

Hemodynamic, Autonomic, and Vascular Effects of Exposure to Coarse Particulate Matter Air Pollution from a Rural Location

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BACKGROUND: Fine particulate matter (PM) air pollution is associated with numerous adverse health effects, including increased blood pressure (BP) and vascular dysfunction. Coarse PM substantially contributes to global air pollution, yet differs in characteristics from fine particles and is currently not regulated. However, the cardiovascular (CV) impacts of coarse PM exposure remain largely unknown.

OBJECTIVES: Our goal was to elucidate whether coarse PM, like fine PM, is itself capable of eliciting adverse CV responses.

METHODS: We performed a randomized double-blind crossover study in which 32 healthy adults (25.9 ± 6.6 years of age) were exposed to concentrated ambient coarse particles (CAP; 76.2 ± 51.5 µg/m³) in a rural location and filtered air (FA) for 2 hr. We measured CV outcomes during, immediately after, and 2 hr postexposures.

RESULTS: Both systolic (mean difference = 0.32 mmHg; 95% CI: 0.05, 0.58; *p* = 0.021) and diastolic BP (0.27 mmHg; 95% CI: 0.003, 0.53; *p* = 0.05) linearly increased per 10 min of exposure during the inhalation of coarse CAP when compared with changes during FA exposure. Heart rate was on average higher (4.1 bpm; 95% CI: 3.06, 5.12; *p* < 0.0001) and the ratio of low-to-high frequency heart rate variability increased (0.24; 95% CI: 0.07, 0.41; *p* = 0.007) during coarse particle versus FA exposure. Other outcomes (brachial flow-mediated dilatation, microvascular reactive hyperemia index, aortic hemodynamics, pulse wave velocity) were not differentially altered by the exposures.

CONCLUSIONS: Inhalation of coarse PM from a rural location is associated with a rapid elevation in BP and heart rate during exposure, likely due to the triggering of autonomic imbalance. These findings add mechanistic evidence supporting the biological plausibility that coarse particles could contribute to the triggering of acute CV events.

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Introduction

Particulate matter (PM) air pollution is a leading cause of global mortality (Lim et al. 2012). Epidemiological, exposure, and toxicological studies altogether provide coherent evidence that fine PM (< 2.5 µm in diameter; PM_{2.5}) can pose cardiovascular (CV) health risks (Brook et al. 2010). PM_{2.5} is derived principally from combustion processes (e.g., coal-fired power plants, vehicle exhaust) and is known to be associated with a wide array of biological responses capable of instigating acute CV events among susceptible individuals. Indeed, a recent American Heart Association scientific statement concluded that the overall evidence linking PM_{2.5} with CV diseases is consistent with a causal relationship (Brook et al. 2010).

On the other hand, the CV health impacts of exposure to “coarse” PM, which is larger in diameter (2.5–10 µm; PM_{10–2.5}), are less conclusive (Brunekreef and Forsberg 2005; Chang et al. 2011; Peng et al. 2008;

Puett et al. 2009; Zanobetti and Schwartz 2009). Coarse PM is an important contributor to worldwide air pollution, typically accounting for 40–60% of overall particulate mass < 10 µm in diameter (Brook et al. 2010; Brunekreef and Forsberg 2005). Not only do its sources and components differ from PM_{2.5}, the composition of coarse PM itself often varies to a greater extent across seasons and locations (i.e., coarse PM is more spatially heterogeneous than PM_{2.5}). Coarse PM represents an assorted mixture of particles (e.g., aerosolized soil/sand or crustal material) most typically generated during mechanical processes such as crushing, grinding, or resuspension of ground material, with sources ranging from farming and roadway dust to construction activities. The major constituents also substantively differ according to nearby activities, landscape features (e.g., desert sands, soil), and local vegetation cover and include metals, crustal material (e.g., silicon, calcium, other natural elements), and

bioaerosols (e.g., pollen, endotoxin) (Brook et al. 2010; Brunekreef and Forsberg 2005).

Short-term exposure to PM_{2.5} has been associated with a variety of adverse CV responses, including vascular dysfunction, altered heart rate variability, and augmented coagulation–thrombosis potential (Brook et al. 2010). In particular, we and others have shown that controlled exposures to PM_{2.5} in the form of concentrated ambient particles (CAP) promotes vasoconstriction and a rapid increase in blood pressure (BP) via altered autonomic balance (Brook et al. 2002, 2009; Huang and Ghio 2009; Lippman and Chen 2009; Urch et al. 2005). Conversely, few studies have assessed the CV responses induced by coarse CAP (Gong et al. 2004; Graff et al. 2009). The effects of coarse CAP on BP and vascular function have never been investigated and remain unknown. Because of differences in sizes, chemical natures, and fates upon inhalation, coarse CAP could potentially elicit responses dissimilar to those induced by fine CAP. In light of the global epidemic of PM-related morbidity and mortality (Brook et al. 2010), along with the important contribution of coarse PM to air pollution throughout many regions (Brunekreef and Forsberg 2005) and the fact that it is not currently a regulated size fraction per se, it is critical to elucidate the potential for adverse CV consequences related to coarse particle exposure. Mechanistic exposure studies help to inform on our present limitations of scientific knowledge regarding the biological plausibility that coarse PM could prompt CV events. As such, we aimed to evaluate the CV responses

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prompted by coarse CAP exposure, with the initial studies designed to test the effects of particles derived from a rural setting.

