Reduced metabolic insulin sensitivity following sub-acute exposures to low levels of ambient fine particulate matter air pollution

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HIGHLIGHTS

► Sub-acute exposure to ambient $PM_{2.5}$ was associated with a worsening of metabolic insulin sensitivity.
► The findings suggest that autonomic imbalance may be a mechanism responsible for PM-induced insulin resistance.
► Sub-acute $PM_{2.5}$ exposure did not promote systemic inflammation, an increase in blood pressure or alter vascular function.
► These findings support the plausibility that $PM_{2.5}$ could potentiate the development of diabetes mellitus.

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ABSTRACT

Epidemiological studies suggest that fine particulate matter ($PM_{2.5}$) may increase the risk for developing diabetes mellitus (DM). To evaluate possible mechanisms explaining these associations, we investigated if sub-acute ambient-level exposures can impair insulin sensitivity. Twenty-five healthy adults living in rural Michigan were transported to an urban location for 5 consecutive days (exposure-block) of daily 4- to 5-hour-long ambient air pollution exposures. Health outcomes, including the homeostasis model assessment of insulin resistance (HOMA-IR) the primary outcome of insulin sensitivity, were measured at 3 time points in relation to exposure-blocks: 7 days prior to start; on the last exposure-day; and 7 days after completion. $PM_{2.5}$ was monitored at the urban exposure site and at community monitors near subjects' residences. We calculated 3 "sub-acute" exposure periods (approximately 5-days-long) starting retrospective from the time of health outcome measurements ($PM_{2.5}$ ranges: 9.7 ± 3.9 to 11.2 ± 3.9 $\mu g \cdot m^{-3}$). A 10 $\mu g \cdot m^{-3}$ increase in sub-acute $PM_{2.5}$ exposures was associated with increased HOMA-IR ($+0.7$, 95% confidence interval (CI) 0.1 to 1.3; $p = 0.023$) and reduced heart rate variability (standard deviation of normal-to-normal intervals [-13.1 ms, 95%CI -25.3 to -0.9; $p = 0.035$]). No alterations in other outcomes (inflammatory markers, vascular function) occurred in relation to $PM_{2.5}$ exposures. Our findings suggest that ambient $PM_{2.5}$, even at low levels, may reduce metabolic insulin sensitivity supporting the plausibility that air pollution could potentiate the development of DM.

1. Introduction

Exposure to fine particulate matter <2.5 μm in diameter ($PM_{2.5}$) air pollution is associated with increased cardiovascular (CV) morbidity

and mortality (Brook et al., 2010). Numerous experiments have elucidated biological mechanisms whereby brief exposures could trigger CV events (Brook et al., 2010). Additional studies have also begun to illustrate how longer-term exposures could further increase CV risk, such as by the promotion of atherosclerosis and hypertension (Brook and Rajagopalan, 2009, 2010). In this regard, we have recently demonstrated a novel adverse health effect of long-term exposures. In an experimental animal model of diet-induced obesity, $PM_{2.5}$ was shown to potentiate metabolic insulin resistance (Sun et al., 2009). In this construct, adverse systemic responses such as inflammation, oxidative stress, autonomic imbalance and endothelial dysfunction may represent "a common soil" that promotes the development of insulin

resistance contemporaneously with a pro-atherogenic state. Indeed, a few epidemiological studies suggest that there may be an association between air pollutants and diabetes mellitus (DM) (Brook et al., 2008; Chuang et al., 2010; Kelishadi et al., 2009; Krämer et al., 2010; Pearson et al., 2010; Puett et al., 2011).

DM is a growing worldwide epidemic promoted by many aspects of modern society (Cornier et al., 2008). We have speculated that an under-recognized societal risk factor may be air pollution (Brook and Rajagopalan, 2009). Given the billions of people continuously exposed and the rapid industrial/urban growth among developing nations (Narayan et al., 2010), even a modest causal-association would be of major public health importance (Nawrot et al., 2011). Hence, our aim was to evaluate mechanisms potentially explaining this relationship in humans by investigating if commonly-encountered $PM_{2.5}$ levels are capable of reducing metabolic insulin sensitivity, which is fundamentally involved in the genesis of DM. We designed this study to discern the impact of 5-day-long cumulative exposures given that slowly changing metabolic parameters of insulin sensitivity (i.e. the homeostasis model assessment of insulin resistance (HOMA-IR)) are more likely to be altered within a subject over this "sub-acute" period (Kelishadi et al., 2009). In addition, we sought to explore several underlying mechanisms potentially involved in the putative instigation of metabolic insulin resistance (i.e. systemic inflammation, autonomic imbalance, endothelial dysfunction).

