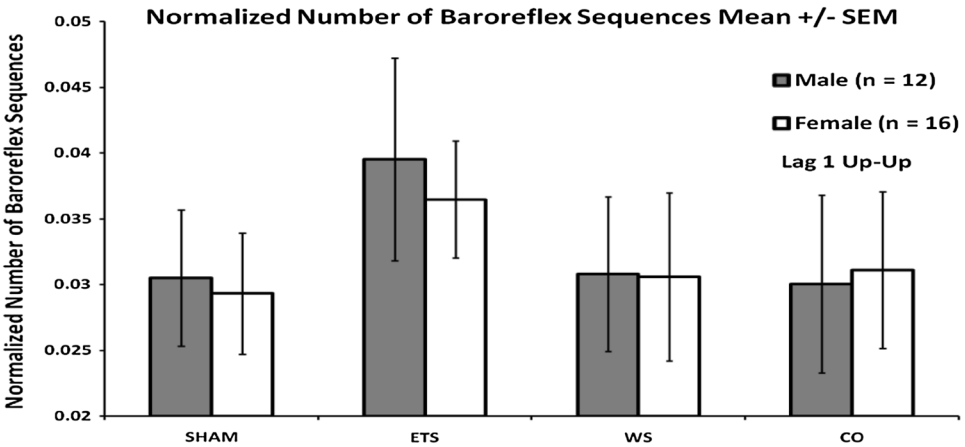

Fig. 4 Mean ± SEM values of the number of baroreflex sequences in response to rising blood pressure (normalized by the number of heart beats per session) during the first 5 min recovery period following exposure to each aerosol for men and women. Gender × treatment, $p < 0.058$

Discussion

The first question addressed by this study was whether we could, in a carefully controlled environment, detect heart rate variability responses from healthy, non-smoking men and women breathing real world concentrations of common particulates (compared to their responses when breathing a water vapor sham)? The second and third questions were whether we could detect vasomotion (blood pressure variability) and/or baroreflex responses under the same conditions? In conjunction with each of these questions, we asked if there were gender differences in cardiovascular responses to these inhaled aerosols? Finally, we needed to answer an underlying question as to whether we had successfully simulated particulate exposures documented in real world environments?

In response to the first question, this study demonstrated significant heart rate variability responses to particulate, but not water vapor (sham), exposure. In particular, we found that, in response to ETS from two cigarettes,

changes in noninvasive indexes of autonomic control of heart rate were smaller, but similar, to those previously reported in response to direct cigarette smoking (Andrikopoulos et al. 1999; Kobayashi et al. 2005) and to exposure to high levels of second hand tobacco smoke (Pope et al. 2001). In addition, ETS and cooking oil exposures were associated with greater low frequency spectral power of heart rate in men than in women breathing the same concentrations, or in the same men, when they breathed water vapor. Finally, the index of parasympathetic control of heart rate declined, and the index of sympathetic control of heart rate rose, during exposure to particulate, but not to sham, aerosols.

Parallel responses occurred in blood pressure indexes of peripheral vasomotion which rose significantly with exposure to particulates, but not to sham, peaking during recovery. In particular, men's SBP_{HF} during recovery from ETS was significantly greater than the women's response or than these men's response to any other aerosol.

Since breathing frequency was unaffected by any aerosol, the increase in men's high frequency spectral power of blood pressure in response to ETS, could come from an increased depth of breathing and/or from decreased heart rate buffering of systolic pressure oscillations. In conjunction with the larger oscillations in men's blood pressure, we found an increase in the numbers of baroreflex sequences during recovery from ETS. Interestingly, even with increased numbers of baroreflex sequences, men's baroreflex was less effective than women's in buffering the ETS-induced oscillations of blood pressure. Specifically, men's low frequency spectral power of systolic pressure increased ~66 % in response to ETS and was accompanied by an ~35 % increase in low frequency spectral power of heart rate. On the other hand, women's low frequency spectral power of blood pressure increased ~19 % and was accompanied by an ~66 % increase in low frequency spectral power of heart rate.

For all particulates, peak spectral powers of heart rate and blood pressure occurred in the recovery period, indicating a delay of 10–20 min after the initiation of the exposure, for these, presumably, neurally involved physiological responses. This delay in the appearance of increased spectral power of blood pressure from the onset of particulate exposure indicated that these were not purely neural responses but were probably linked to circulating factors (like catecholamines). The RR Interval response to ETS demonstrated timing that was also similar to the response time evoked by direct smoking (Kobayashi et al. 2005). This delay in the rise of RRI spectral power was probably coupled to the delayed rise in spectral power of blood pressure. In particular, we speculate that the increase in very low frequency power of RRI in response to particulate exposures, may indicate a regulatory blood pressure response that extended to frequencies below the normal range of heart rate buffering of blood pressure oscillations.

