

TITLE PAGE

Impact of traffic-related air pollution on acute changes in cardiac autonomic modulation during rest and physical activity: a cross-over study.

Running title: Physical activity and TRAP effects on HRV

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ABSTRACT

People are often exposed to traffic-related air pollution (TRAP) during physical activity but it is not clear if physical activity modifies the impact of TRAP on cardiac autonomic modulation. We conducted a panel study among 28 healthy adults in Barcelona, Spain to examine how physical activity may modify the impact of TRAP on cardiac autonomic regulation. Participants completed four two-hour exposure scenarios that included either rest or intermittent exercise in high and low-traffic environments. Time- and frequency-domain measures of heart rate variability (HRV) were monitored during each exposure period along with continuous measures of TRAP. Linear mixed-effects models were used to estimate the impact of TRAP on HRV as well as potential effect modification by physical activity. Exposure to TRAP was associated with consistent decreases in HRV; however, exposure-response relationships were not always linear over the broad range of exposures. For example, each $10 \mu\text{g}/\text{m}^3$ increase in black carbon was associated with a 23% (95% CI: -31, -13) decrease in high frequency power at the low-traffic site whereas no association was observed at the high-traffic site. Physical activity modified the impact of TRAP on HRV at the high-traffic site and tended to weaken inverse associations with measures reflecting parasympathetic modulation ($p \leq 0.001$). Evidence of effect modification at the low-traffic site was less consistent. The strength and direction of the relationship between TRAP and HRV may vary across exposure gradients. Physical activity may modify the impact of TRAP on HRV, particularly at higher concentrations.

KEY WORDS

traffic-related air pollution; particles; heart rate variability; physical activity; noise

INTRODUCTION

Traffic-related air pollution (TRAP) is thought to contribute to cardiovascular morbidity/mortality in part through acute changes in cardiac autonomic modulation (1–3). A change in such modulation (namely a reduction in heart rate variability, HRV) has been linked to increased risks of cardiovascular morbidity and mortality (1). In urban environments, people are often exposed to TRAP during periods of physical exertion through activities such as cycling, walking, or running which often take place in close proximity to vehicle emissions. While exposures such as these during physical activity in urban environments have been associated with acute changes in heart rate variability in healthy adults (4,5), health impact assessments generally suggest that the health benefits of physical activity outweigh the risks associated with exposure to TRAP (6,7). Moreover, a recent study reported that high residential levels of TRAP do not reduce the beneficial impact of physical activity such as participation in cycling, despite implied increases in respiratory uptake and deposition of TRAP, on long-term cardiovascular mortality risk (8). While these findings suggest that physical activity may offset the detrimental impacts of air pollution at the individual level, few studies have specifically evaluated this question. Indeed, little is currently known about how physical activity may modify short-term associations between air pollution and physiological changes thought to play an important role in explaining air pollution health effects.

In this study, we examined the impact of physical activity on the acute relationship between TRAP and heart rate variability in a panel of healthy adults in Barcelona, Spain. The broad range of personal exposures encountered in Barcelona also allowed us to examine the shapes of concentration-response curves for associations between specific traffic pollutants and time- and frequency-domain measures of HRV.

METHODS

Study participants

Healthy non-smoking adults were invited to participate in the current study through an online advertisement. Healthy adults were defined as: ≥ 18 years of age, not knowingly needing any type of medication in the last three months or the near future, not having any previously diagnosed chronic medical conditions (e.g. asthma, arrhythmia, diabetes and chronic heart disease), not knowingly needing anti-allergic treatments in the last three months or the near future, and not having symptoms of an acute respiratory infection in the last two weeks. Individuals taking any regular medications (except for oral anti-contraceptives) or vitamins/supplements were excluded. Women were asked about pregnancy status and were excluded if they reported being pregnant. All participants were asked to abstain from high-intensity exercise for at least 48 hours, from alcohol for at least 24 hours, and from caffeine for at least four hours before baseline measurements.

Study Design

This study employed a repeated-measures cross-over design. Statistical power calculations for the optimal study design for measuring intermediary health of commuter exposures were performed prior to the development of this study protocol. Participants

served as their own controls by performing pre-determined exercise and rest protocols for a two-hour period in high and low-traffic environments on four randomized occasions (i.e. high-traffic during exercise, low-traffic during exercise, high-traffic during rest and low-traffic during rest). This gave a total of four separate exposure scenarios per participant with 24 repeated HRV measurements per scenario, typically two weeks apart. The study took place in Barcelona (Spain) between February and November of 2011. Barcelona is a coastal city with a Mediterranean climate, a high population density (~16 000/km²), and a high motor vehicle density (~6 000/km²) (9). Study days were rescheduled in the case of rain.

To emulate exposures in real-world environments, two sites used by both bicyclists and pedestrians were identified with either high or low levels of traffic-related air pollution. The high-traffic site was located on a pedestrian bridge approximately five metres above a city ring road for motorised traffic; the low-traffic site was a pedestrian-friendly market square. Two participants were simultaneously monitored at the same site over the course of a day. Study days were scheduled on weekdays (Tuesday and Thursday) to capture normal commuter traffic levels.

On study days, participants were requested to arrive at the clinical laboratory by 07:00 using the underground railway, with a fifteen minute period of rest before baseline measurements were taken. At 08:00, participants were transported to the exposure site (typically via a diesel taxi ride, of approximately five minutes duration). For two hours, participants either cycled intermittently on a stationary bike or rested on a seat while TRAP and noise and HRV parameters were continuously measured. For the cycling scenario, participants mounted a cycle ergometer and performed 15-minute intermittent

moderate-intensity exercise defined as a heart rate within 50–70 % of their maximum predicted value according to age and sex (10) (see Supplementary Figure S1). The Ethics Review Committee of the Institut Municipal d'Investigació Mèdica (IMIM) approved the study protocol and written informed consent was obtained from each participant prior to participation.

