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Effects of diesel exhaust inhalation on heart rate variability in human volunteers

Alon Peretz^a, Joel D. Kaufman^{a,b}, Carol A. Trenga^a, Jason Allen^a, Chris Carlsten^{a,b}, Mary R. Aulet^a, Sara D. Adar^a, and Jeffrey H. Sullivan^a

^aDepartment of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington, USA

^bDepartment of Medicine, University of Washington, Seattle, WA, USA

Abstract

Objectives—Particulate matter (PM) air pollution is associated with alterations in cardiac conductance and sudden cardiac death in epidemiological studies. Traffic-related air pollutants, including diesel exhaust (DE) may be at least partly responsible for these effects. In this experimental study we assessed whether short-term exposure to DE would result in alterations in heart rate variability (HRV), a non-invasive measure of autonomic control of the heart.

Methods—In a double-blind, crossover, controlled-exposure study, 16 adult volunteers were exposed (at rest) in randomized order to filtered air (FA) and two levels of diluted DE (100 or 200 $\mu\text{g}/\text{m}^3$ of fine particulate matter) in two-hour sessions. Before, and at four time-points after each exposure we assessed HRV. HRV parameters assessed included both time domain statistics (standard deviation of N-N intervals (SDNN), and the square root of the mean of the sum of squared differences between successive N-N intervals (RMSSD)) and frequency domain statistics (high frequency power (HF), low frequency power (LF), and the LF/HF ratio).

Results—We observed an effect at 3-hours after initiation of DE inhalation on the frequency domain statistics of HRV. DE at 200 $\mu\text{g}/\text{m}^3$ elicited an increase in HF power compared to FA ($\Delta=0.33$; 95% CI: 0.01 to 0.7) and a decrease in LF/HF ratio ($\Delta=-0.74$; 95% CI: -1.2 to -0.2). The effect of DE on HF power was not consistent among study participants. There was no DE-effect on time domain statistics and no significant DE effect on HRV in later time-points.

Conclusions—We did not observe a consistent DE effect on the autonomic control of the heart in a controlled exposure experiment in young participants. Efforts are warranted to understand discrepancies between epidemiological and experimental studies of air pollution's impact on HRV.

Keywords

Air pollution; Heart rate variability; Autonomic nervous system; Diesel exhaust

Address for Correspondence: Joel D. Kaufman. Address: University of Washington Occupational and Environmental Medicine Program, Department of Environmental and Occupational Health Sciences, 4225 Roosevelt Way NE, Suite #302, Box 354695 Seattle, WA 98195 USA; PHONE 206-616-3501; FAX 206-685-3872; Email- joelk@u.washington.edu.

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INTRODUCTION

Acute cardiovascular morbidity and mortality have been consistently associated with ambient fine particulate matter air pollution (PM_{2.5}, particles with aerodynamic diameter 2.5µm or less) in epidemiological studies (Dominici, et al. 2006; Ostro, et al. 2007). PM-induced alterations in the balance of autonomic control of the heart may partly contribute to these associations. Recent epidemiological studies have demonstrated association between PM_{2.5} and a shift of balance in the autonomic control of the heart (Liao, et al. 2004; Park, et al. 2005; Pope, et al. 2004; Romieu, et al. 2005). These associations may be particularly strong for vehicle-related pollutants (Adar, et al. 2007; Riediker, et al. 2004; Schwartz, et al. 2005).

By mass, diesel exhaust (DE) is the primary source of vehicle-derived PM_{2.5} (USEPA 2000). DE is also a substantial contributor to poor air quality in urban environments, accounting for the majority of ultrafine particulate matter (<100 nm). These small particles have a very large surface area, are highly respirable and are excellent carriers for adsorbed inorganic and organic compounds. Therefore, we hypothesized that controlled exposure to DE would result in early alterations in heart rate variability (HRV) measured at 1 and 3 hours from the onset of exposure. Because previous studies have also shown effects of PM_{2.5} on autonomic balance on the order of several hours to a day (Liao, et al. 1999; Park, et al. 2005), we also explored the hypothesis that exposure to DE would effect HRV parameters measured at 6 and 22 hours from initiation of exposure.