Methods

Study outline. The study was a randomized double-blind crossover study comparing the health effects of 2-hr-long exposures to coarse CAP versus filtered air (FA) among healthy adults ($n = 32$) conducted from May 2011 to June 2012. The study was approved by the institutional review board of the University of Michigan, and all study participants signed a written informed consent document during a screening visit when initial blood labs were drawn (fasting lipids, glucose) and a brief history and physical examination were performed. By entry criteria, study participants were healthy, nonsmoking adults living in nonsmoking households, 18–50 years of age without any established CV disease or traditional risk factors (hypertension, treated hyperlipidemia, diabetes). All study participants had screening BP values $< 140/90$ mmHg and fasting glucose levels < 126 mg/dL. No study participant was taking any medication (e.g., statins) or over-the-counter pills (e.g., antioxidants) that might alter vascular function. We also aimed to enroll a subset of qualifying study participants with a body mass index (BMI) of ≥ 30 kg/m² in order to investigate whether those with higher BMI levels were more susceptible to CAP-mediated health outcomes (i.e., demonstrate more robust adverse CV responses) than those with lower BMI values.

Qualifying study participants entered into the randomized double-blind crossover study. There was a 1- to 3-week washout period between exposures, a period which in previous studies has been shown to be adequate to assure that baseline values are not significantly different between exposures (Brook et al. 2009). Study participants came to the facility on each study visit day having fasted for ≥ 8 hr before arriving for the visit. Randomized exposures occurred between 1000 and 1200 hours. Outcomes were not measured before exposures, but were all determined immediately and 2 hr postexposures. The exception was that measures of endothelial function were only measured at the 2-hr postexposure time point because those procedures might disrupt other outcomes. These two time points (immediately and 2-hr postexposures) were selected because previous studies performed by our group (Brook et al. 2002, 2009) and others (reviewed by Lippman and Chen 2009) using fine CAP exposures have demonstrated changes in the outcomes evaluated in the present study within this time period. Study participants rested within the exposure facility between health measurement time periods. During exposures BP, heart rate,

and electrocardiogram (ECG) recordings were measured as described below. An outline figure of the study design is provided in Supplemental Material, Figure S1.

Cardiovascular outcomes. BP and vascular outcomes (including previous reproducibility information) were determined as described previously (Brook et al. 2002, 2009; Urch et al. 2005). All protocols were performed in the order as follows and analyzed by a vascular technician who was blinded to the exposures.

During exposures. After a 10-min rest period after entering the exposure chamber, we measured study participants' left arm brachial BP along with heart rate every 10 min with an appropriate-sized cuff and the arm held at mid-sternal level within the chamber during exposures, using a Spacelabs ambulatory BP 90207 monitor and software package (Spacelabs Healthcare, Snoqualmie, WA, USA). Continuous ECG monitoring was also performed starting immediately upon the participants entering the chamber, using the evo Holter system (Spacelabs Healthcare). We analyzed time and frequency domain heart rate variability (HRV) metrics at 5-min-long epochs using the manufacturer's Pathfinder software system.

Postexposures. After a 5-min rest period lying supine, the average values for three resting supine right upper arm BP and heart rate levels were determined (Omron BP760 device; Omron Healthcare, Lake Forest, IL, USA). Resting basal longitudinal brachial artery diameter (BAD) images were measured at a standardized site on the right upper arm using a portable digital ultrasound system and a 10-mHz linear array transducer (Terason-2000; Terason, Burlington, MA, USA). All images were captured by an ECG triggered on the R-wave. We analyzed digital images using a commercially available software package employing an edge-detection system (Brachial Analyzer; Medical Imaging Applications, Coralville, IA, USA). We measured central aortic hemodynamics using right radial artery tonometry, and large vessel compliance by right carotid-to-femoral pulse wave velocity (PWV). Both outcomes were measured and analyzed using the SphygmoCor device and its software package (AtCor Medical, Sydney Australia). Study participants rested for 2 hr and then the same series of postexposures tests were performed as above. Afterwards, we determined conduit artery endothelial function by brachial artery flow-mediated dilatation (FMD). Repeat resting BAD images were obtained at the same right arm site, followed by continuous arterial imaging for 2 min after reactive hyperemia was induced by upper arm cuff inflation for 5 min. Peak FMD was used as the primary outcome. Microvascular endothelial-dependent vasodilatation [reactive hyperemia index

(RHI)], was measured concomitant with brachial FMD on the ipsilateral hand by finger peripheral arterial tonometry by a commercially available device and its analysis software system (EndoPAT2000; Itamar Medical, Caesarea, Israel). Based on our previous studies (Brook et al. 2002, 2009), we designed the sample size of the present study to evaluate changes in the primary outcomes: *a*) within-chamber changes in diastolic BP, and *b*) BAD and FMD, which were measured post-exposures. Other outcomes were obtained as secondary end points.