2. Materials and methods

We enrolled 25 healthy (age 18–50 years) non-smoking subjects from non-smoking households without known CV disease or risk factors (blood pressure < 140/90 mm Hg; fasting glucose < 126 mg·dl^{-1}; without diagnosis or treatment for hyperlipidemia during screening visits). Subjects with impaired fasting glucose (100–125 mg·dl^{-1}) could be included. Subjects were not taking any known prescription (e.g. statin, anti-hypertensives) or over-the-counter drugs (e.g. anti-oxidants, fish oil) that could alter CV function. The study was approved by the Institutional Review Board of the University of Michigan and each subject provided written informed consent.

2.1. Study outline

Fig. 1 outlines the details of the study that took place during summer months only (June–August) in 2009 and 2010. The primary aim of this study was to investigate if "sub-acute" exposures (defined as 3 separate integrated approximately 5-day-long periods) to ambient $PM_{2.5}$ are associated with HOMA-IR. Other parameters were measured as secondary outcomes. For the purposes of this study, the

rationale for the daily transporting of subjects to an urban location during the exposure block period was to enhance within-subject variability in sub-acute exposure $PM_{2.5}$ levels. In addition, it was anticipated that this would also alter the characteristics of exposures. The effects of specific PM components will be evaluated in subsequent analyses and is beyond the scope of this first paper focusing on $PM_{2.5}$ mass alone.

Each subject lived in a location (within 20 kilometers (km) of Dexter or Tecumseh, Michigan PM monitoring sites and > 400 meters (m) from a major highway) that typically exhibits background levels of $PM_{2.5}$ from 5 to 10 μg·m^{-3} lower than the selected urban exposure site (USEPA, website). Subjects were instructed to remain within the region of their residence throughout the study periods. During the "exposure-block" period they were transported for 5 consecutive days (Monday–Friday) to an urban site located in Dearborn Michigan with $PM_{2.5}$ levels among the highest in the state (Oakes et al., 2010). Each day subjects rested at the same spot (under an open-air shelter if needed) for 4 to 5 h. Subjects were instructed to remain seated in a chair for the exposure time; however, for brief periods they were permitted to have a low level of activity (e.g., walk to restroom, stand to stretch). Exposures began between 9 and 10 am and ended at 2 pm each day. Subjects were driven to and from this location by a research assistant. During the 1 hour-long transportation each way subjects wore a properly fitted N-95 face mask (3M™ model 1860). A similar mask has recently proven effective in removing almost all PM down to a few nanometers during exposure testing to diesel and traffic-related PM (Langrish et al., 2009). Subjects wore the facemask during car travel because it would not be possible to accurately assign a PM exposure during this period without personal monitors; hence we could assign a zero level PM exposure uniformly during travel to all subjects when they wore the mask. On the final day (Friday) of the exposure-block period, subjects ate breakfast at 8 am but remained fasting until completion of the day (~7 h). On this day subjects were transported to the University of Michigan Research Vascular Laboratory (UMRVL), where they had all the health outcomes and blood drawn starting at 3 pm. On the previous Monday (7 days prior to undergoing the exposure-block) and on the following Friday (7 days after completing the exposure-block) subjects also had health outcomes and blood drawn at the UMRVL starting at 3 pm. They were told to remain fasting (~7 h) on each of those days after eating similar breakfasts at 8 am.