METHODS

We conducted a double-blind crossover experiment of DE and filtered air (FA) exposures. In this experiment, each participant was exposed on three different days to each of three conditions: FA, DE calibrated to generate 100µg/m³ of PM_{2.5} (DE₁₀₀), and DE calibrated to generate 200µg/m³ of PM_{2.5} (DE₂₀₀). DE was derived from a 2002 model turbocharged direct-injection 5.9 liter Cummins B-series engine (6BT5.9G6, Cummins, Inc., Columbus, IN). During exposures, PM_{2.5} concentrations were continuously measured and adjusted to maintain steady-state conditions (TEOM 1400a PM_{2.5}, Rupprecht & Patashnick Co., Albany, NY) (for a detailed description of the DE exposure system see the online data supplement. Exposures were randomized by order and separated in time by at least 2 weeks. All participants gave written informed consent. The University of Washington Human Subjects Division approved the consent form and study protocol.

Study participants

Recruited subjects were 18-49 years old with no history of ongoing medical care for heart disease, hypertension, asthma, diabetes, hypercholesterolemia, or other chronic conditions. All subjects were nonsmokers for at least six months and had normal spirometry. Since we were interested in susceptibility by disease status, we separately recruited “healthy” and “metabolic syndrome” participants. Healthy participants had a body mass index (BMI) below 30kg/m², fasting blood sugar below 126mg/dL, resting blood pressure below 130/85 mmHg, and no signs of arrhythmia or ischemia on a screening electrocardiogram (ECG). Metabolic syndrome participants fulfilled any three of the following five criteria: waist circumference ≥102 cm in males and ≥ 88 cm in females; triglycerides ≥ 150 mg/dL; HDL cholesterol < 40 mg/dL in males and < 50 mg/dL in females; systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥ 85 mmHg; and fasting glucose ≥ 100 mg/dL.(Grundy, et al. 2005)

Study protocol

Exposures began at 9am and were two hours in duration; participants were at rest throughout. We used a three-channel ambulatory Holter recorder (Del Mar, Irvine, CA, USA) to monitor

continuous ECG tracings. The electrodes were placed in a modified V1-V5 position. ECG recordings started approximately 1 hour before the onset of exposures and continued throughout the exposure session days (~8-9 hours). ECG recordings were also collected over the entire duration of the follow-up visit (~1 hour) on the morning after exposure days.

Heart rate variability parameters

In order to prevent interference by participant activity, we limited the data for this analysis to standardized 10-minute periods during which subjects were resting supine. These resting periods were conducted at various time-points during the exposure day: before exposure started, at one hour into exposure (1h), and at three hours (3h), six hours (6h), and 22 hours (22h) from the onset of exposure. A single trained analyst, blinded to exposure levels, reviewed and edited the automatically determined readings of QRS complexes in these 10-minute intervals using Del Mar Dartsan software (Model DS-90, Irvine, CA, USA) to obtain all normal-normal (N-N) intervals. Any 10-minute recording with more than 10% ectopic beats and/or artifacts was excluded from the analysis.

For the analysis of HRV we used the middle five minutes of each 10-minute recording period, including only N-N intervals with a successive ratio within 0.8-1.2. We analyzed HRV using software created by The Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland (Niskanen J-P 2004). Generated time domain statistics included the standard deviation of N-N intervals (SDNN), and the square root of the mean squared differences between successive N-N intervals (RMSSD). For frequency domain analysis we used a Fast Fourier Transform (FFT) algorithm. The signal was analyzed with Hanning window for segment lengths of 256 samples with 50% overlapping (Singh, et al. 2004). Spectral data obtained from N-N intervals were analyzed according to the following frequency bands: very-low frequency (VLF, 0.00–0.04 Hz), low frequency (LF, 0.04–0.15 Hz), and high frequency (HF, 0.15–0.40 Hz). Since very-low (and lower) frequency modulation of N-N interval data has no interpretable significance in short ECG recordings (Task-Force 1996), we report the frequency domain measures of LF and HF power in ms^2 , and the LF/HF ratio. To address any irregularity in the N-N interval time series, cubic interpolation at a rate of 4Hz was used before spectrum estimation. To remove the influence of a large low-frequency baseline trend component, a de-trending algorithm was used based on a smoothness priors method (Tarvainen MP 2002).

