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Abstract

Background: Fine particulate matter (PM) air pollution is associated with numerous adverse health effects including increased blood pressure (BP) and vascular dysfunction. However, the cardiovascular (CV) impacts of coarse PM exposure remain largely unknown.

Objectives: Since it substantially contributes to global air pollution (yet differs in characteristics from fine particles and is currently not regulated), it is important to elucidate if coarse PM is itself also capable of eliciting adverse CV responses.

Methods: Thirty-two healthy adults (25.9 ± 6.6 years) were exposed to concentrated ambient coarse particles (CAP) [$76.2 \pm 51.5 \mu\text{g}/\text{m}^3$] in a rural location and filtered air (FA) for 2 hours in a randomized double-blind crossover study. CV outcomes were measured during, immediately and 2 hours post-exposures.

Results: Both systolic (mean difference = 0.32 mm Hg; 95% confidence interval (CI): 0.05, 0.58]; $p = 0.021$) and diastolic (0.27 mm Hg; 95% CI: 0.003, 0.53; $p = 0.05$) BP linearly increased per 10 minutes of exposure during the inhalation of coarse CAP when compared to changes during FA exposure. Heart rate was on average higher (4.1 beats/minutes; 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 were not differentially altered following the exposures (brachial flow-mediated dilatation, micro-vascular reactive hyperemia index, aortic hemodynamics, pulse wave velocity).

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.

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 ($\text{PM}_{2.5}$) can pose cardiovascular (CV) health risks (Brook et al. 2010). $\text{PM}_{2.5}$ 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 $\text{PM}_{2.5}$ 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 ; $\text{PM}_{10-2.5}$) are less conclusive (Brunekreef et al. 2005; Chang et al. 2011; Peng et al. 2008; Puett et al. 2009; Zanobetti et al. 2009). Coarse PM is an important contributor to worldwide air pollution, typically accounting for between 40-60% of overall particulate mass < 10 μm in diameter (Brook et al. 2010; Brunekreef et al. 2005). Not only do its sources and components differ from $\text{PM}_{2.5}$, the composition of coarse PM itself often varies to a greater extent across seasons and locations (i.e., more spatially heterogeneous than $\text{PM}_{2.5}$). Coarse PM represents an assorted mixture of particles (e.g., aerosolized soil/sand or crustal material) most typically generated from mechanical processes (e.g., crushing, grinding, or re-suspension of ground material) with sources ranging from farming, 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 bio-aerosols (e.g., pollen, endotoxin) (Brook et al. 2010; Brunekreef et al. 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; Brook et al 2009, Huang 2009; Lippman 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 on BP and vascular function have never been investigated and remain unknown. Due to differences in sizes, chemical natures and fates upon inhalation, coarse particles 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 et al. 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 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-hour-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 exam was performed. By entry criteria, study participants were healthy nonsmoking adults (living in nonsmoking households) aged 18-50 years without any established CV disease or traditional risk factors (hypertension, treated hyperlipidemia, diabetes). All study participants had screening BP values < 140/90 mm Hg and fasting glucose levels < 126 mg/dL. No subject was taking any medication (e.g., statins) or over-the-counter pills (e.g., anti-oxidants) that might alter vascular function. We also aimed to enroll a subset of qualifying study participants with a body mass index (BMI) ≥ 30 kg/m² in order to investigate if those with higher BMI levels are 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 1-3 week washout period between exposures, which has been shown to be an adequate period to assure that baseline values are not significantly different between exposures in prior studies (Brook et al 2009). Study participants came to the facility on each study visit day having fasted for at least 8 hours prior to arriving for the visit. Randomized exposures occurred from 10 am to noon. Outcomes were not measured prior to exposures, but were all determined immediately and 2-hours post-exposures. The exception was that measures of endothelial function were only measured at the 2-hour post-exposure time point as the procedures might disrupt other outcomes. These 2 time points were selected as prior studies performed by our group (Brook et al. 2002; Brook et al. 2009) and others (as reviewed by Lippman 2009) using fine CAP exposures have demonstrated changes in outcomes evaluated in this current study within this time period. Study participants rested within the exposure facility between health

measurement time periods. In addition, during exposures BP, heart rate, and ECG recordings were measured as described below. An outline figure of the study design is provided in the online supplement (Supplemental Material, Figure S1).