Exposure facility and air pollution measurements. We selected the exposure location (Dexter, MI, USA) in order to deliver coarse PM exposures that are known to be heavily influenced by rural sources. This location is > 10 km from any major freeways and approximately 60 km west of the Detroit, Michigan, metropolitan area. The sources of coarse PM were expected to be dominated by local vegetation, nearby agricultural activities, and rural roadways.

Coarse CAP was generated using a 2-stage virtual impactor system (Demokritou et al. 2002) that concentrates ambient coarse particles (predominantly 2.5–10 μ m) without altering their composition or chemical nature. The mobile air research laboratory, AirCARE-2, and the CAP human exposure facility are described in detail elsewhere (Brook et al. 2009; Harkema et al. 2004) and briefly in the Supplemental Material, "AirCARE-2 exposure facility," p. 2). Randomized blinded exposures were delivered to study participants seated within an air-tight chamber via a facemask with an air flow rate of 25–28 L/min. Gaseous pollutants (e.g., ozone) remained at or below ambient levels and were not concentrated. Intra-chamber carbon dioxide levels were lowered by a scrubber and monitored for safety reasons and did not differ between exposure types. During FA exposures, a high-efficiency particulate filter was inserted at the inlet of the concentrator. Coarse CAP mass levels were continuously monitored during exposures downstream of the concentrator using a personal DataRAM monitor (Thermo Scientific, Waltham, MA, USA), and particle size distributions were measured with a 3321 APS instrument (TSI Inc., St. Paul, MN, USA). Outdoor and within-chamber temperatures and relative humidity were also monitored. Chamber temperature was maintained at approximately 24°C during exposures. CAP filter samples were collected immediately upstream of the chambers on 47-mm Teflon filters (Pall, Ann Arbor, MI, USA) at a flow rate of 6 L/min. The samples were analyzed gravimetrically using a microbalance (MT-5; Mettler Toledo, Columbus, OH, USA) in a temperature/humidity-controlled clean laboratory as

described in the Federal Reference Method [U.S. Environmental Protection Agency (EPA) 2011a].

Statistical methods. Summary statistics were computed for continuous measures as mean \pm SD as well as median [interquartile range (IQR)], and for categorical variables as frequency and proportion (%). All outcomes were visually evaluated and analyzed for normality of distribution using the Shapiro–Wilk Normality Test.

Analyses of end points measured post-exposures. Following the nature of data collection (crossover study design), the matched pairs of measurements obtained postexposures (post-CAP vs. post-FA) were compared using paired *t*-tests (for normally distributed data) and matched Wilcoxon tests for not-normally distributed data (i.e., FMD, RHI).

Analyses of end points repetitively measured during exposures. The longitudinal measurements (i.e., BP, heart rate values) repetitively obtained during exposures were modeled in mixed-effect models to evaluate for any exposure–time interaction. We included random effects to account for within-subject correlation. The outcome of this model (*b*₃, parameter from the model below) is the average change in parameters (BP, heart rate) between exposure types (CAP vs. FA) over 10-min time periods during exposures [model 1: Outcome_{*it*} = *b*₀ + *b*_{*i*} + (*b*₁ × time_{*it*}) + (*b*₂ × CAP_{*it*}) + (*b*₃ × CAP_{*it*} × time_{*it*}), where *i* represents the *i*th individual, *t* represents the *t*th repeated measurement, *b*_{*i*} represents the random intercept for participant *i*, CAP represents exposure (1 if CAP, 0 if FA), and time represents the duration of exposure at the time of measurement *t*]. HR values initially increased at the start of CAP exposures but did not further increase over time. As such, this model was not significant for heart rate (i.e., no effect modification by time during exposures). Thus, we also compared the average heart rate values throughout the entire exposure time periods (Wald tests in mixed models) without interactions terms for time. The outcome of this model is the average difference in parameters (BP, heart rate) throughout the entire exposure (model 2: *b*₂ parameter from the previous model and excluding the *b*₃ parameter in the model when not significant). The changes in HRV parameters that occurred for each study participant during individual exposures were calculated by subtracting the measurements obtained during the last 5 min at the end of the exposures from those during the first 5 min at the start of exposures. We compared these HRV changes between exposure types (CAP vs. FA) by mixed model analyses.

Exposure–response analyses. The associations between the average PM mass during the 2-hr exposures with BP and heart

rate changes were first evaluated by linear mixed models. Potential nonlinear exposure–response relationships (i.e., between coarse CAP mass and CV outcomes) were also examined by a nonparametric regression model LOESS.

Effect modification analyses. The potential effect of BMI on the CV outcome changes induced by the exposure types was evaluated by mixed models by including an interaction term for BMI (continuous values; above vs. below the median value; > 30 vs. < 30 kg/m²). We performed all analyses using the statistical software package R (version 2.14.1; <http://www.r-project.org>).