Statistical Analysis

All statistical testing was based on two-tailed tests with $\alpha=0.05$. Descriptive data are presented as a mean \pm standard error to the mean (SEM) unless specified otherwise. Statistical analyses were performed using STATA 9.1 (StataCorp LP, College Station TX).

All HRV parameters were log-transformed prior to statistical analysis as they were highly skewed. Changes in HRV were normalized as the difference between each HRV parameter at a given time-point after exposure onset relative to the same HRV parameter, pre-exposure. Exposure-related changes in HRV at each time point were computed for DE_{200} or DE_{100} by contrasting to the FA levels using paired *t*-tests. Due to our small study population, we computed the percent difference at 1h and 3h from the pre-exposure non-transformed levels of the HRV parameters that were significantly affected by DE exposure, to assess whether the effect was consistent among study participants. We also tested our results for effect modification by subject-related characteristics, and for period and carryover effects, using interaction terms in an ANOVA model.

RESULTS

A total of 23 participants (6 healthy and 17 metabolic syndrome) completed all three levels of exposure. Seven were excluded from further analyses: one participant did not meet the inclusion criteria for N-N intervals taken from the ECG measurement at pre-exposure to DE₂₀₀; six participants had missing ECG measurements at pre-exposure to either FA, or DE₂₀₀. Characteristics of the 16 participants (3 healthy and 13 metabolic syndrome) with acceptable HRV measurements are presented in Table 1.

The average exposure levels to PM_{2.5} approximated the target concentrations. In addition, we maintain low concentrations of the gases carbon monoxide (CO) and nitrogen dioxide (NO₂) (Table 2).

Pre-exposure HRV statistics were not significantly different between exposure sessions or between healthy and metabolic syndrome participants within exposure session (Table 3). Although metabolic syndrome participants generally exhibited slightly lower HRV measures than healthy individuals within the same exposure session, we analyzed the DE effect on HRV statistics without sorting by health status due to the small number of healthy participants with available data. As a sensitivity analysis we also conducted analysis limited to the metabolic syndrome participants. Results were similar to those from both study populations combined.

Analysis of exposure-related changes of HRV parameters at all time periods are presented in Figure 1. These results revealed no consistent differences at 1h between DE and FA. Table 4 shows the effect of exposure to DE₁₀₀ and DE₂₀₀ on HRV Parameters at 3h. While there was no significant effect for any HRV parameter from DE₁₀₀, we noted a significant increase in HF power over pre-exposure levels after DE₂₀₀ (0.26) as compared to FA (-0.07) ($\Delta = 0.33$; 95% CI: 0.01 to 0.7). At this same time-point, there was a decrease in LF/HF ratio from pre-exposure levels following DE₂₀₀ (-0.54) as compared to an increase after exposure to FA (0.2) ($\Delta = -0.74$; 95% CI: -1.2 to -0.2).

Changes in the frequency domain HRV parameters observed at 3h remained generally consistent for DE₂₀₀ across the later time points with evidence of increased HF, decreased LF, and a decreased LF/HF ratio after DE as compared to FA (Figure 1). At 22h following exposure to FA, frequency domain parameters showed a trend toward pre-exposure baseline values that was not observed after exposure to DE₂₀₀. However, exposure-related changes in frequency domain statistics at 6h and 22h were not statistically different between exposures to DE₂₀₀ and FA. In addition, no dose response was observed in these later time points. No significant effect of DE was observed on heart rate (data not shown) and on time domain measurements at any time-points. Age, gender, BMI, fasting plasma lipid levels, smoking history, carryover, and period effects did not modify the association between DE and frequency domain statistics in all time points.