Cardiovascular outcomes

BP and vascular outcomes (including prior reproducibility information) were determined as described in more detail previously (Brook et al 2002; Brook et al 2009; Urch et al 2005). All protocols were performed in the order as follows and analyzed by a vascular technician blinded to the exposures. During exposures: After a 10 minute rest period after entering the exposure chamber, left arm brachial BP along with heart rate were measured every 10 minutes with the appropriate sized cuff and arm held at mid-sternal level within the chamber during exposures using a Spacelabs ambulatory BP 90207 monitor and software package (Spacelabs; <http://www.spacelabshealthcare.com/en/>). Continuous electrocardiogram monitoring was also performed starting immediately upon entering the chamber using the evo Holter system (Spacelabs; <http://www.spacelabshealthcare.com/en/>). Time and frequency domain heart rate variability (HRV) metrics were analyzed at 5-minute long epochs using the manufacturers' Pathfinder software system. Post-exposures: After a 5-minute rest period lying supine the average values for 3 resting supine right upper arm BP and heart rate levels were determined (Omron BP760 device). 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; <http://www.terason.com/>). All images were captured by an electrocardiogram triggered on the R-wave. Digital images were analyzed using a commercially available software package employing an edge-detection system (Brachial Analyzer, Medical Imaging Applications; <http://www.mia-llc.com/>). Central aortic

hemodynamics were measured using right radial artery tonometry. Large vessel compliance was measured 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; <http://www.atcormedical.com/sphygmocor.html>). Next, study participants rested for 2 hours and then the same series of post-exposures tests were performed as above. Afterwards, conduit artery endothelial function was determined 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 minutes after reactive hyperemia was induced by upper arm cuff inflation for 5 minutes. Peak FMD was used as the primary outcome. Micro-vascular 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; <http://www.itamar-medical.com/>). Based upon our prior studies (Brook et al 2002; Brook et al 2009), the sample size of the study was designed to evaluate changes in the primary outcomes: within chamber changes in diastolic BP; and BAD and FMD measured post-exposures. Other outcomes were obtained as secondary endpoints.

Exposure facility and air pollution measurements

We selected the exposure location in order to deliver coarse PM exposures that are known to be heavily influenced by rural sources (Dexter, Michigan) (http://www.epa.gov/castnet/javaweb/site_pages/ANA115.html; 2013). This location is >10 KM from any major freeways and approximately 60 km west of the Detroit 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) which concentrates ambient coarse particles (predominantly from 2.5-10 μm) without altering their composition and 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 online supplement (see Supplemental Material, AirCARE-2 Exposure Facility). Randomized blinded exposures were delivered to study participants seated within an air-tight chamber via a facemask with an air flow rate between 25-28 liters/minute. Gaseous pollutants (e.g., ozone) remain at or below ambient levels and were not concentrated. Intra-chamber CO_2 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), and particle size distributions were measured with a 3321 APS instrument (TSI Inc., St Paul, MN). Outdoor and within chamber temperatures and relative humidity were also monitored. Chamber temperature was maintained at approximately 24 degrees Celsius during exposures. CAP filter samples were collected immediately upstream of the chambers on 47-mm Teflon filters (Pall, Ann Arbor, MI) at a flow rate of 6 L/min. The samples were analyzed gravimetrically using a microbalance (MT-5 Mettler Toledo, Columbus, OH) in a temperature/humidity-controlled clean laboratory as described in the Federal Reference Method (USEPA. 1997. Reference method for the determination of fine particulate matter as $\text{PM}_{2.5}$ in the atmosphere. EPA 40 CFR Part 50. Washington DC; <http://vlex.com/vid/fine-particulate-matter-atmosphere-19784843>)

Statistical methods

Summary statistics were computed for continuous measures as mean \pm standard deviation (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 endpoints measured post-exposures: Following the nature of data collection (cross-over study design), the matched pairs of measurements obtained post-exposures (post-CAP vs. post-FA) were compared using paired t-tests (for normally-distributed data) and matched Wilcoxon tests for not normally-distributed data (FMD, RHI). Analyses of endpoints repetitively measured during exposures: The longitudinal measurements (BP, heart rate values) repetitively obtained during exposures were modeled in mixed-effect models to evaluate for any exposure-time interaction. Random effects were included to account for within subject correlation. The outcome of this model (b3; parameter from the model below) is the average change in parameters (BP, heart rate) between exposure-types (CAP versus FA) over 10 minute 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*: b2 parameter from the