Exposure monitoring

Real-time concentrations of ultrafine particles ($<0.1 \mu\text{m}$) (UFPs) (CPC; Model 3007, TSI, MN, USA), black carbon (BC) (Model AE51, Magee Scientific, CA, USA), and fine particulate matter ($\text{PM}_{2.5}$) (DusTrak Model 8532, TSI, MN, USA) were continuously monitored during each exposure period. Oxides of nitrogen (NO_x ; NO, NO_2) were also monitored but are not reported because concentrations were strongly correlated with BC and UFPs ($r>0.73$). All monitoring devices were table-mounted adjacent to study participants to capture personal exposures and all air pollution measurements were collected as one-minute means. Data corrections and calibration factors were applied according to manufacturer's instructions (see Supplementary Information: calibration/correction factors).

A tripod-mounted weather station (Model WMR200, Oregon Scientific, OR, USA) was used to collect 5-minute mean ambient temperature and relative humidity, and environmental noise (LA_{eq} , in dB(A)) was monitored using a tripod-mounted sound level meter (Model SC160, CESVA, ESP) with a $\pm 1.0 \text{ dB}$ tolerance and a range of 30 to 137 dB. The noise meter was positioned adjacent to the participants at 1.5 m above ground and at least at 1.5 m away from any reflecting surface. Noise measurements were

collected as one-minute means. Technicians also used two manual handheld traffic counters to measure traffic counts at each exposure site.

Heart rate and heart rate variability

Holter monitors (Model CardioLight, Gem-Med, ESP) were used to continuously monitor heart rate (HR) and heart rate variability (HRV) during each 2-hour exposure period. Raw data were sent to the holter supplier (Gem-med) for clinical analysis and interpretation. Time-domain [standard deviation of normal-to-normal intervals (SDNN) and root mean square of successive differences in adjacent NN intervals (RMSSD)] and frequency-domain [low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.40 Hz) power, plus the ratio of LF to HF (LF:HF)] measures of HRV were determined as five-minute moving averages.

Statistical Analyses

Linear mixed-effects models with random subject intercepts and independent covariance structure were used to evaluate the impacts of TRAP on acute changes in HRV. All HRV measures were log-transformed to normalize distributions. All analyses used 15-minute mean exposure periods which included the five-minute period of HRV evaluation as well as the 10 minutes prior to HRV evaluation. Five-minute exposure periods were also evaluated but gave similar results (not shown). All coefficients reflect percent changes in HRV outcomes per 10 000 particles/cm³ increase in UFPs or per 10 µg/m³ increase in BC or PM_{2.5}. Each air pollutant was evaluated in a separate model. A separate manuscript will examine the independent impact of noise on HRV and thus effect estimates for noise are beyond the scope of (and not presented in) this study.

Ambient temperature and mean HR were included in all models regardless of statistical significance. Further, general additive mixed models were performed to study the linearity of the effect estimate of ambient temperature and mean HR. In cases of non-linearity ($p < 0.05$) for these two variables, their quadratic terms were introduced into the model. Other covariates examined as potential confounding factors included relative humidity, noise, day of the week, and month. These covariates were only included if they improved model fit according to the Bayesian Information Criterion, or had a meaningful impact on model coefficients (as a change of 10% or more) for individual air pollutants.

Potential interactions between TRAP and physical activity were examined by including first order interaction terms between TRAP exposures (individually) and an indicator variable for physical activity in the models. Stratified models were also examined according to the activity status of a given exposure period (i.e. rest/exercise). Exercise intensity across exposure scenarios were examined using the Scheffe method for multiple-comparison post-estimation. Given the broad range of exposures encountered, models were also examined within each exposure site (i.e. high and low-traffic) to verify that the magnitude and direction of associations were consistent across the range of exposures. The statistical significance of first order interaction terms between site and individual air pollutants were examined to identify potential differences in air pollutant impacts on HRV across sites. In addition, the shapes of exposure-response relationships for individual air pollutants were examined using restricted cubic splines with the Stata function *mkspline2* with 4 knots at evenly spaced percentiles of exposure. Models with 3 knots were also examined but AIC and BIC criteria sometimes indicated better fit for

models with 4 knots (data not shown). All statistical analyses were conducted using Stata/SE (v12.0; StataCorp LP, USA). Statistical significance was defined as $p < 0.05$.

RESULTS

Twenty-eight healthy adults completed the four exposure scenarios after excluding two participants (upon completion of the first exposure scenario) due to ectopic supraventricular tachyarrhythmia. Participant baseline characteristics are presented in Table 1 and descriptive data for air pollution exposures are presented in Table 2. Air pollution exposures varied dramatically across sites with mean exposures at the high-traffic site exceeding values at the low-traffic site by 4–7 times. Fifteen minute mean noise exposures were not correlated with air pollution exposures at either the low ($-0.079 \leq r \leq 0.0096$) or the high-traffic sites ($-0.16 \leq r \leq 0.035$) but individual air pollutants were correlated. Specifically, UFP and black carbon exposures were correlated at both the low ($r = 0.77$) and high-traffic ($r = 0.67$) sites whereas correlations of these pollutants with $PM_{2.5}$ were lower ($0.22 \leq r \leq 0.62$).