To assess whether the increased HF power was consistent among study participants, we plotted the percent difference of HF power from the pre-exposure level for each participant for both DE₂₀₀ and DE₁₀₀ at 1h and 3h (Figure A online data supplement). Only 7 (1 healthy and 6 metabolic syndrome) out of 16 participants showed an increased HF power at DE₂₀₀ as compared to FA. Six showed an increased HF at DE₁₀₀ as compared to FA. No consistent dose response relationship was observed. The same approach used for LF/HF ratio, revealed that only 9 out of 16 participants showed a decreased ratio at DE₂₀₀ as compared to FA.

DISCUSSION

In this study we did not find a consistent effect of DE inhalation on the autonomic modulation of the heart as assessed by heart rate variability measures. Although we showed a significant

increment of HF power at 3h following DE₂₀₀ compared to FA, only 7 of 16 participants demonstrated this effect (supplementary Figure A). No dose-response relationship was found for increasing concentrations of DE, and the effect was opposite of the most typically observed effect of air pollutants. Moreover, the significant decrease in LF/HF ratio at 3h following DE₂₀₀ compared to FA was driven by a small number of participants. Our inconsistent effect of DE on HF power and LF power does not allow inference regarding a shift in the balance of the autonomic control of the heart.

HRV has been used frequently in air pollution research as a signal of cardiovascular effect, and to explore for potential pathophysiological mechanisms by which air pollution may lead to cardiovascular mortality and morbidity. Epidemiological studies in susceptible populations-especially panel study designs-have shown a relationship between air pollution and decreased HRV (Adar, et al. 2007; Chan, et al. 2004; Gold, et al. 2000; Liao, et al. 1999; Liao, et al. 2004; Park, et al. 2005) as well as increased cardiac arrhythmia with exposure to components of ambient particulate air pollution (Dockery, et al. 2005; Peters, et al. 2000; Rich, et al. 2006; Sarnat, et al. 2006). The previously observed effects of air pollutants on HRV and arrhythmia are not entirely consistent across studies. Potential factors that may explain these discrepancies include age and health status of study participants, composition of particles and gases, and use of different methodologies for HRV analysis.

Compared to observational approaches such as panel study designs, an experimental approach as used in this study-with rigorous control over exposure situations and potentially confounding factors-would be expected to be highly tuned to finding an exposure-related effect if one exists. Our inability to find a decrease in HRV after DE inhalation in young individuals without known cardiovascular disease is consistent with prior controlled exposure studies to concentrated ambient particles (CAPS) (Devlin, et al. 2003; Gong, et al. 2004) and ultrafine carbon particles (Frampton, et al. 2004). Moreover, while HRV was decreased in the lion's share of published epidemiological studies that involved elderly participants, HRV effects of air pollution in young healthy individuals did not consistently demonstrate this decline in HRV (Chan, et al. 2004; Devlin, et al. 2003; Frampton, et al. 2004; Gong, et al. 2004; Magari, et al. 2001; Riediker, et al. 2004; Scharrer, et al. 2007). It has long been recognized that autonomic function changes with increasing age, as demonstrated by a decrease in HRV even in individuals without pre-existing cardiovascular or autonomic disease (Liao, et al. 1995; O'Brien, et al. 1986). It is possible that the response of the autonomic nervous system to a stimulus such as pollutant inhalation may be different between elders who are healthy or possess underlying unknown cardiovascular disease and our study population. Although our population included individuals with metabolic syndrome, the absence of pre-existing cardiovascular disease or autonomic neuropathy in our population may decrease the utility of HRV measures. The significant range of HRV responses in routine cardiac modulation in our study population may have overwhelmed the small adverse DE effect on autonomic modulation.

The composition of the inhaled pollutants may also contribute to the discordance of our findings with previously published data. DE is not the only source of pollution and co-pollutants such as ozone may potentially make diesel particles more injurious to biological tissues (Bosson, et al. 2007; Madden, et al. 2000). Therefore, although our system creates a realistic combustion-derived exposure, which is highly relevant to urban air pollution, especially traffic-related exposure, it may not replicate the situations seen in observational studies of HRV.