2.2. Exposure measurement and assignment methods

The exposure assignment method for each subject over the 3 time periods is outlined in Fig. 1. Details regarding $PM_{2.5}$ measurement

	"Pre-exposure block" sub-acute period											"Exposure-block" sub-acute period							"Post-exposure block" sub-acute period				
Study day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	TH	F	SA	SU	M	TU	W	TH	F	SA	SU	M	TU	W	TH	F	SA	SU	M	TU	W	TH	F
Exposure	L†	L	L	L	L*							C†	C	C	C	C*			L†	L	L	L	L*
Outcomes measured					Visit #1											Visit #2							Visit #3

*Exposure estimates end at 2 pm (1 hr prior to health outcome measurement)

†Exposure estimates start at midnight
L=Local residence monitor levels used (closer of Dexter or Tecumseh sites to their house)

C=Combined Dearborn + local residence monitor levels used
  Each day is subjects' time-averaged exposures comprised of :
    Dearborn exposure (9 or 10 am to 2 pm; per subject's time spent at the site)
    1 hour before/after Dearborn exposures (wearing N95 facemask= 0 exposure to $PM_{2.5}$ assigned for these periods.

Local monitor used from 8 or 9 am back until 3 pm previous day (except day #12: starts at midnight)

Fig. 1. Outline of the study design. Overview of the study flow and of the sub-acute exposure estimate methods.

methods and aspects of the 3 exposure monitoring site locations are provided in the online supplement methods.

2.3. Health outcomes evaluated

All health outcomes assessments were performed on 3 separate visits (Fig. 1; study days: 5, 16, 23) at the UMRVL starting at 3 pm. Subjects were instructed to fast for approximately 7 h (since 8 am) on each day. The order of testing was the same for each patient and all visits.

Subjects had automated seated blood pressure and heart rate measured (Omron 780) in triplicate on their right arm resting at heart level. After resting supine for 10–15 min, subjects had 6 min of resting supine heart rate variability recorded and analyzed for the standard deviation of normal-to-normal intervals (SDNN) as well as frequency domain metrics (evo Holter monitor; Pathfinder software; Spacelabs). Afterward, right radial artery tonometry pulse wave analyses for aortic hemodynamic profiles followed by arterial pulse wave velocity for arterial compliance were performed (SyphgmoCor; AtCor Medical). Microvascular endothelial function and nitroglycerin-mediated dilatation were next performed on the right hand using the EndoPAT2000 (Itamar Medical). Further details of the individual protocols are provided in the online supplement methods.

Blood outcomes were drawn last. Insulin and glucose values were drawn in triplicate (5 minute intervals) per optimal HOMA-IR protocol (Wallace et al., 2004; Muniyappa et al., 2008). Along with adiponectin, these blood aliquots were stored in −70 °C freezer for batched analyses at the Michigan Diabetes Research and Training Center Laboratory (http://www.med.umich.edu/mdrtc/cores/ChemCore/index.html). HOMA-IR was calculated from the average of the 3 measurements at each time period using the following formula: $[\text{glucose } (\text{mg} \cdot \text{dl}^{-1}) \times \text{insulin } (\mu\text{U} \cdot \text{ml}^{-1})]/405$. Lower values correspond to greater insulin sensitivity. HOMA-IR is a validated metric of insulin sensitivity used in repeated measures studies to demonstrate changes across time periods within individuals (Wallace et al., 2004; Muniyappa et al., 2008).

All other aliquots of blood were sent to the laboratory of Dr. Rajagopalan at Ohio State University for analyses of inflammatory markers. These included sub-types of peripheral blood mononuclear cells (PBMC) and an array of circulating blood cytokine levels. Details regarding these methods are outlined in the online supplement methods.

2.4. Statistical methods

Data are presented as mean ± standard deviation unless otherwise stated. Comparisons across the mean values from the 3 time periods in Table 1 were performed by repeated measures analysis of variance. Models for determining the overall associations between $PM_{2.5}$ exposure across the 3 periods and each temporally-corresponding health outcomes (including HOMA-IR as the primary outcome) were performed using generalized estimating equations (GEE) to account for the within-subject correlation due to repeated measures. The best fit GEE model was selected based on quasilikelihood independence criteria. Models were performed unadjusted and secondarily adjusted for a few predetermined parameters that alter HOMA-IR (body mass index, age, sex) given the limited sample size. We started with adding BMI and age and excluded either variable if it was individually non-significant ($p > 0.1$) to provide the most parsimonious model.