Group averaged, partial coherences between blood pressure and heart rate, with respiration partialed out, were examined in a previous study for the same time periods and exposures to these aerosols (Jayanthi et al. 2007). Those results indicated peak coherencies of magnitude between 0.4 to 0.6 between systolic pressure and heart rate in frequency regions known to characterize parasympathetic (~0.23 Hz) and combined parasympathetic and sympathetic (~0.08 Hz) regulation of heart rate. Men's partial coherence was slightly, but not significantly, greater than women's. Coherencies between respiration and blood pressure (heart rate partialed out) and respiration and heart rate (blood pressure partialed out) were flat across the frequency range with an average value of ~0.2. The low coherence in these last two measures gives strength to the higher coherence seen in the blood pressure/heart rate relationship and strongly supports the importance of baroreflex activity in buffering responses to particulate exposures in both low and high frequency domains.

Epidemiologic studies have determined that concentrations of fine and ultrafine (<2.5 μm, $PM_{2.5}$) components of ambient air pollution are directly related to cardiovascular mortality (Bhatnagar 2006; Pope et al. 2006; Schwartz et al. 2002) and can be measured by heart rate variability (Gold et al. 2000). Speculation as to mechanisms linking cardiovascular disease to fine particle air pollution indicate that disruption of nitric oxide pathways resulting in damaged blood vessels may lead to development of cardiovascular diseases (Bhatnagar 2006). That study further made the case that cigarette smoking, exposure to ETS and exposure to fine particle air pollution were characterized by similar vascular responsiveness and could each be driven by the same mechanism(s). Support for Bhatnagar's hypothesis is found in a study of vascular reactivity and endothelial function which documented impaired vascular function response to short term exposure to concentrations of ambient fine particles that mimic the urban environment (Brook et al. 2002). The heart rate and blood pressure variability responses of our subjects to particulate, but not sham, exposures support the hypothesis that exposure to workplace-relevant levels of airborne particulates directly decreased the index of parasympathetic control of heart rate, increased the index of sympathetic control of heart rate and increased peripheral vasomotion in healthy, non smoking, adult men and women. Baseline gender differences reported in this study were not different from those we previously reported (Evans et al. 2001a).

Cardiovascular risks from long term exposure to ETS are well documented in the Office on Smoking and Health (US) (2006), but the threshold exposure necessary to detect biomarkers of cardiovascular responses to short term

exposures has not been clearly established. Increased sympathetic control of autonomic balance regulating heart rate was clearly established for a group of 25 (9 males and 16 females) subjects breathing a very high concentration of ETS (average number of lit cigarettes ~9) during two, 2-h sessions seated in an airport smoking room, compared to their response to two, 2-h sessions in nonsmoking areas (Pope et al. 2001). The present study lowers the threshold to detect such responses.

The present study adds seven findings to those of the Pope study: (1) documentation that detectable heart rate variability responses were observed in response to short exposures to lower levels of ETS concentrations, when observations were made under tightly controlled conditions, (2) other inhaled particulates evoke a similar (to ETS) heart rate variability response, (3) ETS and other inhaled particulates stimulate increased peripheral vasomotion, (4) responses to particulates peaked 15–20 min after initiation of exposure, (5) gender differences in sympathetic control of heart rate were greater in response to ETS and cooking oil fumes than to wood smoke or sham exposures, (6) men's exaggerated systolic pressure response to ETS in the breathing frequency range may result from a decreased ability of their baroreflex to regulate blood pressure in response to ETS and (7) responses to particulates evoked an overall increase in indexes of sympathetic vasomotor activity that, in addition to being gender dependent, may also be concentration dependent.