previous model and excluding the b3 parameter in the model when not significant). The changes in HRV parameters that occurred for each subject during individual exposures were calculated by subtracting the measurements obtained during the last 5 minutes at the end of the exposures from to those during the first 5 minutes at the start of exposures. These HRV changes were compared between exposure types (CAP versus FA) by mixed model analyses. Exposure response analyses: The associations between the average PM mass during the 2-hour exposures with BP and heart rate changes were first evaluated by linear mixed models. Potential non-linear exposure-response relationships (i.e., between coarse CAP mass and CV outcomes) were also examined by a nonparametric regression model L_Ocal regr_{ESS}ion (LOESS). Effect modification analyses: The potential effect of BMI upon the CV outcome changes induced by the exposure-types were evaluated by mixed models by including an interaction term for BMI (continuous values; above versus below the median value; above versus below 30 kg/m²). All analyses were performed using the statistical software package R (version 2.14.1).

Results

The subject characteristics are presented in Table 1. Six study participants had a BMI \geq 30 kg/m². The mean coarse (PM_{10-2.5}) concentrations (average levels over the 2-hour exposure period) were significantly higher during CAP compared to FA exposures (Table 2). Both systolic (mean difference = 0.32 mm Hg; 95% confidence interval (CI): 0.05, 0.58; p = 0.021) (Figure 1A) and diastolic (0.27 mm Hg; 95% CI: 0.003, 0.53; p = 0.05) BP levels (Figure 1B) linearly increased per 10 minutes of exposure during the inhalation of coarse CAP when compared to changes during FA exposure (*model 1*). In addition, systolic (0.74 mm Hg; 95% CI: -0.13, 1.60; p = 0.096) and diastolic BP (1.1 mm Hg; 95% CI: 0.27 to 2.00] mm Hg, p = 0.010), as well as heart rate (4.1 beats/minute; 95% CI: 3.06, 5.12; p < 0.0001), were on average higher throughout

coarse CAP versus FA air exposures (*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). 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 prior to controlled exposures also did not show any significant results (see Supplemental Material, Mixed Models for Effect Modification by Ambient PM).

The associations between the changes in BP and heart rate per 10 minutes 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 compared to FA exposures (Table 3). However, most of the other study outcomes measured immediately and/or 2 hours following exposures were not differentially affected by coarse CAP compared to FA (Table 4)..

Finally, the interaction terms for BMI (the pre-specified 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 levels were not different for the day prior to controlled exposures (see Supplemental Material, Table S1).

Discussion

Our findings show for the first time that the inhalation of coarse PM is associated with the triggering of a rapid increase in BP and heart rate over a 2 hour period among healthy adults. The exposure was also related to concomitant alterations in HRV consistent with the genesis of acute autonomic imbalance (i.e., reduced cardiac parasympathetic activity). This constellation of responses mirrors those we previously observed following exposures to fine CAP (Brook et al. 2002; Brook et al 2009). However, coarse PM exposure was not associated with changes in conduit or micro-vascular endothelial-dependent vasodilatation, arterial compliance, or central aortic hemodynamics for up to 2 hours after exposure. Taken together, these alterations in clinically important intermediate biological endpoints support the plausibility that coarse PM could potentially be capable of promoting acute CV events.

Comparisons to previous studies

There have only been 2 published studies regarding the CV effects of controlled exposure to coarse PM (PM_{10-2.5}). 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 4 and 24 hours after exposure. More recently, Graff et al. (2009) observed similar reductions in HRV 20 hours following 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 accord with our current findings; however, neither prior CAP study evaluated the effects on HRV during the exposure period. In addition, no previous study has reported the impact of coarse PM upon arterial hemodynamics and vascular function parameters.