3. Results

We enrolled a total of 25 subjects (14 subjects during 2009 and 11 during 2010). The mean age and body mass index (BMI) were 38 ± 12 years and $25.7 \pm 4.5 \text{ kg} \cdot \text{m}^{-2}$, respectively. The cohort consisted of 17 females and 8 males. Other subject-related parameters are presented in Table 1 with the mean results from each of the 3 separate weeks presented separately. The average exposure duration each day at the Dearborn urban site was 239.5 ± 2.3 and 300.4 ± 0.3 min for summers 1 and 2, respectively. The mean $PM_{2.5}$ level that occurred during just the 4–5 hour-long acute exposures at the Dearborn urban site was $11.5 \pm 4.8 \ \mu\text{g} \cdot \text{m}^{-3}$. The $PM_{2.5}$ values for the 3 sub-acute integrated 5-day-long exposure periods are presented in Fig. 2.

Table 1 presents the results of the major health outcomes measured at the end of the 3 separate visit periods. There were no significant differences in microvascular endothelial function (reactive hyperemia index), blood pressures, aortic hemodynamics, arterial stiffness (pulse wave velocity), and heart rate variability metrics between the 3 time points. However, glucose and HOMA-IR were significantly lower during the exposure-block week visit when $PM_{2.5}$ levels also trended lower (lower HOMA-IR implies greater insulin sensitivity). Among all other CV endpoints and biomarkers measured, the only significant differences were found for interleukin (IL)-6, IL-1β, and IL-8 levels that were actually higher during the exposure-block week when

Table 1
Cardio-metabolic health outcomes measured among 25 Michigan residents in relation to fine particulate matter air pollution exposures in an urban environment.

	Pre-exposure block period (visit 1: study day #5)	Exposure block period (visit 2: study day #16)	Post-exposure block period (visit 3: study day #23)	p[a]
Microvascular function				
Reactive hyperemia index (RHI)	2.20 ± 0.72	2.05 ± 0.43	1.85 ± 0.40	0.14
Arterial compliance and hemodynamics				
Brachial systolic blood pressure (mm Hg)	116 ± 13	118 ± 14	120 ± 14	0.13
Brachial diastolic blood pressure (mm Hg)	75 ± 10	76 ± 9	77 ± 9	0.17
Heart rate (beats·min^{-1})	72 ± 12	68 ± 10	72 ± 11	0.08
Central aortic systolic blood pressure (mm Hg)	106.9 ± 12.6	107.5 ± 13.3	109.1 ± 14.5	0.25
Augmentation index at HR 75 (%)	13.8 ± 13.5	14.1 ± 11.2	14.6 ± 10.7	0.83
Pulse wave velocity (m·s^{-1})	6.7 ± 1.3	6.5 ± 1.3	6.7 ± 1.4	0.96
Heart rate variability				
Standard deviation of normal-to-normal beats (SDNN) (ms)	57.3 ± 34.6	65.1 ± 29.2	61.1 ± 29.7	0.30
High frequency peak (ms^2)	1217 ± 2274	1544 ± 3762	1225 ± 2014	0.73
Low frequency peak (ms^2)	996 ± 1420	1168 ± 1364	1120 ± 1020	0.71
Low/high frequency ratio	2.1 ± 2.2	2.4 ± 2.6	2.2 ± 2.2	0.38
Metabolic blood biomarkers				
Glucose (mg·dl^{-1})[b]	84.0 ± 8.0, n = 24	74.3 ± 6.3, n = 23	78.5 ± 7.1, n = 23	0.001
Insulin (μU·ml^{-1})[b]	15.1 ± 5.8, n = 24	12.5 ± 4.6, n = 24	15.1 ± 6.4, n = 24	0.08
Homeostasis model assessment of insulin resistance (HOMA-IR)[b]	3.26 ± 1.53, n = 24	2.39 ± 0.98, n = 24	2.79 ± 1.24, n = 23	0.05
Adiponectin (ng·ml^{-1})	7021 ± 3539	6652 ± 4082	6705 ± 3274	0.46

All data are presented as mean ± SD, n = 25.

[a] p value represents significance of differences between the three measures determined using a general linear model repeated measures analysis of variance.

[b] Data < 25 samples in Table 1 values represent the results excluding extreme outliers for insulin and glucose (changes > 2 SD from mean probably representing incomplete fasting state).

HOMA-IR was lower. (Online supplement: Supplement Table 1.) All additional outcomes outlined in the Materials and methods section are available in the online supplement.