In total, after clinical analysis and removal of unrealistic data (as interpreted by the Gem-Med clinical cardiologist/s), a minimum of 1 700 simultaneous repeated measurements were available for both time-domain (SDNN and RMSSD) and frequency-domain (HF, LF, and LF:HF) HRV parameters. Mixed-effect models describing the relationship between TRAP and HRV are shown in Table 3. All final models were adjusted for body mass index, ambient temperature, noise, and linear and quadratic terms for heart rate. In general, adjusting for noise did not have a large impact on air pollution coefficients but small increases/decreases were observed in some models (see

Supplementary Table S1). In addition, all models are presented separately for the low and high-traffic sites as significant air pollution-site interactions ($p < 0.05$) were detected for the majority of outcomes and exposure-response relationships were not always linear across the broad range of exposures examined. Specifically, inverse associations between TRAP and HRV were often stronger at lower concentrations and then moderated or turned positive at higher concentrations (Figures 1–3); this was particularly true for $PM_{2.5}$. Heart rate slightly although significantly varied across low and high-traffic sites in the rest protocol (Mean \pm S.D. = 70 ± 9 vs 74 ± 11 bpm, respectively; $p < 0.001$) and the exercise protocol (103 ± 19 vs 105 ± 18 bpm; $p = 0.045$).

UFPs and BC were associated with significant decreases in SDNN, RMSSD, LF, and HF at the low-traffic site whereas smaller inverse (or slightly positive) associations were observed at the high-traffic site. UFP-site interactions were not statistically significant for frequency-domain measures of HRV; analyses over the entire range of exposures (i.e. from both sites combined) for these outcomes suggested inverse associations with LF (-1.1%, 95% CI: -1.7, -0.41) and HF (-1.2%, 95% CI: -1.8, -0.56) and a small positive, non-significant association with LF:HF (0.15%, 95% CI: -0.41, 0.70). The interaction between black carbon and site was not statistically significant for LF:HF and a small positive association was observed for the dataset as a whole (0.14%, 95% CI: -1.2, 1.4). However, each $10 \mu\text{g}/\text{m}^3$ increase in black carbon exposure was associated with a 10% (95% CI: -0.17, 22) increase in LF:HF at the low-traffic site whereas a smaller inverse association was observed at the high traffic site. Moreover, in this same model, the impact of black carbon on LF:HF was statistically significant when noise was not included (percent change: 10%, 95% CI: 0.13, 22).

PM_{2.5} was associated with significant decreases in RMSSD and HF at the low-traffic site whereas positive associations were observed for SDNN, RMSSD and HF at the high-traffic site as the slopes of exposure-response relationships were positive at higher concentrations (Figure 1). PM_{2.5}-site interactions were not statistically significant for frequency-domain measures of HRV; when analyzed across the entire range of exposures PM_{2.5} was associated with non-significant decreases in LF (-0.53%, 95% CI: -2.1, 1.1) and HF (-0.67%, 95% CI: -2.2, 0.93) and a small positive change in LF:HF (0.15%, 95% CI: -1.2, 1.5).

While not all air pollution-site interactions were statistically significant, the directions and magnitudes of associations between TRAP and HRV were often different between sites and as a result all analyses related to effect modification by physical activity were conducted within sites. In general, limited evidence of effect modification by physical activity was observed for the low-traffic site, although associations between UFPs and BC and time-domain measures of HRV (SDNN and RMSSD) were stronger during physical activity (see Supplementary Table S2). In particular, UFPs (-5.5%, 95% CI: -10, -0.65) and BC (-19%, 95% CI: -31, -3.5) were associated with significant decreases in RMSSD during physical activity but not during rest and interactions were statistically significant ($p \leq 0.001$). Alternatively, physical activity at the high-traffic site tended to reduce the impact of TRAP on HRV (Figure 4). Specifically, UFPs, black carbon and PM_{2.5} were all associated with significant reductions in RMSSD during rest whereas no associations were observed during physical activity. A similar pattern was observed for HF, suggesting that physical activity may offset the impact of TRAP on parasympathetic modulation of the heart at higher exposure levels.

DISCUSSION

In this study we examined the impact of TRAP on acute changes in cardiac autonomic modulation during rest and physical activity. In general, our findings suggest that TRAP has an important impact on HRV and that these changes take place within minutes of exposure. Moreover, our findings suggest that the relationship between TRAP and HRV is not linear over a broad range of exposures and that sharper decreases occur on the lower end of the exposure spectrum with magnitudes decreasing or changing direction at higher exposure levels. Finally, to our knowledge this is the first study to specifically evaluate the impact of physical activity on the relationship between TRAP and HRV. Interestingly, our findings suggest that physical activity may offset the detrimental impact of TRAP on parasympathetic modulation of the heart at high exposure levels. A different effect was observed at lower exposures levels, however, with a stronger relationship observed between TRAP and RMSSD during physical activity. As few studies have specifically evaluated the impact of physical activity on the relationship between TRAP and HRV, these findings should be replicated in future studies. Nevertheless, our findings suggest that physical activity and the range of exposure levels should be considered when interpreting studies of TRAP and HRV as the magnitudes of observed associations may be sensitive to these factors.

A number of studies have observed inverse relationships between TRAP and HRV and our findings are consistent with these previous observations (4,5,11–13). More importantly, our study suggests that exposure-response relationships between the TRAP and HRV are not linear across a broad range of exposure. In particular, we noted that

inverse associations between TRAP and HRV were often stronger at lower concentrations and then moderated or turned positive at higher concentrations. This was particularly true for PM_{2.5} although evidence of non-linearity was also observed for UFPs and BC. The multi-centre ULTRA study found that equivalent increases in fine PM could be positively associated with HRV, but not in each of its sites with equivalent increases which suggests that effects may differ according to the sources of PM at a particular site (14). While findings have not been consistent in previous studies, a strong and consistent increase in HRV in association with fine PM (but not gaseous co-pollutants also measured) has been observed among healthy young men, suggesting increased vagal activity possibly due to a local inflammatory reaction in the lungs (15). As fine particles are not expected to penetrate as deeply as UFPs and BC, PM_{2.5} effects may be isolated from the cardiovascular and nervous systems, with this penetration potential difference more pronounced when performing physical activity.