The background on the magnitude of arterial baroreflex buffering of the cardiovascular response to inhaled particulates is sparse. Increased spectral power of both blood pressure and heart rate have been reported in subjects smoking cigarettes (Hayano et al. 1991; Narkiewicz et al. 1998; Andrikopoulos et al. 1999). The report of Narkiewicz et al. (1998) specifically addressed the importance of baroreflex buffering of direct effects of cigarette smoking on blood pressure, muscle and skin sympathetic nerve activities, heart rate, and biochemical marker responses to cigarette smoking. In one arm of the study, baroreflex responses were clamped by maintaining blood pressure near control levels throughout smoking exposure and recovery from that exposure. They determined that, with baroreflex buffering removed, muscle sympathetic nerve activity (SNA) increased 50-fold and heart rate increased 37 beats/min in response to smoking a single cigarette, compared to the minimal changes in those variables when the same subjects performed sham smoking. They also speculated that since muscle SNA activity was robust in comparison to the catecholamine response, those activities represented different aspects of sympathetic outflow. Finally, they verified that skin sympathetic outflow was not baroreflex mediated since it was increased to the same amount by cigarette smoking whether or not changes in blood pressure were involved. Their study clearly demonstrated a major point that we are also making: the challenge in detecting cardiovascular biomarkers of responses to ETS and, presumably, other inhaled particulates, lies in presenting a stimulus strong enough to overcome reflex buffering while still maintaining reasonable levels of exposure. We can only speculate that the minimal but significant, increases in men's systolic blood pressure (4 mmHg) and heart rate (1 beats/min) and the increased oscillations in systolic pressure, following ETS exposure, would have been considerably greater had our study been conducted in subjects whose reflex activities were blocked by clamping blood pressure at control levels. It is also likely that the cardiopulmonary branch of the baroreflex was the operative buffering mechanism invoked, since heart rate did not decrease in response to the small increase in blood pressure in response to ETS and the other particulates. Although the responses measured in our study are those that would be expected for an individual in a real life scenario, the actual load of particulate exposure would be a function of minute ventilation (i.e. concentration times volume in a unit time). However, our objective was to quantify the physiological response that would be elicited in an individual and not dose response per se and therefore we did not adjust for particulate exposure load.

In answer to the underlying question about relevance of this study's exposure concentrations to real world particulate exposures, we determined that the mean values of exposures (Coil, 990, ETS, 335, and wood smoke, 210 μg/m^3) in our study were comparable to those reported by studies in real world environments. For example, Balakrishnan et al. (2004) reported 24 h in-kitchen concentrations ranging from ~100–1,500 μg/m^3 when wood was used as a cooking fuel in Indian households. Lee et al. (2001) reported peanut oil cooking fumes in a metropolitan Hong Kong Korean barbeque restaurant ranging from about 700–2,900 μg/m^3. Maskarinec et al. (2000) reported gravimetric ETS-specific area concentrations ranging up to 393 μg/m^3 in smoking areas of 53 bars in Knoxville, TN, the area from which our subjects were recruited. Our median ETS exposure was 146 μg-h/m^3 and represented 23.5 % of the total measured median exposures to what is defined as respirable suspended particulate matter. This compares reasonably well to results obtained in other studies (Tomkins et al. 2006; Jenkins et al. 2005) for non-smoking subjects living in smoking homes. Of course, our subjects were exposed over a shorter time period with median ETS levels less than 50 % of those typically experienced by individuals living with smokers during a 16 h, away-from-work period, (Jenkins et al. 1996). The similarities in our subjects' responses to the wide range (~200–1,000 μg/m^3) of particulate levels points to a stress response to inhaled particulates that should be explored further.

Conclusions

Taken together, data from our study indicate that heart rate and systolic blood pressure oscillations in both low and high frequency ranges are sensitive indicators of cardiovascular, autonomic reactions to airborne particulate exposures. Interactions between systolic blood pressure and heart rate indicated that baroreflex activity in low and high (and, probably, very low) frequency ranges actively buffered particulate-induced increases in vasomotor activity so that increases in mean blood pressure and heart rate were kept to a minimum. Finally, the effect of our low level, short term, exposure to ETS that increased spectral power of high (respiratory) frequency systolic pressure more in men compared to women, and in men compared to all other aerosols, implies a gender specific response to ETS that warrants further exploration.

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Conflict of interest JME, ARP, RHI, QZ and CFK have no competing interests. RAJ was a Distinguished Research and Development staff member at Oak Ridge National Laboratory until his retirement in 2004. He acted as an expert witness in tobacco industry-related litigation from 1997 to 2008.

Ethical standard Prior to conduct of any part of this study, all subjects signed a consent form, approved by both the Oak Ridge Site Wide and University of Kentucky Institutional Review Boards (IRBs). The conduct of these studies complied with all regulations stipulated by these institutions.

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