Numerous differences between fine and coarse PM exist beyond their aerodynamic diameters including patterns of respiratory tract deposition upon inhalation, sources, and chemical compositions (Brook et al. 2010; Brunekreef et al. 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 to those we observed following fine CAP (Brook et al. 2002; Brook et al. 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, likely via autonomic imbalance. However, in the present study $PM_{10-2.5}$ was not related to a further impairment in aortic, conduit, or micro-vascular 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 demonstrated the day following 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 did not impair micro-vascular 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 pro-oxidative chemicals, such as combustion-derived particles (e.g., from urban settings) (Brook et al. 2009) and/or smaller ultra-fine PM (e.g., diesel) (Lucking et al 2011) are more apt to promote systemic endothelial dysfunction due to 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 activating 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 hours do not eliminate the possibility that other biological pathways may also be activated (or become manifest/relevant) at later time periods post-exposure. We previously observed that FMD was blunted in a delayed fashion starting 20-24 hours following fine CAP exposure in an urban setting (i.e., Toronto) (Brook et al 2009). Additionally, more prolonged exposures (e.g., 24-48 hours) at ambient PM_{2.5} concentrations in the Detroit area have also been associated with higher BP levels, but typically starting from 1-2 days later (Brook et al 2011; Dvonch et al. 2009). We have speculated that PM causes a large portion of acute CV effects that occur within hours as a consequence of rapid autonomic imbalance, whereas those observed at a later time period (≥ 1 day post-exposure) 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). Whilst CAP experiments can inform on the health impacts induced by acute exposures, additional study types

(e.g., prospective cohorts) are required to explore the potential for additional adverse effects induced by longer-term coarse PM exposures.

Coarse particles and CV health

The epidemiological studies evaluating the associations between coarse PM and CV diseases have provided mixed results (Brunekreef et al. 2005; Chang et al. 2011; Peng et al. 2008; Puett et al. 2009; Zanobetti et al. 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 et al. 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 et al. 2005). Based upon 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 our study (76.2 $\mu\text{g}\cdot\text{m}^3$). Hence, 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 to 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 regards to health outcomes remains unclear and depends upon if mass per se (or associated factors such as the concentration of certain components) versus 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.

While all particles $< 10 \mu\text{m}$ in diameter (PM_{10}) and $\text{PM}_{2.5}$ are both regulated in the U.S. by the National Ambient Air Quality Standards (<http://www.epa.gov/air/criteria.html>), the coarse PM ($\text{PM}_{10-2.5}$) size fraction itself is not currently a regulated air pollutant because of remaining scientific knowledge gaps. 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. Whilst 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 endpoints. 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). Since 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 prior studies (Graff et al 2009; Gong et al 2004, 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 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 findings only directly apply to coarse PM derived from a rural setting. Given the fact that coarse PM is heavily impacted by nearby sources (more so than $\text{PM}_{2.5}$ which is often a more

homogenous regional pollutant from a mass concentration standpoint) (Brunekreef et al 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 evaluating 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/character may prompt dissimilar health responses. However, given the fact that several different particle types (rural coarse CAP in the current study and fine CAP derived from 2 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 are likely to do the same. Ongoing exposures will answer this issue and determine if urban coarse PM, more likely to be composed of particles enriched in pro-oxidative 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 this 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).

While mean coarse PM levels were significantly lower during FA compared to CAP exposures, 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 prior to the series of concentrators may have reduced its efficiency as compared to some of our prior studies using a fine CAP system (Brook et al 2009). Nonetheless, on every occasion each individual subject was exposed to a higher PM level during their CAP compared to their own respective FA exposure scenario (see Supplemental Material, Table S2). In addition, most FA exposures had coarse PM levels $< 10 \mu\text{g}/\text{m}^3$ (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 > 2 hours post-exposures; therefore it is possible that some degree of vascular dysfunction occurred in a delayed fashion and was missed by our study. Ongoing analyses of data and future studies will explore this issue as well as explore the potential for several other adverse cardio-metabolic health effects induced by coarse CAP. We also did not evaluate the potential effects of environmental factors (e.g., ambient pollution, traffic, noise) prior to the controlled exposures or during the 2-hour period following the randomized exposures on our study endpoints. In previous studies when baseline (i.e., pre-exposure) CV endpoints were determined; they were not different 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 not likely 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 24-hours prior to controlled exposures in future studies to directly assess this issue. Finally, some concentration of particles between 0.1-2.5 μm in diameter has previously been reported using the virtual impactor system in our study (Moffet et al 2004). The lower diameter cut-point for concentration is not exact and hence it is possible that some of the health effects we observed are attributable to fine particles which 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 (~ 75%) within the CAP is likely to be within the coarse PM ($\text{PM}_{10-2.5}$) 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 U.S. and perhaps billions worldwide encounter coarse PM from varying sources. It is therefore critical to determine if exposure is associated with adverse health effects. Our results show that rural coarse PM exposure is associated with a triggering of 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.