Results

Table 1 presents the study participants' characteristics. Six study participants had a BMI \geq 30 kg/m². The concentrations of the mean coarse PM (average levels over the 2-hr exposure period) were significantly higher during CAP exposures compared with FA exposures (Table 2). Both systolic (mean difference = 0.32 mmHg; 95% CI: 0.05, 0.58; *p* = 0.021) (Figure 1A) and diastolic BP (0.27 mmHg; 95% CI: 0.003, 0.53; *p* = 0.05) (Figure 1B) linearly increased per 10 min of exposure during the inhalation of coarse CAP when compared with changes during FA exposure (model 1). In addition, systolic (0.74 mmHg; 95% CI: −0.13, 1.60; *p* = 0.096) and diastolic BP (1.1 mmHg; 95% CI: 0.27, 2.00; *p* = 0.010), as well as heart rate (4.1 bpm; 95% CI: 3.06, 5.12; *p* < 0.0001), were on average higher throughout coarse CAP exposure versus FA air exposure (model 2) (Figures 1 and 2). Controlling for outdoor and intra-chamber temperatures in the mixed models did not alter the significance of these results (see Supplemental Material,

“Mixed models controlling for ambient and chamber temperature,” p. 3). An evaluation for potential effect modification of the CAP-induced changes in BP and heart rate by ambient PM_{2.5} levels during the day before controlled exposures also did not show any significant results (see Supplemental Material, “Mixed models for effect modification by ambient PM,” pp. 3–4).

The associations between the changes in BP and heart rate per 10 min of exposures with mean coarse PM levels during exposures (CAP alone; and CAP and FA limbs combined) were not significant when analyzed by linear models. We thus explored the associations of the dose–response relationships during CAP exposures using nonlinear models (see Supplemental Material, Figures S2 and S3).

Several HRV metrics including the high frequency (HF) power and the low frequency/HF ratio were significantly altered during coarse CAP exposures compared with FA exposures (Table 3). However, most of the other study outcomes measured immediately and/or 2 hr after exposures were not differentially affected by coarse CAP compared with FA (Table 4).

Finally, the interaction terms for BMI (the prespecified characteristic) were not significant when evaluated for potential effect modification on study outcomes. However, given the limited number of study participants and BMI ranges, this study likely did not have adequate power to fully evaluate the impact of obesity on health responses. There were no other significant effect modifiers of the BP and HRV changes. Ambient PM_{2.5} and PM₁₀ (\leq 10 μ m) levels were not different for the day before controlled exposures (see Supplemental Material, Table S1).

Table 1. Participant characteristics (*n* = 32; 16 female study participants).

Variable	Mean \pm SD	Minimum	Maximum	Median	25th percentile	75th percentile
Age (years)	25.9 \pm 6.6	18.0	46.0	24.0	22.0	27.0
Weight (kg)	78.4 \pm 16.3	55.9	111.4	75.7	64.0	89.2
Height (m)	1.7 \pm 0.1	1.6	2.0	1.7	1.6	1.8
BMI (kg/m ²)	26.3 \pm 5.7	18.3	43.5	24.2	23.0	28.8
Fasting glucose (mg/dL) ^a	86.9 \pm 6.9	70.0	103.0	87.0	83.0	90.5
Total cholesterol (mg/dL)	163.9 \pm 31.4	104.0	244.0	163.0	145.8	180.5
HDL-C (mg/dL)	55.4 \pm 15.9	25.0	91.0	54.5	45.2	63.2
LDL-C (mg/dL)	88.8 \pm 26.1	49.0	135.0	88.0	66.8	105.0
Triglycerides (mg/dL)	106.0 \pm 80.9	40.0	401.0	75.5	57.0	118.2

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

^aMissing data for 1 participant.

Table 2. Coarse PM concentrations (μ g/m³) during exposures.

Exposure	Mean \pm SD	Minimum	Maximum	Median	25th percentile	75th percentile
FA	10.1 \pm 7.1	2.6	27.4	6.8	6.8	10.4
Coarse CAP	76.2 \pm 51.5*	10.3	246.5	68.9*	41.2	98.4

The values represent the average concentration over the entire 2-hr period of exposures. Mass levels below the detection limit (6.8 μ g/m³) were recorded at this value for analyses (*n* = 15, FA exposures only).

**p* < 0.01 for differences of mean or median levels between exposure types compared by paired *t*-tests and Wilcoxon ranked sum tests, respectively.

Discussion

We observed the inhalation of coarse PM was associated with the triggering of a rapid increase in BP and heart rate over a 2-hr period among healthy adults. The exposure was also related to concomitant alterations in HRV that were consistent with the genesis of acute autonomic imbalance (i.e., reduced cardiac parasympathetic activity). This constellation of responses mirrors those we previously observed after exposures to fine CAP (Brook et al. 2002, 2009). However, coarse PM exposure was not associated with changes in conduit or microvascular endothelial-dependent vasodilatation, arterial compliance, or central aortic hemodynamics for up to 2 hr after exposure. Taken together, these alterations in clinically important intermediate

biological end points support the plausibility that coarse PM could be capable of promoting acute CV events.