Table 2 presents the sum results for all of the significant associations between the sub-acute $PM_{2.5}$ exposure levels (all 3 time periods together) and the temporally-corresponding health outcomes. A $10~\mu g \cdot m^{-3}$ increase in sub-acute 5-day $PM_{2.5}$ level was associated with a reduction in SDNN, consistent with lower overall heart rate variability primarily due to reduced parasympathetic activity (adjusted for age and body mass index), as well as an increase in insulin and HOMA-IR (adjusted and unadjusted models). This means that higher pollution levels were related to lower insulin sensitivity. For an approximate 1 standard deviation change in sub-acute $PM_{2.5}$ exposure level ($3.5~\mu g \cdot m^{-3}$), the adjusted HOMA-IR would increase by 0.25 (95% CI 0.04–0.46). No other CV outcome or blood biomarker (cytokines, PBMC) beyond HOMA-IR and SDNN was associated with the 5-day $PM_{2.5}$ exposure levels (Table 2).

SDNN was also associated with changes in HOMA-IR across all 3 time periods ($\beta = -0.13$ per 10 ms decrease, $p = 0.035$), suggesting that reduced overall heart rate variability (principally lower parasympathetic activity) was related to lower insulin sensitivity. When SDNN and 5-day $PM_{2.5}$ were jointly considered as predictors of HOMA-IR in unadjusted models, SDNN was significant ($\beta = -0.14$ per 10 ms increase, $p = 0.022$) while the effect of 5-day $PM_{2.5}$ level was reduced ($\beta = 0.64$ per $10~\mu g \cdot m^{-3}$ increase, $p = 0.063$). Hence, a portion of the effect of $PM_{2.5}$ on HOMA-IR was possibly mediated via changes in SDNN. Importantly, $PM_{2.5}$ levels during the post-exposure period time window alone were significantly related to HOMA-IR values measured at the corresponding 3rd time point visit ($\beta = 0.43$ per $10~\mu g \cdot m^{-3}$ increase, $p = 0.03$). Exposure levels during period 2 (the exposure block period alone) showed a strong similar trend of an association with HOMA-IR values ($\beta = 0.28$ per $10~\mu g \cdot m^{-3}$ increase, $p = 0.19$). There were no other significant associations between any other health outcomes (e.g. vascular and inflammatory mediators) and sub-acute exposures or HOMA-IR changes.

4. Discussion

Exposures to $PM_{2.5}$ over sub-acute periods even at relatively low concentrations were associated with reduced metabolic insulin sensitivity among healthy adults. This is the first study to show that this occurs even in relation to $PM_{2.5}$ levels meeting daily ($<35~\mu g \cdot m^{-3}$) U.S.
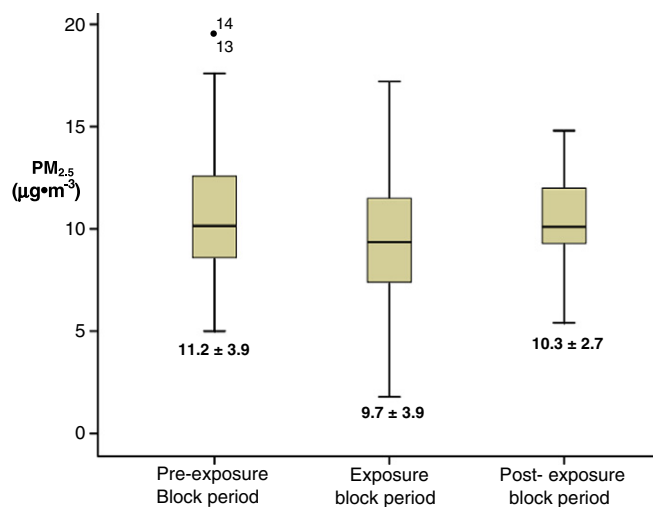

Fig. 2. Time-averaged fine particulate matter levels during 5-day periods prior to each outcome measurement. Box and whiskers represent the inter-quartile ranges and 2 standard deviations of $PM_{2.5}$ ($\mu g \cdot m^{-3}$), respectively. Lines in the box are medians and text data in the figure are the means and standard deviations. Individual points represent outliers with individual patient numerical identifier.

Table 2
Associations between cardio-metabolic parameters and sub-acute fine particulate matter air pollution exposures measured among 25 Michigan residents.