The different methods of HRV analyses in prior air pollution studies (long-term (24 hours) (Adar, et al. 2007; Magari, et al. 2002; Pope, et al. 2004; Pope, et al. 1999) and short-term (5 minutes)) (Devlin, et al. 2003; Frampton, et al. 2004; Gold, et al. 2000; Gong, et al. 2004; Liao, et al. 2004; Park, et al. 2005; Sullivan, et al. 2005) can lead to different inferences for PM effect on HRV (Task-Force 1996). To allow for maximal comparability between exposures, we opted

for short-term standardized HRV analysis rather than long-term analysis, thus eliminating influence by factors such as variation in posture, physical and mental activity, and sleep. As a result, our results cannot be compared directly to all other published studies of HRV and pollution.

The timing of outcome assessments also differs between studies, and reflects uncertainty regarding the time course of the cascade of effects which might culminate in the health effects of PM. While PM appear to exert health effects within hours of exposure (Mills, et al. 2005; Peters, et al. 2001), we sought to find early effects of DE on HRV. Alternatively, PM exposure may lead to an initial pulmonary inflammatory response with subsequent cascade of delayed effects (hours or even days) on the autonomic nervous system (Haefeli, et al. 1993; Nel, et al. 2001; van Eeden, et al. 2001; Weisensee, et al. 1993). We did not demonstrate a late effect of DE on HRV; however, we noticed long-term trends in frequency domain parameters compared to pre-exposure values. Although this trend lacked statistical significance, it may represent the onset of a late autonomic effect. In fact, Park et al. demonstrated the strongest effect on HRV (decreased HRV) was with a 48-hour moving average of PM_{2.5} (Park, et al. 2005). If future experimental studies are conducted, it would be prudent to explore late autonomic effects.

Strengths and limitations

This study's strength is in the experimental design, unlike most published studies that have used an observational design from which it is much more difficult to determine causal relationships. Nonetheless, several limitations may hinder inference from this study. First, after applying our exclusion criteria on HRV data, only a small and nonhomogenous study population was analyzed. We did not find significant baseline differences in HRV statistics between the two sub-populations of the study. Second, we assessed HRV in short recording intervals. This may have affected our ability to identify any difference in time domain statistics between the exposure sessions (Task-Force 1996), and we may have sacrificed some power to detect associations by limiting the number of outcome measures in our dataset. In addition, this approach may have limited our ability to directly compare our results with those of other studies.

CONCLUSIONS

In a controlled exposure experiment, we were not able to show a consistent effect of diesel exhaust inhalation on heart rate variability. To better understand and confirm prior observations of PM effects on HRV principally derived from non-experimental designs, controlled exposure experimental studies in different susceptible populations, using relevant exposures and standardized analysis of ECG recordings, are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The study protocol was approved by the University of Washington Human Subjects Division.