Comparisons to previous studies. There have been only two published studies regarding the CV effects of controlled exposure to coarse PM. Gong et al. (2004) demonstrated among 16 healthy and asthmatic adults that coarse CAP exposure is related to elevations in heart rate and decreases in HRV at 4 and 24 hr after exposure. More recently, Graff et al. (2009) observed similar reductions in HRV at 20 hr after coarse CAP exposure among 14 healthy adults. Two panel studies have also shown that short-term exposure to coarse PM even at lower outdoor ambient concentrations is related to reductions in HRV (Lipsett et al. 2006; Yeatts et al. 2007).

These autonomic nervous system changes are in accord with the findings of the present study; however, neither previous CAP study evaluated the effects on HRV during the exposure period. Furthermore, no previous study has reported the impact of coarse PM on arterial hemodynamics and vascular function parameters.

Numerous differences between fine and coarse PM exist beyond their aerodynamic diameters, including patterns of respiratory tract deposition on inhalation, sources, and chemical compositions (Brook et al. 2010; Brunekreef and Forsberg 2005). Despite these dissimilarities, coarse CAP exposure was associated with a rapid increase in BP and heart rate, reduced HRV, and a trend (albeit non-significant, Table 4) toward conduit artery vasoconstriction (reduced BAD)—responses comparable with those we observed after fine CAP exposure (Brook et al. 2002, 2009). This suggests that the size and/or characteristics of the inhaled pollutant particles per se are not vital determinants of their capacity to trigger this specific set of acute hemodynamic alterations, which likely occurs via autonomic imbalance. However, in the present study, coarse PM was not related to further impairment in aortic, conduit, or microvascular function. This differs from the blunting of resistance arteriole endothelial function reported after diesel exhaust particle inhalation (Lucking et al. 2011) and the reduced brachial FMD we observed the day after fine CAP exposure in Toronto (Brook et al. 2009). On the other hand, fine plus ultrafine CAP (20 nm to 3 μ m in diameter) from a maritime region near Edinburgh, Scotland, did not impair microvascular endothelial function (Mills et al. 2008). Fine CAP derived from regional-transported PM_{2.5} in Michigan also did not blunt brachial FMD (Brook et al. 2009). Altogether, the results suggest that certain characteristics of the inhaled particles are likely prime determinants of their capacity to elicit arterial dysfunction (unlike BP and HRV). It is probable that PM rich in prooxidative chemicals, such as combustion-derived particles [from, e.g., urban settings (Brook et al. 2009)] and/or

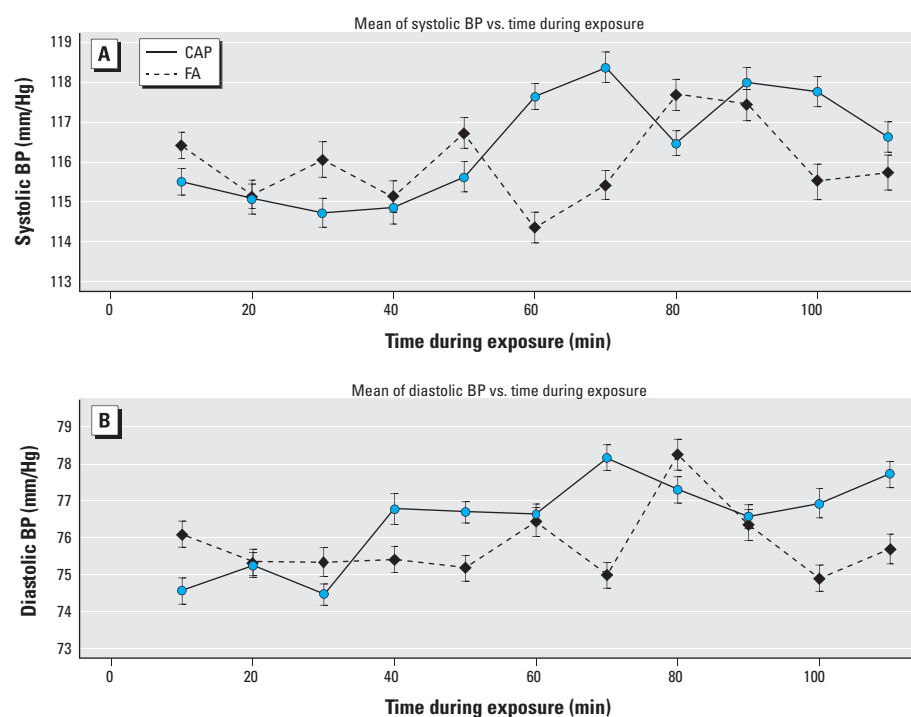


Figure 1. Systolic (A) and diastolic (B) blood pressure levels during exposures. The figure presents the mean \pm SE of BP values measured every 10 min during exposures after a 10-min rest period after the study participants entered the chamber.