Outcome	β[a]	95% CI	p	β[b]	95% CI	p
SDNN (ms)	−11.60	−24.0 to 0.9	0.070	−13.1	−25.3 to −0.9	0.035
Glucose ($mg \cdot dl^{-1}$)	4.6	−1.0 to 10.3	0.109	5.4	0.5 to 10.3	0.029
Insulin ($\mu U \cdot ml^{-1}$)	3.1	0.2 to 6.1	0.036	2.9	0.2 to 5.6	0.034
HOMA-IR	0.8	0.11 to 1.5	0.024	0.7	0.1 to 1.3	0.023

Associations of outcomes per $10~\mu g \cdot m^{-3}$ increase in $PM_{2.5}$ level.
No changes in GEE associations occurred between HOMA-IR and $PM_{2.5}$ exposures whether outlying values for insulin or glucose (thus calculated HOMA-IR) were included or excluded (per Table 1). The association results presented in Table 2 include all values.
95%CI, 95% confidence interval and SDNN, standard deviation of normal-to-normal beats.
[a] β estimate from the GEE (unadjusted).
[b] β estimate from the GEE adjusted for age + body mass index (SNDD); age (glucose); and body mass index (insulin, HOMA-IR). The parameters included in the adjusted models were pre-selected as stated in the Materials and methods section and thereafter included because they showed a trend for associations with the health outcome of interest and/or modified the association with exposure. Given the limited sample size (n = 25) we adjusted for only up to 2 variables in a model. No major outcome differences were found adjusting for other factors.

National Ambient Air Quality Standards (NAAQS) (Brook et al., 2010). These results support the emerging evidence that $PM_{2.5}$ might contribute to the risk for developing DM (Brook et al., 2008; Chuang et al., 2010; Kelishadi et al., 2009; Krämer et al., 2010; Pearson et al., 2010; Puett et al., 2011), given the fundamental role of insulin resistance in its etiology (Cornier et al., 2008). Even if the physiological effect is small compared to traditional risk factors (e.g. obesity), since billions of people are continuously exposed (Narayan et al., 2010) this health effect has the potential to translate into significant population-attributable risks for DM (Nawrot et al., 2011).

4.1. Previous studies

Few epidemiological studies have explored the relationship between air pollutants and DM (Brook et al., 2008; Chuang et al., 2010; Kelishadi et al., 2009; Krämer et al., 2010; Pearson et al., 2010; Puett et al., 2011). We demonstrated that NO_2, a pollutant often associated with traffic pollution, was associated with DM among women in Ontario (Brook et al., 2008). Studies from Germany (traffic pollutants) (Krämer et al., 2010) and a large cross-sectional analysis of DM prevalence in the U.S. ($PM_{2.5}$ levels) (Pearson et al., 2010) also supported a positive association. On the other hand, another recent study reported mixed results and less consistent relationships (Puett et al., 2011).

Given the small number of studies and mixed results, the presence of a true biological relationship could be more strongly supported by the demonstration of an accompanying mechanistic basis. Only 2 studies have evaluated metrics of insulin sensitivity in relation to PM exposures in humans (Kelishadi et al., 2009; Chuang et al., 2010). In Taiwan, elevations in annual $PM_{2.5}$ levels (mean $35.3 \pm 15.9~\mu g \cdot m^{-3}$) were related to increases in glucose and hemoglobin-A1c (Chuang et al., 2010). Among adolescents living in Isfahan Iran, very high weekly PM_{10} concentrations (mean $111–157~\mu g \cdot m^{-3}$) were positively associated with HOMA-IR levels (Kelishadi et al., 2009). Though perhaps of importance for developing nations, these concentrations are almost never encountered in the U.S. (4–10 fold higher than typical daily levels) (Brook et al., 2010). These 2 reports showing adverse alterations in HOMA-IR therefore do not directly support the epidemiological associations between PM and DM found in Europe and the U.S. (Brook et al., 2008; Krämer et al., 2010; Pearson et al., 2010; Puett et al., 2011) given that the PM levels were extremely high. Our findings substantively add to these 2 previous papers by demonstrating that $PM_{2.5}$, even low

concentrations meeting NAAQS and encountered by billions of people, are still associated with adverse perturbations in insulin sensitivity. Moreover, our study was designed to specifically investigate the association between $PM_{2.5}$ and HOMA-IR.