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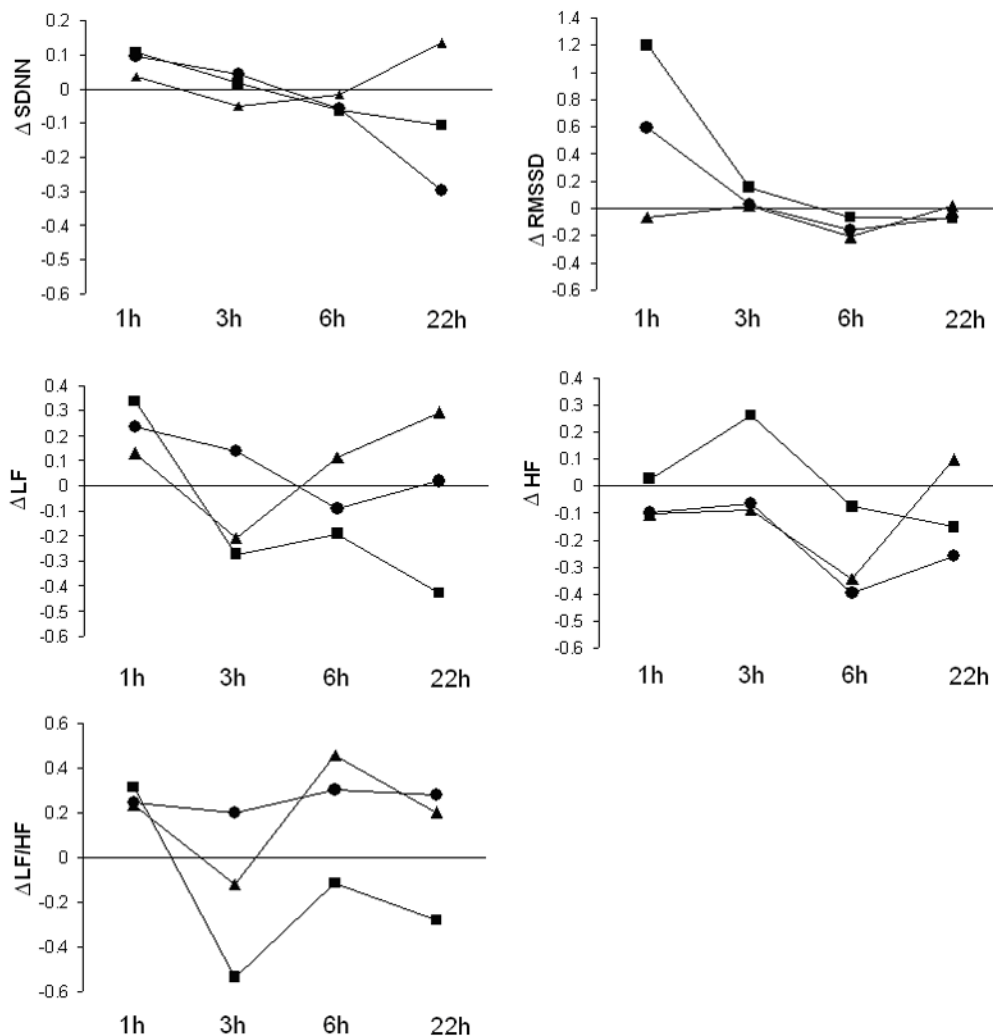


Figure 1. Mean change in log transformed HRV parameters at each time point from pre-exposure levels for exposure to filtered air (FA) (●), diesel exhaust at 100 μg/m³ (▲), or diesel exhaust at 200 μg/m³ (■).

Table 1

Study participants' characteristics by health status

Characteristic	Healthy	Metabolic Syndrome
Participants (<i>n</i>)	3	13
Age, mean in yr (<i>range</i>)	32 (24-39) ^a	41 (31-48) ^a
Gender (F) (<i>n</i>)	0	5
Race		
Caucasian (<i>n</i>)	2	10
Other (<i>n</i>)	1	3
Body Mass Index (kg/m ²)	24.7 (0.9) ^a	39.2 (1.8) ^a
Total Cholesterol (mg/dl)	157.3 (23.9) ^a	183.2 (6.1) ^a
Triglycerides (mg/dl)	90.3 (19.8)	168.9 (34.6)
Glucose (mg/dl)	90.3 (2) ^a	101.9 (3) ^a
Smoking history (<i>n</i>) (P/Y) ^b	0	5 (3.5)

^a Statistically significant differences between healthy and metabolic subjects at p<0.05;

^b P/Y- average pack-year calculated as (number of cigarettes per day X number of years smoked)/20; where not specified, values are mean (SEM)

Table 2

Average PM_{2.5} mass concentrations* and gas concentrations[†] measured during two-hour exposure sessions of 16 participants.

Pollutant	Filtered air	Diesel exhaust at 100 $\mu\text{g}/\text{m}^3$	Diesel exhaust at 200 $\mu\text{g}/\text{m}^3$
PM _{2.5} ($\mu\text{g}/\text{m}^3$)	4.16	101.95	206.03
NO ₂ (ppb)	15.44	20.57	28.29
NO (ppb)	38.29	947.89	1629.31
CO (ppm)	0.29	0.47	0.74

* From TEOM - discrete 10-min averaging intervals;

[†] 1-min averaging intervals.