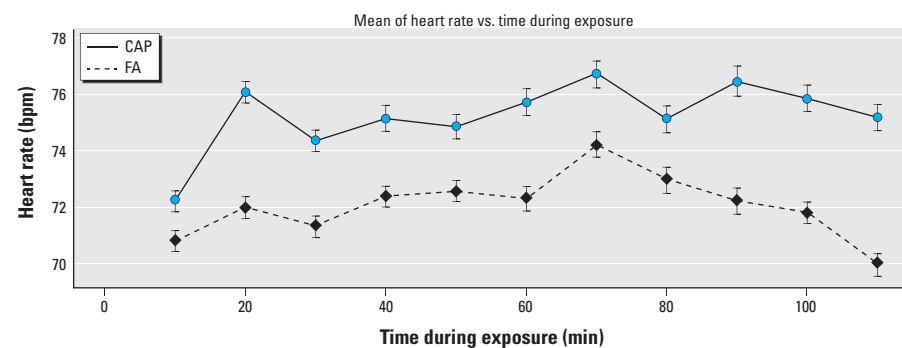


Figure 2. Heart rate levels during exposures. The figure presents the mean \pm SE of heart rate values measured every 10 min during exposures after a 10-min rest period after the study participants entered the chamber.

Table 3. Changes in heart rate variability parameters [mean (95% CI)] during exposures.

HRV outcome	Change in outcome during CAP vs. FA exposures	p-Value ^a
Log SDNN (msec)	-0.087 (-0.19, 0.02)	0.107
Log HF peak (msec ²)	-0.42 (-0.13, -0.71)	0.006
Log LF peak (msec ²)	-0.068 (-0.30, 0.17)	0.58
LF/HF ratio	0.24 (0.07, 0.41)	0.007

Abbreviations: HF, high frequency power; LF, low frequency power; SDNN, SD of the normal-to-normal R-R intervals.

^aCompared between exposure types (CAP vs. FA) by mixed model analyses.

smaller ultrafine PM [e.g., diesel exhaust particles (Lucking et al. 2011)] are more apt to promote systemic endothelial dysfunction because of their potentially greater capacity to trigger oxidative stress and inflammation *in vivo* (Brook et al. 2010).

Biological mechanisms. Several factors support that PM-mediated acute autonomic imbalance was responsible for the observed hemodynamic changes. The BP increase was rapid, transient, and occurred in conjunction with an increase in heart rate—a marker of augmented sympathetic tone (Palatini 2011). Both changes were also manifest during concomitant alterations in HRV metrics (Table 3) supporting vagal withdrawal and/or relative sympathetic activation (Lahiri et al. 2008). There was also no evidence that other pro-hypertensive pathways were activated, such as a direct PM-mediated impairment in endothelial-dependent vasodilatation or arterial compliance. These findings in sum support that the inhaled particles likely perturbed autonomic balance via the activation of afferent pulmonary autonomic reflexes (Brook et al. 2010); however, the precise neural pathways involved remain to be fully elucidated.

The responses observed over a total of 4 hr do not eliminate the possibility that other biological pathways may also be activated (or become manifest or relevant) at later postexposure time periods. We previously observed that FMD was blunted in a delayed fashion starting 20–24 hr after fine CAP exposure in an urban setting (i.e., Toronto) (Brook et al. 2009). In addition, more prolonged exposures (e.g., 24–48 hr) at ambient PM_{2.5} concentrations in the Detroit (Michigan, USA) area have also been associated with higher BP levels, but typically starting from 1 to 2 days later (Brook et al. 2011; Dvonch et al. 2009). We have speculated that PM causes a large portion of the acute CV effects that occur within hours as a consequence of rapid autonomic imbalance, whereas those observed at a later time period (≥ 1 day postexposure) are more likely

prompted by additional and/or separate slower-acting pathways such as inflammation and oxidative stress-induced endothelial dysfunction (Brook and Rajagopalan 2009). Although CAP experiments can inform on the health impacts induced by acute exposures, additional study types (e.g., prospective cohort studies) are required to explore the potential for additional adverse effects induced by longer-term coarse PM exposures.

Coarse PM and CV health. The epidemiological studies evaluating the associations between coarse PM and CV diseases have provided mixed results (Brunekreef and Forsberg 2005; Chang et al. 2011; Peng et al. 2008; Puett et al. 2009; Zanobetti and Schwartz 2009). Whether coarse PM increases CV risk is important to settle for several reasons, perhaps most importantly because this air pollutant is a near ubiquitous issue and not currently regulated (Brunekreef and Forsberg 2005). Coarse particles comprise a sizeable fraction of total PM air pollution when measured by mass per volume of air. In many regions, it can account for > 50–70% of total PM mass levels < 10 μm in diameter (Brook et al. 2010; Brunekreef and Forsberg 2005). Based on estimations from recent global air pollution data (van Donkelaar et al. 2010) it is likely that billions of people worldwide may be exposed to high levels of coarse PM at concentrations that are comparable to, or even exceed on occasion, those in the present study (76.2 $\mu\text{g}/\text{m}^3$). Therefore, our findings have relevance to the “real-world” setting. On the other hand, it must be recognized that the number of coarse particles is small (e.g., several orders of magnitude lower) compared with ultrafine particle counts (typically derived from nearby combustion sources) given similar overall mass concentrations of pollution (Brook et al. 2010). The importance of this difference in regard to health outcomes remains unclear and depends on whether mass per se (or associated factors such as the concentration of certain components) or the

number of actual particles inhaled (or associated factors such as greater surface area/toxicity of smaller particles) is the prime determinant of adverse health effects.