4.2. Potential mechanisms

Pathways whereby $PM_{2.5}$ could potentially instigate insulin resistance include the consequences of systemic inflammation (cellular- or cytokine-mediated), a hypothalamic–adrenal stress response, tissue-level oxidative stress altering insulin signaling cascades, direct actions on insulin signaling by pollutant constituents reaching the circulation, reduced nitric oxide bioavailability, blunted tissue perfusion due to endothelial dysfunction or vasoconstriction, altered high density lipoprotein particle function, and/or relative augmentation of sympathetic activity (Brook et al., 2010; Brook and Rajagopalan, 2009, 2010). At present, the precise mechanism for our findings must remain speculative. Nonetheless, we have previously demonstrated in an animal model an adverse effect of $PM_{2.5}$ exposure over several weeks on insulin sensitivity by promoting systemic and cellular inflammatory responses in tissues such as the visceral adipose cells (Sun et al., 2009). In addition to direct actions of cytokines (Cornier et al., 2008), inflammatory mechanisms may also involve activation of pattern recognition receptors such as Toll-like receptor 4 by PM constituents (e.g. LPS) or oxidized fatty acids/phospholipids (Kampfrath et al., 2011; Shi et al., 2006). However, all the circulating inflammatory markers or modulators we evaluated (monocyte sub-types, cytokines, and adiponectin) were not related to $PM_{2.5}$ exposures or changes in HOMA-IR. While a role for other mediators or longer durations of exposures cannot be excluded, this does not support systemic inflammation as the likely etiology for our findings.

The duration of this study was abbreviated compared to the chronic exposures in our animal studies (Sun et al., 2009). This suggests that alternative biological mechanisms capable of more quickly altering insulin signaling might be involved. The lack of effect of PM exposures on endothelial function argues against an important mechanistic role played by impaired nitric oxide signaling or vasoconstrictive changes. It remains possible that the methodology utilized did not capture the endothelial or hemodynamic changes relevant to perturbations in whole-body insulin sensitivity (i.e. perfusion of large skeletal muscle beds) (Cornier et al., 2008). On the other hand, changes in SDNN (i.e. reduced overall heart rate variability primarily reflecting lower parasympathetic nervous system activity) across the 3 time periods were inversely related to insulin sensitivity. Sub-acute $PM_{2.5}$ exposure was concomitantly associated with a decrease in SDNN. In a multivariate model, the statistical association between sub-acute $PM_{2.5}$ levels and HOMA-IR was partially explained by lower SDNN. The results suggest that air pollution-mediated parasympathetic withdrawal (i.e. autonomic imbalance favoring sympathetic activity) may be a contributing mechanism to the rapid worsening of insulin sensitivity. Indeed, alterations in autonomic balance are well-known to mediate changes in insulin sensitivity (Cornier et al., 2008; Egan, 2003). However, we cannot exclude the possibility that the changes in HOMA-IR and SDNN occurred in parallel and were not mechanistically inter-linked. Further studies will be needed to better elucidate the mechanisms whereby $PM_{2.5}$ could alter metabolic insulin sensitivity in humans. Employing more sophisticated measures of insulin sensitivity (e.g. glucose clamp, glucose tolerance tests) could more firmly corroborate our initial findings and better establish a hepatic versus muscle source of the metabolic derangement.

4.3. Strengths and limitations

While many panel studies have reported the health effects of different exposure lag periods during post hoc analyses, we believe this to be the first protocol that was a priori designed to investigate the effect of an integrated cumulative 5 day-long exposure to ambient $PM_{2.5}$ on CV responses and insulin sensitivity. Hence, this represents one of the first attempts to evaluate the physiological effects of "sub-acute" periods of exposure. The reason for our selection of this sub-acute period was that changes in HOMA-IR were thought to likely require several days to occur in response to higher PM exposures.

Mean sub-acute $PM_{2.5}$ levels were slightly non-significantly lower during the exposure-block period. This occurred due to lower regional levels near the subjects' residences due to temporal variances (these integrated averages were heavily influenced by the 17 to 18 h per day of local regional monitor concentrations) and as an effect of the zero level of exposure while wearing the facemask for 2 h/day. Regardless, this had no consequence upon our primary aim or statistical methods which was to evaluate the effect of 3 pooled sub-acute time periods of $PM_{2.5}$ exposures upon insulin sensitivity. The travel to the urban location was a method employed in this study design solely to foster greater within-subject variability in PM exposures across the 3 time periods. Moreover, $PM_{2.5}$ levels measured during the post-exposure period alone were significantly associated with HOMA-IR. These secondary observations add internal validity and support for the authenticity of the overall study findings.