Few other studies have specifically examined the shape of exposure-response relationships between TRAP and HRV, although Wu and associates (12) also noted a non-linear relationship between PM_{2.5} and HRV among taxi drivers in Beijing. Like this study, we observed evidence of a possible threshold with inverse associations between PM_{2.5} and HRV indices occurring at concentrations below approximately 50 µg/m³ and positive slopes observed above this concentration. Reasons for differences in exposure-response shapes between studies are not clear, but variation in particle composition, gas constituents of TRAP and/or population characteristics may play a role in explaining this heterogeneity. It should be recognised that gases and secondary organics of TRAP may also have effects (and amplify those of PM) on HRV at high levels, such as was seen with

NO_x in Chapel Hill among healthy, young adults (16). Unlike PM_{2.5} and BC, the exposure-response curve of UFP and HRV measures in general was quite linear. As these pollutants were all correlated it is possible that these curves reflect the impact of multiple rather than individual pollutants and thus to some extent reflect the TRAP mixture. The observation that PA performance at low and high-traffic sites affects the impact of TRAP on HRV measures may be due to different dose potentials, such as with UFP having a higher toxicity due to its greater penetration and deposition capability into the cardiovascular and nervous systems compared to PM_{2.5} and BC (17). That is, the overall dose of each pollutant may not increase equally with higher pulmonary ventilation rates, as seen previously for some pollutants such as ozone (5), however further research needs to explore this suggestion.

Increased pulmonary ventilation with physical activity leads to increased inhalation of air and therefore greater inhaled concentration (and potential dose) of TRAP (18). However, despite this possible increased dose, our findings suggest that physical activity may also offset the impact of TRAP on parasympathetic modulation of the heart at higher exposure levels. The underlying biological mechanisms explaining this finding are not entirely clear although laboratory experiments have shown that physical activity generally decreases HRV measures owing to increased sympathetic input (19). The common noise/stress hypothesis suggests that acute noise effects may occur when the performance of certain activities such as concentration or relaxation are disturbed (20). Thus, performing physical activity may decrease the impact of TRAP exposure on HRV by lowering the potential for disturbance through distraction compared to resting. The observation that physical activity decreased this impact more saliently in the high-traffic,

compared to the low-traffic, site may be due to the higher noise level experienced with more traffic and therefore the higher potential for effect modification (reduction) by exercise as a distraction. It should be noted that physical activity level, indicated by heart rate, slightly but statistically-significantly varied between exposure scenarios, possibly due to the known physiological stress response with higher noise exposure expected at the high compared to low traffic site (21). Further investigation is required to replicate these findings and characterize interactions between TRAP and physical activity and their combined impact on cardiac autonomic modulation; however, our findings suggest that activity level should be considered when interpreting the impact of TRAP on HRV. Moreover, it is important to note that physical activity has many important health benefits including decreased mortality risk (22). In addition, recent evidence suggests that the beneficial effect of physical activity on reducing mortality risk is attenuated by exposure to TRAP over the long-term (8).

In a subsidiary analysis we observed that adjusting TRAP models for noise generally did not affect associations with HRV outcomes. Noise itself has been found to elicit short-term stress responses in healthy adults, such as activation of the sympathetic division of the autonomic nervous system, reducing HRV (23). Additionally, noise has been seen to amplify the effect of TRAP on HRV, in that the effects of TRAP on HRV were stronger at higher compared to lower noise levels (11). One contributing factor to our null observation with noise may be the lack of correlation between it and TRAP (as our principal exposure) in this study; however, as mentioned previously, we will perform additional analyses in a separate study with noise as the principal exposure to investigate this further.

While our study had many important advantages including a large number of repeated measurements per subject during rest and physical activity, personal exposure measurements including noise, and a broad range of exposures, it is important to note several limitations. First, given the strong correlation between UFPs and BC it was difficult to estimate the individual effects of these pollutants. This was less of a concern for PM_{2.5}, but in general the coefficients presented likely reflect a broader mixture of “traffic pollution” as opposed to single pollutants. On the other hand, correlations between noise and TRAP were low and our findings suggest that the impact of TRAP on HRV was not confounded by noise. Secondly, respiratory/ventilation rate was not measured and thus we could not measure differences in inhaled doses of TRAP between low and high-traffic sites in this study. HR, however, was measured and did not indicate a large difference in effort between sites. Previous studies of HRV have not specifically measured respiratory or ventilation rate, however future work could consider this to allow extrapolation of capacity for accurate inhaled dose measurements. Our study did not address the commensurate electrophysiological or biochemical changes which, alongside HRV metrics, could also be modulated by TRAP and contribute to short-term cardiovascular risk; future work may specifically assess this to provide further insight into the previously observed associations between TRAP, exercise and clinical outcomes such as cardiac repolarisation or vascular dysfunction (13). On this note, our findings may only translate across to young, healthy individuals experiencing short-term exposures and not necessarily to individuals of older age or with chronic diseases such as diabetes and ischemic heart disease. Finally, as exposure-response functions were not linear across the broad range of exposures it was necessary to analyze models separately

for the low and high-traffic sites. This decreased the number of measurements available with strata and thus reduced statistical power to detect important relationships between TRAP and HRV as well as effect modification by physical activity. Nevertheless, we noted several important relationships in our analyses and to our knowledge this is the first study to examine how physical activity may impact the acute effects of TRAP on HRV.