Although PM₁₀ and PM_{2.5} are both regulated in the U.S. by the National Ambient Air Quality Standards (U.S. EPA 2011b), the coarse PM size fraction itself is not currently a regulated air pollutant because of gaps remaining in the scientific knowledge. To provide evidence supporting “causality” that a specific pollutant associated with adverse health effects in epidemiological studies is indeed a responsible agent, it is essential to demonstrate substantiation for the biological plausibility of a relevant mechanism of harm. Although our findings do not directly demonstrate that coarse PM triggers CV events, taken together they do support the “biological plausibility” of such a contention given the demonstration of adverse changes in relevant physiological intermediate end points. Though the observed CV alterations were small in magnitude and thus unlikely to pose direct risks to healthy people, individuals with underlying vulnerable atherosclerotic plaques and/or who are susceptible to heart failure, stroke, or arrhythmias could have the risk for a CV event promoted by the acute changes in BP, heart rate, and autonomic imbalance (Brook et al. 2010). Because millions of susceptible people are likely impacted by coarse PM, even a very small absolute increase in CV risk can translate into substantial global public health concerns (Lim et al. 2012). In this context and given the current results that support and expand upon the findings from previous studies (Gong et al. 2004; Graff et al. 2009; Lipsett et al. 2006; Yeatts et al. 2007), more attention and research regarding the adverse health effects of the coarse PM fraction are warranted.

Strengths and limitations. This study was the first double-blind controlled exposure of coarse PM versus FA that evaluated the effects on hemodynamics and vascular function. It is important to highlight that our present

Table 4. Study outcomes and their differences (mean \pm SD) measured postexposures.

Outcome	Coarse CAP		FA		Difference between coarse CAP and FA		<i>p</i> -Value ^a	<i>p</i> -Value ^b
	Immediate-post	2 hr-post	Immediate-post	2 hr-post	Immediate-post	2 hr-post		
Brachial SBP (mmHg)	109.2 \pm 11.7	108.4 \pm 11.3	108.7 \pm 12.3	108.8 \pm 11.3	0.63 \pm 7.81	−0.60 \pm 7.05	0.66	0.65
Brachial DBP (mmHg)	72.3 \pm 8.4	68.1 \pm 9.0	72.0 \pm 9.3	68.7 \pm 8.4	0.50 \pm 7.51	−0.57 \pm 5.99	0.72	0.61
Heart rate (bpm)	62.9 \pm 9.9	65.0 \pm 9.0	60.6 \pm 8.9	61.1 \pm 9.0	2.33 \pm 7.67	3.57 \pm 8.34	0.12	0.03
Aortic SBP (mmHg)	97.4 \pm 11.2	95.0 \pm 11.4	97.5 \pm 12.0	95.7 \pm 12.2	−0.10 \pm 7.50	−0.83 \pm 6.54	0.94	0.49
AP (mmHg)	3.3 \pm 3.3	2.2 \pm 3.4	3.4 \pm 3.9	2.6 \pm 4.9	−0.24 \pm 2.65	−0.62 \pm 2.90	0.63	0.26
Aix@75 (%)	5.9 \pm 12.3	2.1 \pm 13.0	5.0 \pm 14.5	1.1 \pm 16.9	0.41 \pm 8.50	−0.14 \pm 9.96	0.80	0.94
PWV (m/sec)	6.8 \pm 1.6	6.6 \pm 1.4	6.7 \pm 1.5	6.7 \pm 1.6	−0.08 \pm 1.10	−0.33 \pm 1.01	0.96	0.19
BAD (cm)	3.62 \pm 0.60	3.64 \pm 0.62	3.68 \pm 0.66	3.70 \pm 0.64	−0.07 \pm 0.23	−0.06 \pm 0.23	0.12	0.15
FMD-peak (%) ^c		9.4 \pm 4.1		9.0 \pm 3.7		0.35 \pm 4.51		0.68
RHI		2.1 \pm 0.5		2.0 \pm 0.5		0.08 \pm 0.59		0.57

Abbreviations: Aix@75, augmentation index at a heart rate of 75 beats/min; AP, augmentation pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure. Supplemental Material (Table S3) provides all the results as median (IQR). Positive values represent higher values post-CAP exposures (e.g., heart rate was higher, whereas BAD trended to be smaller).