References

- Brook RD, Brook JR, Urch B, Vincent R, Rajagopalan S, Silverman F. 2002. Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation* 105:1534-1536.
- Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G, et al. 2009. Insights into the Mechanisms and Mediators of the Effects of Air Pollution Exposure on Blood Pressure and Vascular Function in Healthy Humans. *Hypertension* 54:659-667.
- Brook RD, Rajagopalan S. 2009. Particulate matter air pollution and blood pressure. *J Am Soc Hypertens* 3:332-350.
- Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez-Roux A, et al. 2010. Particulate matter air pollution and cardiovascular disease. An update to the scientific statement from the American Heart Association. *Circulation* 121:2331-2378.
- Brook RD, Bard RL, Burnett RT, Shin HH, Vette A, Croghan C, et al. 2011. Differences in Blood Pressure and Vascular Responses Associated with Ambient Fine Particulate Matter Exposures Measured at the Personal versus Community Level. *Occup Environ Med* 68:224-230.
- Brunekreef B, Forsberg B. 2005. Epidemiological evidence on effects of coarse airborne particles on health. *Eur Respir J* 26:309-318.
- Chang HH, Peng RD, Dominici F. 2011. Estimating the acute health effects of coarse particulate matter accounting for exposure measurement error. *Biostat* 12:637-652.
- Demokritou P, Gupta T, Ferguson S, Koutrakis P. 2002. Development and laboratory characterization of a prototype coarse particle concentrator for inhalation toxicology studies. *Aerosol Sci* 33:111-123.
- Dvonch JT, Kannan S, Schulz AJ, Mentz G, House J, Benjamin A, et al. 2009. Acute Effects of Ambient Particulate Matter on Blood Pressure: Differential effects across urban communities. *Hypertension* 53:853-859.
- Gong H, Linn WS, Terrell SL, Clark KW, Gellter MD, Anderson KR, et al. 2004. Altered heart-rate variability in asthmatic and healthy volunteers exposed to concentrated ambient coarse particles. *Inhalant Toxicol* 16:335-343.

- Graff DW, Cascio WE, Rappold A, Zhou H, Huang Y-CT, Devlin RB. 2009. Exposure to concentrated coarse air pollution particles causes mild cardiopulmonary effects in healthy young adults. *Environ Health Perspect* 117:1089-1094.
- Harkema JR, Keeler GJ, Wagner JG, Morishita M, Timm E, Hotchkiss J, et al. 2004. Effects of inhaled urban air particulates on normal and hypersecretory airways in rats. Health Effects Institute Research Report 120. Health Effects Institute, Boston MA.
- Hashizume M, Ueda K, Nishiwaki Y, Michikawa T, Onozuka D. 2010. Health effects of Asian dust events: a review of the literature. *Nihon Eiseigaku Zasshi* 65:413-421.
- Huang YC, Ghio AJ. 2009. Controlled human exposures to ambient pollutant particles in susceptible populations. *Environ Health* 8: 33.
- Karanasiou A, Moreno N, Moreno T, Viana M, de Leeuw F, Querol X. 2012. Health effects from Sahara dust episodes in Europe: literature review and research gaps. *Environ Int* 47: 104-114.
- Lahiri MK, Kannankeril PJ, Goldberger JJ. 2008. Assessment of autonomic function in cardiovascular disease. *J Am Coll Cardiol* 51:1725-1733.
- Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. 2012. A comparative risk assessment of the burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380:2224-2260.
- Lippman M, Chen LC. 2009. Health effects of concentrated ambient air particulate matter (CAP) and its components. *Crit Rev Toxicol* 39:865-913.
- Lipsett MJ, Tsai FC, Roger L, Woo M, Ostro BD. 2006. Coarse particles and heart rate variability among older adults with coronary artery disease in the Coachella Valley, California. *Environ Health Perspect* 114:1215-1220.
- Lucking AH, Lundback M, Barath SL, Mills NL, Sidhu MK, Langrish JP, et al. 2011. Particle traps prevent adverse vascular and prothrombotic effects of diesel engine exhaust inhalation in men. *Circulation* 123:1721-1728.
- Mills NL, Robinson SD, Fokkens PHB, Leseman DLAC, Miller MR, Anderson D, et al. 2008. Exposure to concentrated ambient particles does not affect vascular function in patients with coronary heart disease. *Environ Health Perspect* 116:709-715.