The present paper does not identify the $PM_{2.5}$ components and sources responsible for the observed effects; though detailed data in this regard and other pollutants (e.g. ozone) were collected as part of the study. These data will be used in future analyses to investigate the role of PM composition and interactions with other co-pollutant on outcomes. Interestingly, several recent studies have reported on this issue; for example, a recent animal inhalation study in a nearby exposure site location in Detroit indicated that modulation of cardiac function was most strongly linked to local industrial sources including iron/steel manufacturing (Rohr et al., 2011). Similarly, determining if there are factors altering subjects' susceptibilities (e.g. obesity) will require future studies. Details regarding the time course, the dose–response, any interaction with other time-varying parameters (e.g. personal-level ambient temperature), and the effect of other particle sizes (coarse $PM_{10-2.5}$) will also require further experiments.

We cannot exclude the possibility for exposure estimation errors which is an inherent limitation to all panel studies. We believe that there is no evidence for a systematic flaw given that levels during the post-exposure period alone were significantly associated with HOMA-IR. It is also possible that particle constituent differences between the exposure periods, particularly at the urban site, may have had additional unaccounted for effects upon health outcomes including HOMA-IR. Future examination of the same-day health effects (which were acquired on a daily basis during the exposure block period only) that occurred in relation to a wider variety of air pollutants (which were measured in detail only at the exposure site) may help elucidate this issue further. We also cannot exclude the potential for significant correlations of unmeasured air pollutants with PM and hence that some of the health actions attributed to PM were (at least partially) mediated by other co-varying pollutants. However, we believe it is unlikely that this accounts for the majority of our reported associations, particularly since many of the gaseous pollutants (e.g., NO_2) are more spatio-temporally heterogeneous than our metric of $PM_{2.5}$ exposure. Whilst some pollutants are indeed known to be correlated with PM levels, we believe their correlation with 5-day long $PM_{2.5}$ is unlikely to be robust enough to account for the majority of the positive association between $PM_{2.5}$ mass concentration and HOMA-IR. We acknowledge that further studies investigating the sources, components and effects of other co-pollutants on insulin sensitivity are warranted. Regardless, the possible effect of unmeasured co-pollutants does not diminish the veracity and importance of the observed association between higher $PM_{2.5}$ (mass concentration per se whether or not it is the primary or only responsible pollutant) and reduced insulin sensitivity.

Finally, though HOMA-IR is a reasonable measure of insulin sensitivity for epidemiological studies and can be used to evaluate for within-subject metabolic alterations (Wallace et al., 2004; Muniyappa et al., 2008), we acknowledge that superior tests for assessing insulin sensitivity are available. This was the first study we have undertaken to investigate the metabolic perturbations induced by $PM_{2.5}$ in humans. Hence, the metric of insulin sensitivity necessarily provided preliminary mechanistic evidence. Given these supportive findings, we are now performing studies using a more invasive and sophisticated metric in our ongoing air pollution exposure studies (i.e., frequently sampled intravenous glucose tolerance test). The results of these studies will help corroborate and further add to our current findings. In the current study we did not have detailed time–activity and dietary assessments of the subjects each day of study participation. Ongoing studies will gather this information which may be important by impacting basal insulin sensitivity and/or by interacting with air pollution to affect metabolic outcomes.

5. Conclusions

Sub-acute exposures over 5 days to $PM_{2.5}$, even at low concentrations commonly-encountered throughout the world, were associated with reduced insulin sensitivity. This metabolic perturbation was most likely explained by unfavorable alterations in autonomic balance. Our findings support the contention that in addition to the traditionally-implicated factors of modern society (e.g. obesity, poor diet); exposure to $PM_{2.5}$ may be an independent contributing risk factor for developing DM.

Disclosures

No relevant conflict of interest.

Author contributions

1. Researched data; 2. Wrote the manuscript; 3. Reviewed/edited the manuscript; 4. Contributed to the discussion; 5. Contributed to research design.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.07.034.

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