In conclusion, exposure to TRAP has a rapid impact on cardiac autonomic modulation in healthy adults and the magnitude and direction of these changes is not linear across a broad range of exposures. More research is needed to better define the relationship between the levels of TRAP, physical activity and cardiac autonomic regulation. In general, however, inverse associations between TRAP and HRV were stronger at lower concentrations and moderated at higher exposures. Physical activity may modify the impact of TRAP on HRV, and in particular may offset the detrimental impact of TRAP on parasympathetic control of the heart at higher concentrations.

LIST OF ABBREVIATIONS

BC, black carbon; BMI, body mass index; BP, blood pressure; bpm, beats per minute; HF, high frequency power; HR, heart rate; HRV heart rate variability; LAeq, A-weighted decibels (dB) of sound pressure; LF, low frequency power; LF:HF, ratio of low to high frequency power; NO_x, nitrogen oxides (NO, nitrogen oxide; NO₂, nitrogen dioxide); PM_{2.5}, particulate matter ≤ 2.5 μm (fine); RMSSD, root mean square of successive differences in adjacent NN intervals; SDNN, standard deviation of normal to normal intervals; TRAP, traffic-related air pollution; UFP, ultrafine particles.

CONFLICT OF INTEREST

The authors declare that they have no actual or potential conflicting, financial or non-financial, interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to the reporting of the work described in this article: TCH, SW, MF, DM, DW and MN guided data analyses and interpretation; NK, GCT, AdN and MN oversaw the planning and conduct of the work.

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SUPPLEMENTARY INFORMATION

Supplementary information is available at Journal of Exposure Science and Environmental Epidemiology's website.

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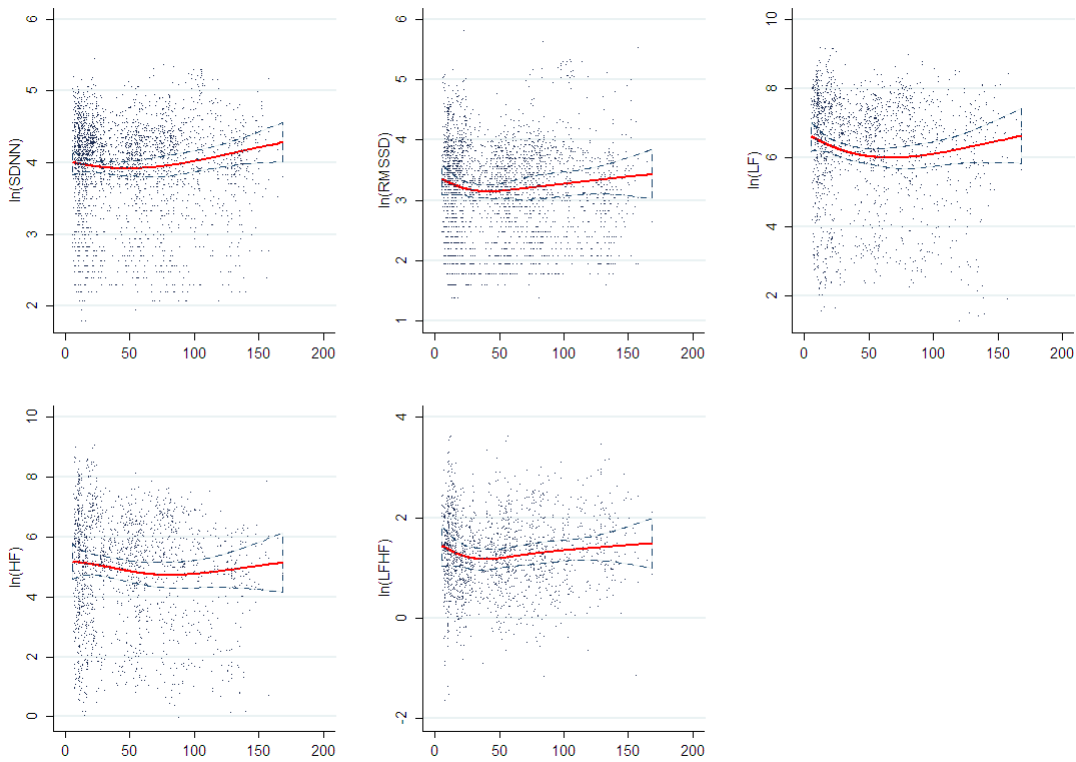
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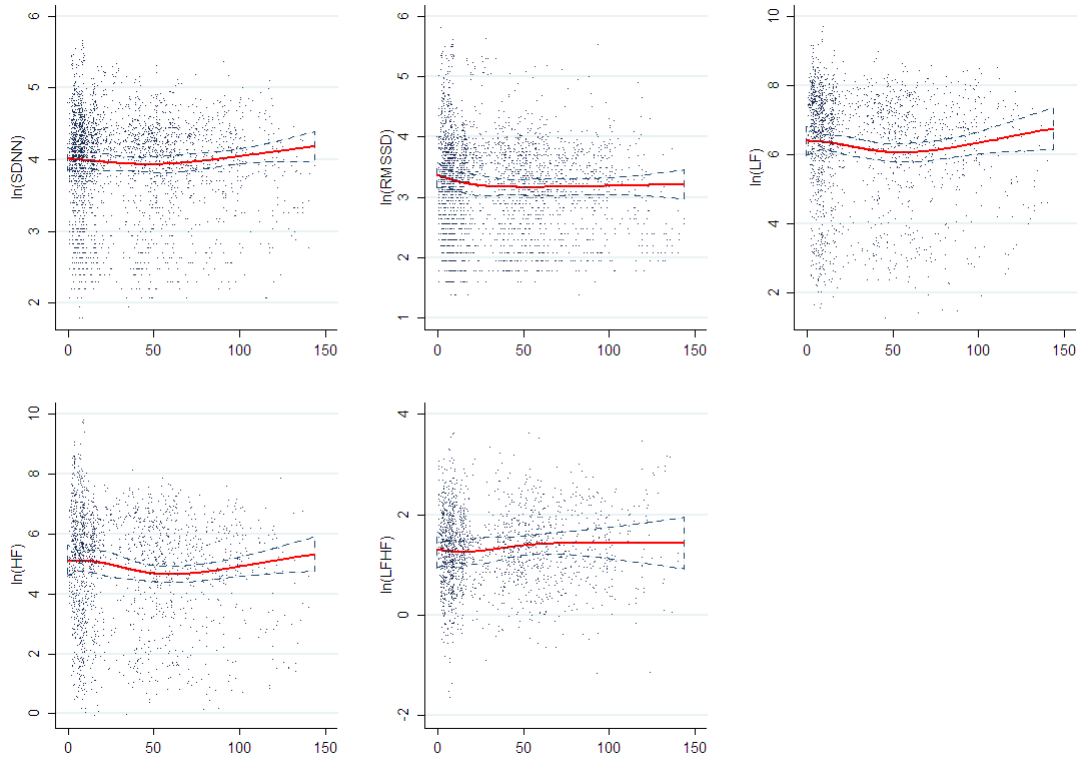
FIGURES