^aComparisons of immediate-post exposures (CAP vs. FA). ^bComparisons of 2 hr-post exposures (CAP vs. FA). Paired *t*-tests were used for all statistical comparisons except for FMD and RHI (Wilcoxon Ranked Sum Tests). ^cOther FMD metrics (60-sec time point dilatation; 2-min mean dilatation) were also not significantly different between exposures.

findings directly apply only to coarse PM derived from a rural setting. Given the fact that coarse PM is heavily impacted by nearby sources (more so than $PM_{2.5}$, which is often a more homogenous regional pollutant from a mass concentration standpoint) (Brunekreef and Forsberg 2005), the particulate composition at the study site we selected was likely principally influenced by prevalent local agricultural activities (emissions that may also be more difficult to control). In order to address this issue, ongoing analyses are underway to evaluate the specific particle sources and components most responsible for mediating the observed CV changes. We are also performing complimentary exposures in an urban setting to investigate the potential that coarse particles of a differing source or character may prompt dissimilar health responses. However, given the fact that several different particle types [rural coarse CAP in the present study and fine CAP derived from two different settings (Brook et al. 2009)] have now been shown to elicit similar BP and heart rate responses, we believe that coarse PM from an urban setting is likely to do the same. Ongoing exposures will answer this issue and determine whether urban coarse PM, more likely to be composed of particles enriched in prooxidative chemicals (e.g., metals) from nearby industrial sources, might also elicit further adverse health responses (e.g., vascular dysfunction). Nevertheless, to our knowledge this study is the first demonstration that coarse PM from any source is capable of directly elevating BP and heart rate during inhalation.

We acknowledge that the present study does not provide information regarding the responsible coarse PM components. Planned analyses of the collected filters during coarse exposures will provide important insights into the coarse PM components (e.g., elemental/organ carbon; trace elements/metals) most strongly associated with (i.e., likely responsible for eliciting) the observed CV health responses. Source apportionment analyses are also planned, which will help elucidate the most germane sources and whether or not the current findings relate specifically to rural coarse PM, or more broadly to coarse PM derived from multiple different (e.g., urban) source types. This is an important topic because coarse PM represents a heterogeneous group of particles. For example, dust storms, which are principally due to windblown coarse PM comprised of crustal material, are major public health issues throughout the world. However, the epidemiological evidence linking dust from deserts and agricultural sources to excess CV morbidity remains mixed (Hashizume et al. 2010; Karanasiou et al. 2012; Schwartz et al. 1999).

Although mean coarse PM levels were significantly lower during FA exposure

compared with CAP exposure, we recognize that the levels were above ambient outdoor concentrations during some of the FA exposures. The position of our HEPA filter in the coarse exposure facility before the series of concentrators may have reduced its efficiency as compared with some of our previous studies using a fine CAP system (Brook et al. 2009). Nonetheless, on every occasion each individual participant was exposed to a higher PM level during his or her CAP exposure period compared with his or her own respective FA exposure scenario (see Supplemental Material, Table S2). In addition, most FA exposures had coarse PM levels that were $< 10 \mu g/m^3$ (see Supplemental Material, Figure S4). Given the nature of the main statistical analyses comparing within-subject changes in biological outcomes, we believe our findings remain valid despite this limitation.

We did not evaluate vascular outcomes at > 2 hr postexposures; therefore, it is possible that some degree of vascular dysfunction occurred in a delayed fashion and was missed in the present study. Ongoing analyses of data and future studies will explore this issue as well as the potential for several other adverse cardio-metabolic health effects induced by exposure to coarse CAP. We also did not evaluate the potential effects of environmental factors (e.g., ambient pollution, traffic, noise) before the controlled exposures or during the 2-hr period after the randomized exposures on our study end points. In previous studies when baseline (i.e., preexposure) CV end points were determined, they did not differ between study days given the crossover design and were not significant determinants of the subsequent responses to FA and CAP (Brook et al. 2009). Therefore, it is unlikely that unmeasured environmental factors could account for the differential BP and heart rate responses between exposure types. Nonetheless, we plan to evaluate personal PM exposure levels for the 24 hrs preceding controlled exposures in future studies in order to directly assess this issue. Finally, some concentration of particles between 0.1 and 2.5 μm in diameter has previously been reported using the virtual impactor system employed in the present study (Moffet et al. 2004). The lower diameter cut point for concentration is not exact and it is therefore possible that some of the health effects we observed are attributable to fine particles that may have also been enriched above ambient levels (albeit by a smaller factor than coarse PM). However, a previous study has verified that the majority of the mass (approximately 75%) within the CAP is likely to be within the coarse PM size fraction (Moffet et al. 2004). Our ongoing analyses of the particle constituents and size distributions will help to further elucidate this important issue.

Conclusions

Coarse PM is not currently a regulated air pollutant. Nonetheless, millions of people in the United States, and perhaps billions worldwide, encounter coarse PM from varying sources. It is therefore critical to determine whether exposure is associated with adverse health effects. Our results show that rural-origin coarse PM exposure is associated with triggering an acute increase in BP and heart rate, likely via autonomic imbalance. This supports the plausibility that exposure to higher levels of coarse PM from a rural setting might be capable of promoting acute CV events, particularly among susceptible individuals, and provides further biological evidence for considering its independent regulation. Further research regarding the adverse CV health impacts of coarse PM is warranted.

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