- Moffet RC, Shields LG, Berntsen J, Devlin RB, Prather KA. 2004. Characterization of an ambient coarse particle concentrator used for human exposure studies: aerosol size distributions, chemical composition, and concentration enrichment. *Aerosol science and technology*, 38:1123-1137.
- Palatini P. 2011. Role of elevated heart rate in the development of cardiovascular disease in hypertension. *Hypertension* 58:745-50.
- Peng PD, Chang HH, Bell ML, McDermott A, Zeger SL, Samet JM, et al. 2008. Coarse particulate matter air pollution and hospital admissions for cardiovascular and respiratory diseases among Medicare patients. *JAMA* 299:2172-2179.
- Puett RC, Hart JE, Yanosky JD Paciorek C, Schwartz J, Suh H, et al. 2009. Chronic fine and coarse particulate exposure, mortality, and coronary heart disease in the Nurses' Health Study. *Environ Health Perspect* 117:1697-1701.
- Schwartz J, Norris G, Larson T, Sheppard L, Claiborne C, Koenig J. 1999. Episodes of high coarse particle concentrations are not associated with increased mortality. *Environ Health Perspect* 10:339-342.
- Urch B, Silverman F, Cory P, Brook JR, Lukic KZ, Rajagopalan S, et al. 2005. Acute blood pressure responses in healthy adults during controlled air pollution exposures. *Environ Health Perspect* 113:1052-1055.
- Van Donkelaar A, Martine RV, Brauer M, Kahn R, Levy R, Verduzco C, et al. 2010. Global estimates of ambient fine particulate matter concentrations from satellite-based aerosol optical depth: Development and application. *Environ Health Perspect* 118:847-855.
- Yeatts K, Svendsen E, Creason J, Alexis N, Berbs M, Scott J, et al. 2007. Coarse particulate matter (PM_{2.5-10}) affects heart rate variability, blood lipids, and circulating eosinophils in adults with asthma. *Environ Health Perspect* 115:709-714.
- Zanobetti A, Schwartz J. 2009. The effect of fine and coarse particulate matter air pollution on mortality: A national analysis. *Environ Health Perspect* 117:898-903.

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

Variable	Mean ± SD	Minimum	Maximum	Median	25th percentile	75th percentile
Age (years)	25.9 ± 6.6	18.0	46.0	24	22	27
Weight (kg)	78.4 ± 16.3	55.9	111.4	75.7	64.0	89.2
Height (m)	1.7 ± 0.1	1.6	2.0	1.7	1.6	1.8
Body mass index (kg/m)	26.3 ± 5.7	18.3	43.5	24.2	23.0	28.8
Fasting glucose (mg/dL) ^a	86.9 ± 6.9	70.0	103.0	87.0	83.0	90.5
Total cholesterol (mg/dL)	163.9 ± 31.4	104.0	244.0	163.0	145.8	180.5
HDL-C (mg/dL)	55.4 ± 15.9	25.0	91.0	54.5	45.2	63.2
LDL-C (mg/dL)	88.8 ± 26.1	49.0	135.0	88.0	66.8	105.0
Triglycerides (mg/dL)	106.0 ± 80.9	40.0	401.0	75.5	57.0	118.2

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation.

^aMissing 1 subject data point.

Table 2. PM_{10-2.5} concentrations during exposures.

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

CAP, concentrated ambient particles; SD, standard deviation.

PM_{10-2.5} concentrations are in $\mu\text{g}/\text{m}^3$ and determined by Teflon filter-based gravimetric mass measurements. The values represent the average concentration over the entire 2-hour period of exposures. Mass levels below the detection limit ($6.8 \mu\text{g}\cdot\text{m}^3$) were recorded at this value for analyses (n = 15, filtered air exposures only).

*P values < 0.01 for differences of mean or median levels between exposure types compared by paired t-tests and Wilcoxon Ranked Sum Tests, respectively.

Table 3. Changes in heart rate variability parameters during exposures.

HRV Outcome	Change in outcome during CAP exposures versus FA	p^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

Results are presented as mean (95% confidence interval).

HRV, heart rate variability; CAP, coarse concentrated ambient particles; FA, filtered air; SDNN, standard deviation of the normal-to-normal R-R intervals; HF, high frequency power; LF, low frequency power.

^ap values are the comparisons of the changes in HRV outcomes that occurred during the CAP versus FA exposures. The changes in HRV parameters that occurred for each subject during individual exposures were calculated by subtracting the measurements obtained during the last 5 minutes at the end of the exposures from those during the first 5 minutes at the start of exposures. These HRV changes were compared between exposure types (CAP versus FA) by mixed model analyses.

Table 4. Study outcomes and their differences measured post-exposures.