Figure 1. Exposure-response relationship between fine particulate matter concentration and heart rate variability measures



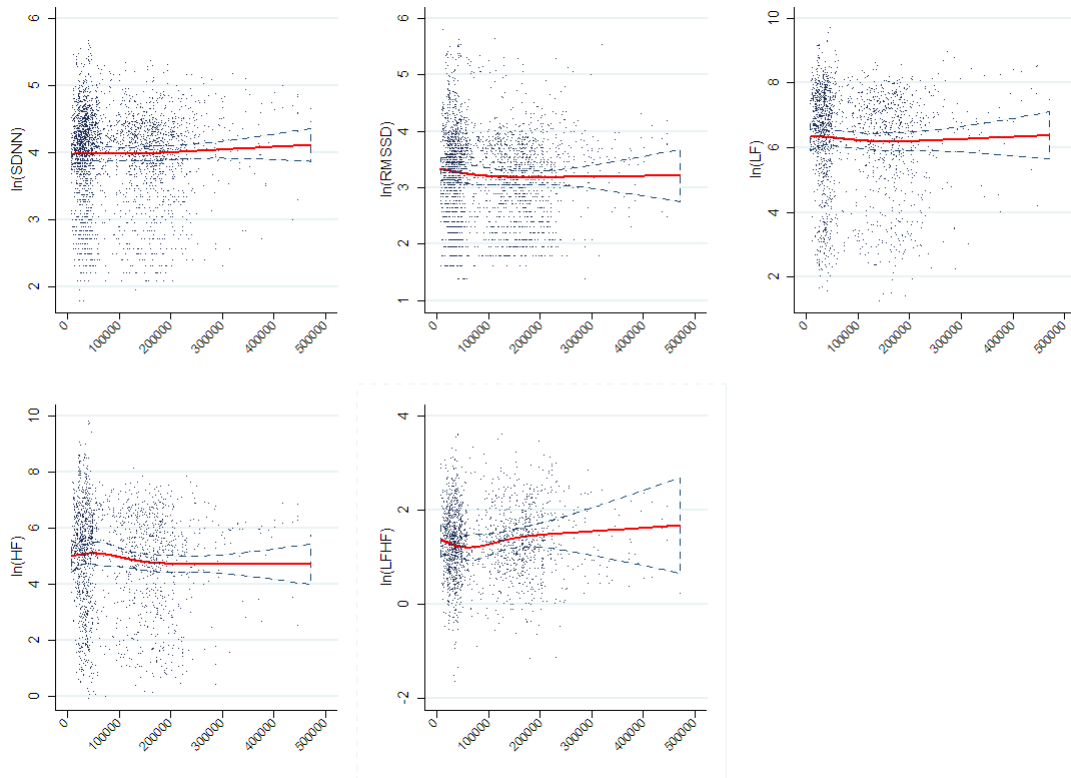
All linear mixed-effect models are adjusted for ambient temperature, noise, body mass index, and linear and quadratic terms for heart rate. Restricted cubic spline models were performed to illustrate the exposure-relationship between $PM_{2.5}$ ($\mu\text{g}/\text{m}^3$) and all heart rate variability measures, produced using the Stata function `mkspline2` with 4 knots at evenly spaced percentiles of exposure. Models with 3 knots were also examined but AIC and BIC criteria sometimes indicated better fit for models with 4 knots (data not shown).