Table 3

Geometric means (95% CI) of HRV measurements prior to exposure of healthy (n=3) and metabolic syndrome (n=13) participants.

HRV parameter	Filtered Air	100 $\mu\text{g}/\text{m}^3$ Diesel exhaust	200 $\mu\text{g}/\text{m}^3$ Diesel exhaust
SDNN (ms)			
<i>All participants</i>	29.6(25.4-34.5)	28.5(24-33.8)	30.0 (24.4-36.8)
<i>Healthy</i>	33.1(26.2-41.6)	34.5(21-56.9)	30.4(20.3-45.5)
<i>Metabolic syndrome</i>	28.8(23.8-34.9)	27.2(22.3-33.3)	29.9(23-38.7)
RMSSD (ms)			
<i>All participants</i>	26.7(21-34)	27.0(21-34.6)	26.9(21.6-33.5)
<i>Healthy</i>	34.2(28.3-41.3)	30.9(16.6-57.4)	28.7(16.9-48.7)
<i>Metabolic syndrome</i>	25.2(18.8-33.9)	26.1(19.2-35.6)	26.5(20.2-34.8)
LF (ms²)			
<i>All participants</i>	200.4(141.3-284.1)	181.6(127.8-258.1)	222.7(132.8-373.5)
<i>Healthy</i>	230.7(48.5-1097.3)	284.0(41.9-1924.1)	266.5(67.9-1045.9)
<i>Metabolic syndrome</i>	193.9(128.5-292.8)	163.8(112.7-238.1)	213.7(112.9-404.7)
HF (ms²)			
<i>All participants</i>	120(69.5-207.2)	106(65.7-171.1)	114.8(71-185.6)
<i>Healthy</i>	188.3(81.8-433.6)	150.5(27.7-816.9)	120(52.9-272)
<i>Metabolic syndrome</i>	108.2(55.3-211.6)	97.8(55.3-173.1)	113.7(62.1-207.8)
LF/HF ratio			
<i>All participants</i>	1.7(1-2.9)	1.7(1-2.9)	1.9(1.2-3.2)
<i>Healthy</i>	1.2(0.2-9.2)	1.9(0.1-33.8)	2.2(0.7-6.9)
<i>Metabolic syndrome</i>	1.8(0.9-3.5)	1.7(0.9-3.1)	1.9(1-3.4)

Table 4

Changes^a in Heart rate variability (HRV) from pre-exposure to 3 hours after initiating exposure, log transformed, mean (SEM)

HRV parameter	Filtered air	100µg/m ³ Diesel exhaust	200µg/m ³ Diesel exhaust	Average DE effect ^b (95% CI)
SDNN	0.04 (0.05)	-0.05 (0.07)	0.01 (0.09)	DE ₁₀₀ : -0.09 (-0.27 to 0.84) DE ₂₀₀ : -0.03 (-0.2 to 0.2)
RMSSD	0.03 (0.04)	0.02 (0.1)	0.15 (0.1)	DE ₁₀₀ : -0.01 (-0.23 to 0.22) DE ₂₀₀ : 0.13 (-0.07 to 0.3)
LF	0.14 (0.12)	-0.21 (0.15)	-0.27 (0.2)	DE ₁₀₀ : -0.35 (-0.71 to 0.02) DE ₂₀₀ : -0.41 (-0.9 to 0.1)
HF	-0.07 (0.08)	-0.09 (0.22)	0.26 (0.19)	DE ₁₀₀ : -0.02 (-0.45 to 0.4) DE ₂₀₀ : 0.33 (0.01 to 0.7) ^c
LF/HF ratio	0.2 (0.12)	-0.12 (0.24)	-0.54 (0.18)	DE ₁₀₀ : -0.32 (-0.81 to 0.17) DE ₂₀₀ : -0.74 (-1.2 to -0.2) ^d

^aHRV (post-exposure) – HRV (pre-exposure);

^b difference between changes at filtered air and changes at 100µg/m³ diesel exhaust (DE₁₀₀) or 200µg/m³ diesel exhaust (DE₂₀₀);

^c p<0.05;

^d p<0.01