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

CAP, concentrated ambient particles; SBP, systolic blood pressure; DBP, diastolic blood pressure; AP augmentation pressure; Aix@75, augmentation index at a heart rate of 75 beats/min; PWV, pulse wave velocity; BAD, brachial artery diameter; FMD-peak, flow-mediated dilatation (peak value) (conduit vascular function); RHI, reactive hyperemia index (micro-vascular function).

Results are mean ± standard deviation. The online Supplemental Material (Table S3) provides all the results as median (interquartile range [IQR]).

The differences are presented as mean ± standard deviation (CAP minus FA results). Positive values represent higher values post-CAP exposures (e.g., heart rate was higher while BAD trended to be smaller post-CAP exposures).

^ap values are comparisons of immediate-post exposures (CAP vs. FA). ^bp values are comparisons of 2 hours-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.

Figure legends

Figure 1. Systolic (A) and diastolic (B) blood pressure levels during exposures. The figure presents the mean \pm standard error of blood pressure values measured every 10 minutes during exposures following a 10 minute rest period after the study participants entered the chamber.

Figure 2. Heart rate levels during exposures. The figure presents the mean \pm standard error of heart rate values measured every 10 minutes during exposures following a 10 minute rest period after the study participants entered the chamber.

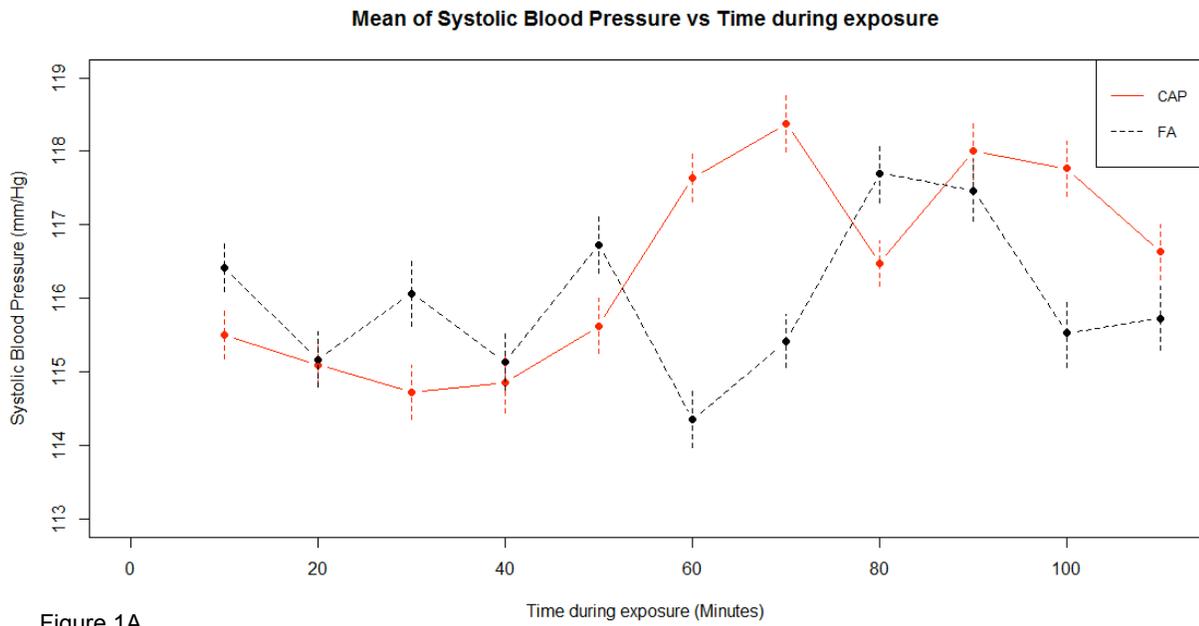


Figure 1A

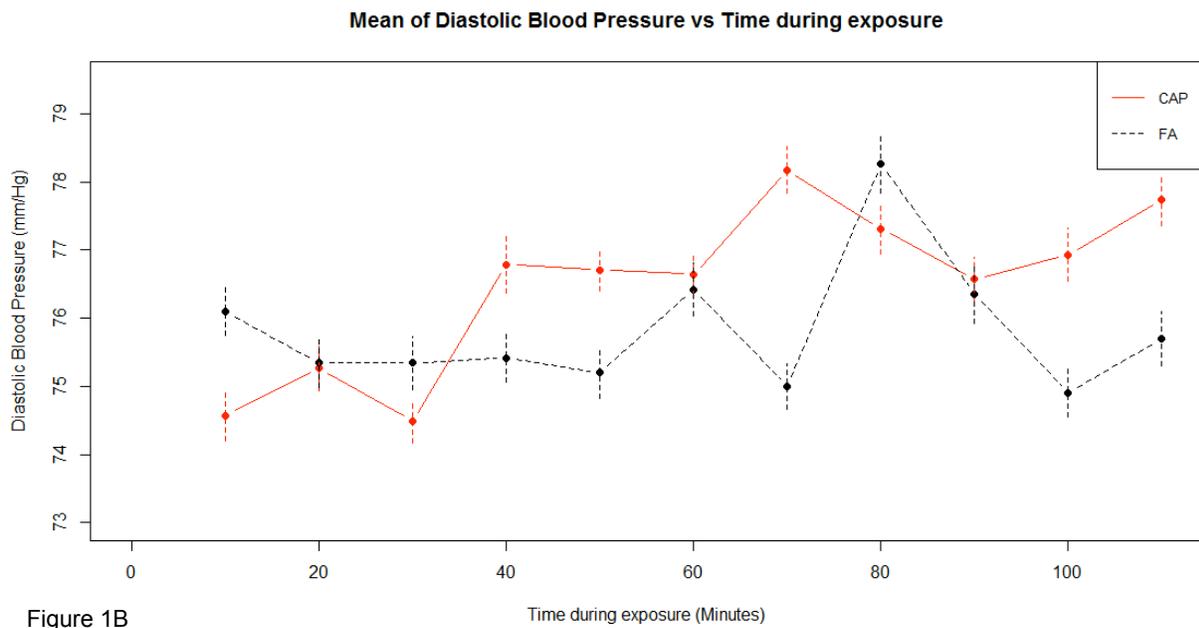


Figure 1B

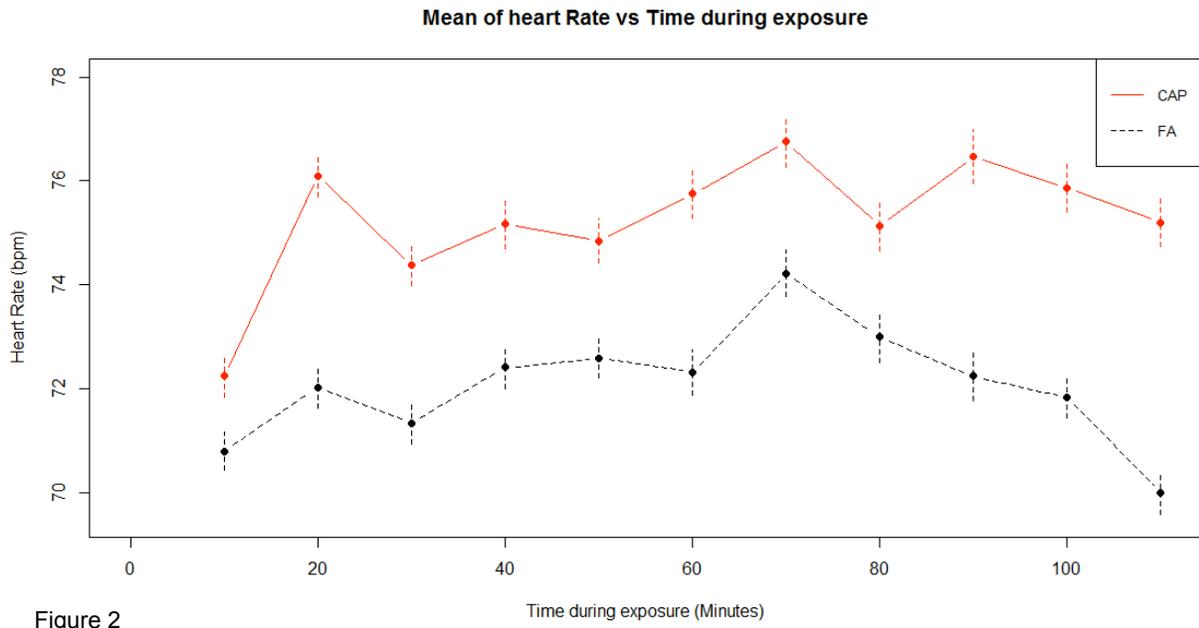


Figure 2