Figure 2. Exposure-response relationship between black carbon concentration and heart rate variability measures



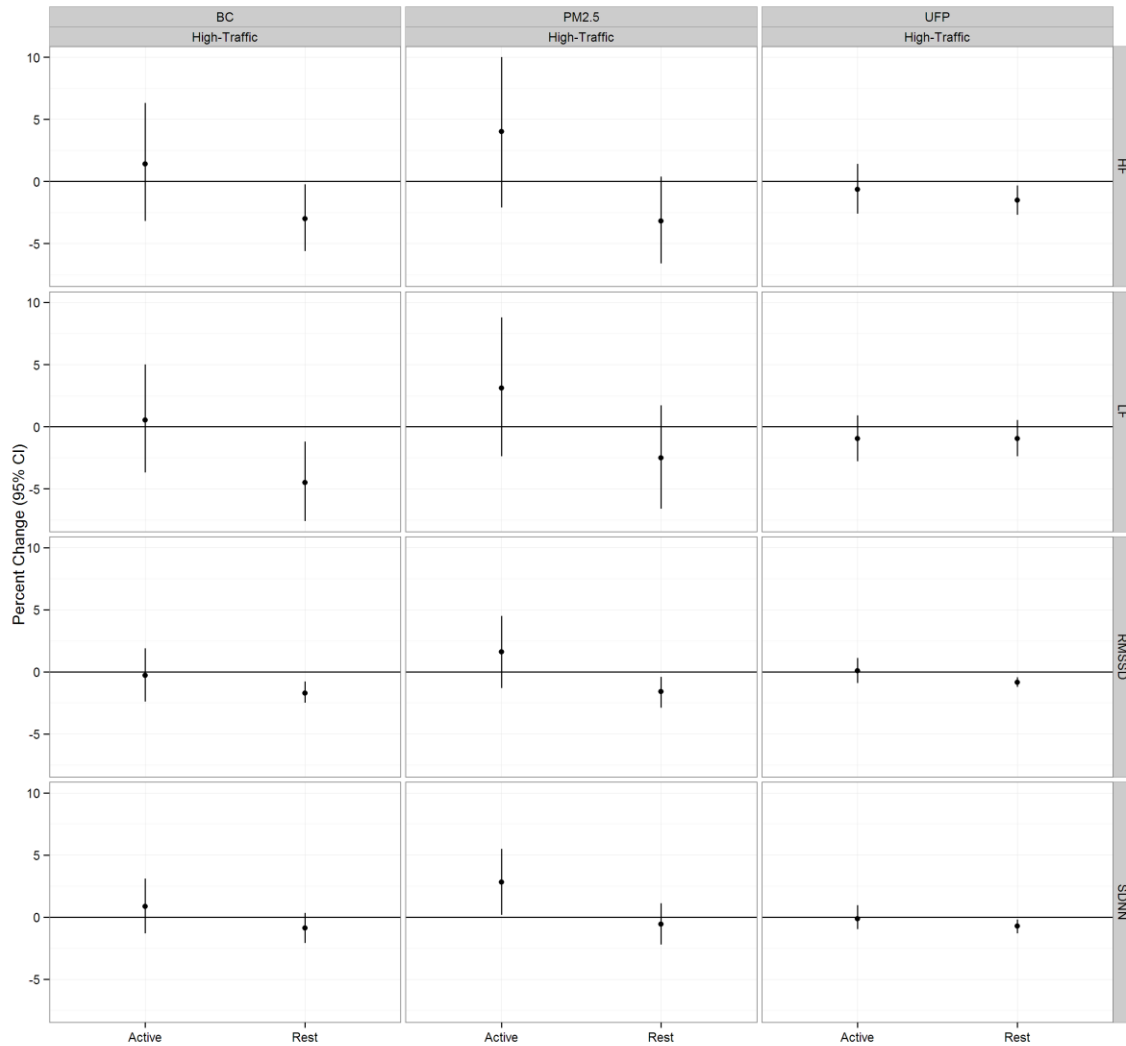
All linear mixed-effect models are adjusted for ambient temperature, noise, body mass index, and linear and quadratic terms for heart rate. Restricted cubic spline models were performed to illustrate the exposure-relationship between black carbon ($\mu\text{g}/\text{m}^3$) and all heart rate variability measures, produced using the Stata function `mkspline2` with 4 knots at evenly spaced percentiles of exposure. Models with 3 knots were also examined but AIC and BIC criteria sometimes indicated better fit for models with 4 knots (data not shown).

Figure 3. Exposure-response relationship between ultrafine particle concentration and heart rate variability measures



All linear mixed-effect models are adjusted for ambient temperature, noise, body mass index, and linear and quadratic terms for heart rate. Restricted cubic spline models were performed to illustrate the exposure-relationship between UFPs ($\text{particles}/\text{cm}^3$) and all heart rate variability measures, produced using the Stata function `mkspline2` with 4 knots at evenly spaced percentiles of exposure. Models with 3 knots were also examined but AIC and BIC criteria sometimes indicated better fit for models with 4 knots (data not shown).

Figure 4. Impact of physical activity on the relationship between traffic-related air pollution and heart rate variability



BC, black carbon; PM_{2.5}, particulate matter < 2.5 μm diameter; UFP, ultrafine particles; HF, high frequency power; LF, low frequency power; RMSSD, root mean square of successive differences in adjacent NN intervals; SDNN, standard deviation of normal to normal intervals.

All linear mixed-effect models are adjusted for body mass index, ambient temperature,

and linear and quadratic terms for heart rate. All coefficients reflect percent changes in HRV outcomes per 10 $\mu\text{g}/\text{m}^3$ of BC/PM_{2.5} or per 10 000/cm³ of UFP increases. Each air pollutant was evaluated in a separate model. Models were stratified according to the activity status of a given exposure period (i.e. active/rest {physical activity}). Models of the high-traffic site only are illustrated here; the directions and magnitudes of associations between traffic-related air pollution and heart rate variability were often different between sites, and in general, limited evidence of effect modification by physical activity was observed for the low-traffic site (see Supplementary Table S2).

TABLES

Table 1. Participant characteristics at baseline* (n=28)

Parameter	Mean (SD) / %	Range
Age (years)	34 (8)	(21 – 52)
Gender (% male)	54%	
BMI (kg/m ³)	23.1 (3.0)	(18.2 – 29.6)
Systolic BP (mm Hg) ^a	98.4 (10.4)	(78.7 – 121.3)
Diastolic BP (mm Hg) ^a	64.3 (8.4)	(50.7 – 88.0)
Heart Rate (bpm) ^a	67.7 (7.7)	(48.0 – 86.0)
Heart Rate Variability		
SDNN (ms)	80.4 (39.9)	(33 – 281)
RMSSD (ms)	45.7 (36.9)	(12 – 241)
LF (ms ²)	1 400 (2 072)	(0 – 15 375)
HF (ms ²)	781 (1 999)	(0 – 15 234)
LF:HF	4.01 (3.35)	(0.28 – 16.5)

BMI, body mass index; BP, blood pressure; SDNN, standard deviation of normal to normal intervals; RMSSD, root mean square of successive differences in adjacent NN intervals; LF, low frequency power; HF, high frequency power; LF:HF, ratio of low to high frequency power.

^a Based on all four study days

* Baseline measures of BP and heart rate variability were collected after a 15-minute period of rest in-clinic prior to leaving for the traffic exposure site.

Table 2. Descriptive Statistics for Traffic, Noise, and Traffic-Related Air Pollutants

Parameter	Total		Low traffic site		High traffic site	
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
<i>TRAP</i> ^a						
UFP ($\times 10^3/\text{cm}^3$)	98.3 (81.9)	57.6 (7.1–472)	32.6 (12.5)	32.4 (7.1–64.7)	167 (65.5)	161(54.5–472)
BC ($\mu\text{g}/\text{m}^3$)	34.8 (32.9)	19.4 (0–144)	8.59 (5.14)	7.84 (0–25.5)	62.2 (26.9)	56.9 (16.5–144)
PM _{2.5} ($\mu\text{g}/\text{m}^3$)	52.5 (39.8)	47.2 (5.08–168)	19.8 (12.8)	16.1 (5.08–74.8)	83.4 (31.1)	77.1 (28.8–168)
<i>Noise</i> ^a						
L _{Aeq} (dB)	59.5 (5.4)	59.8 (33.0–68.8)	55.1 (3.8)	55.5 (33.0–64.6)	64.3 (0.93)	64.2 (61.2–68.8)
<i>Meteorology</i> ^b						
Temperature (°C)	23.0 (4.4)	23.1 (13.1–35.3)	22.0 (3.8)	22.7 (14.1–32.2)	24.1 (4.73)	24.9 (13.1–35.3)
Humidity, Relative (%)	60.3 (11.8)	60.5 (29–91)	64.1 (11.3)	66.0 (38–91)	56.0 (10.9)	56.0 (29–88)
<i>Traffic Counts</i> ^c						
Motorbikes	9 (9)	6 (0–35)	1 (1)	1 (1–5)	16 (6)	15 (1–35)
Cars	41 (39)	9 (0–110)	4 (2)	4 (0–12)	80 (13)	81 (39–110)
Buses/Trucks	6 (6)	3 (0–24)	1 (1)	0.5 (0–5)	11 (4)	11 (3–24)

TRAP, traffic-related air pollution; UFP, ultrafine particles; BC, black carbon; PM_{2.5}, particulate matter < 2.5 μm diameter; L_{Aeq}, A-weighted decibels (dB) of sound pressure.

^a Based on 15-minute measurements; ^b Based on one-minute measurements (taken every 10 minutes); ^c Based on 5-minute measurements.

Table 3. Mixed-Effect Models for the relationship between traffic-related air pollution and HRV

Exposure	HRV outcome [Percent Change (95% CI)]									
	Time-domain				Frequency-domain					
	SDNN (ms)		RMSSD (ms)		LF (ms ²)		HF (ms ²)		LF:HF	
	Low Traffic site	High Traffic site	Low Traffic site	High Traffic site	Low Traffic site	High Traffic site	Low Traffic site	High Traffic site	Low Traffic site	High Traffic site
UFP ^a	-4.9 (-7.1, -2.7) p<0.001	-0.52 (-0.96, -0.08)	-6.8 (-8.7, -4.8) p<0.001	-0.79 (-1.2, -0.36)	-5.9 (-10, -1.1) p=0.091	-0.57 (-1.5, 0.41)	-7.0 (-11, -2.3) p=0.067	-0.74 (-1.7, 0.25)	1.0 (-3.1, 5.2) p=0.948	0.17 (-0.66, 1.0)
PM _{2.5} ^b	-1.4 (-3.7, 1.0) p=0.002	1.7 (0.52, 3.0)	-5.7 (-7.8, -3.5) p<0.001	1.8 (0.56, 3.0)	-2.3 (-7.3, 2.9) p=0.19	1.4 (-1.2, 4.1)	-5.1 (-9.9, -0.11) p=0.063	3.3 (0.61, 6.0)	2.9 (-1.6, 7.6) p=0.545	-1.8 (-3.9, 0.44)
BC ^b	-14 (-19, -9.1) p<0.001	-0.15 (-1.2, 0.94)	-19 (-23, -15) p<0.001	-1.6 (-2.6, -0.56)	-14 (-24, -3.8) p=0.005	0.17 (-2.1, 2.5)	-23 (-31, -13) p<0.001	1.6 (-0.66, 4.0)	10 (-0.17, 22) p=0.139	-1.3 (-3.2, 0.60)

^a Per 10 000/cm³; ^b Per 10 µg/m³

All models are adjusted for body mass index, ambient temperature, noise, and linear and quadratic terms for heart rate. P-values are for first order interaction terms between traffic site and a given air pollutant.