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Reduced Metabolic Insulin Sensitivity Following Sub-acute Exposures to Low Levels of Ambient Fine Particulate Matter Air Pollution

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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-

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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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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 µg•m⁻³). A 10 µg•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 msec, 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.

Keywords

Metabolic syndrome; insulin sensitivity; PM_{2.5}; heart rate variability; endothelial function

1. Introduction

Exposure to fine particulate matter < 2.5µm 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, Rajagopalan, 2009; Brook, Rajagopalan 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, 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; Kramer, 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, et al 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; without diagnosis or treatment for hyperlipidemia during screening visits). Subjects with impaired fasting glucose (100–125 mg/dL) 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.

2.2 Study Outline

Figure 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 5–10 $\mu\text{g}\cdot\text{m}^{-3}$ 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 hours. 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 or 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 (Langrisk, 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 hours). 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 hours) 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 Figure 1. Details regarding PM_{2.5} measurement 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 (Figure 1; study days: 5, 16, 23) at the UMRVL starting at 3 PM. Subjects were instructed to fast for approximately 7 hours (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 minutes, subjects had 6 minutes 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 –70C 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: [glucose (mg/dL) × insulin (μU•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 (Walace 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 \pm 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 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 quasilielihood 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 ± 4.5 kg·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 minutes 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 ± 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 Figure 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-1 β , and IL-8 levels that were actually higher during the exposure-block week when HOMA-IR was lower. (Online supplement: Supplement Table 1). All additional outcomes outlined in the 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 $\mu\text{g}\cdot\text{m}^{-3}$ 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 $\mu\text{g}\cdot\text{m}^{-3}$), 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 msec 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 msec increase, $p=0.022$) while the effect of 5-day PM_{2.5} level was reduced ($\beta=0.64$ per 10 $\mu\text{g}\cdot\text{m}^{-3}$ 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 $\mu\text{g}\cdot\text{m}^{-3}$ 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 $\mu\text{g}\cdot\text{m}^{-3}$ 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 ($<35 \mu\text{g}\cdot\text{m}^{-3}$) U.S. 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; Kramer, 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; Kramer, et al 2010; Pearson, et al 2010; Puett, et al 2011). We demonstrated that NO₂, a pollutant often associated with traffic pollution, was associated with DM among women in Ontario (Brook, et al 2008). Studies from Germany (traffic pollutants) (Kramer, 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 2008); Chuang, et al 2010). In Taiwan, elevations in annual PM_{2.5} levels (mean $35.3 \pm 15.9 \mu\text{g}\cdot\text{m}^{-3}$) were related to increases in glucose and hemoglobin-A1c (Chuang, et al 2010). Among adolescents living in Isfahan Iran, very high weekly PM₁₀ concentrations (mean $111\text{--}157 \mu\text{g}\cdot\text{m}^{-3}$) were positively associated with HOMA-IR levels (Kelishadi, et al 2008). 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; Kramer, 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, Rajagopalan 2009; Brook, Rajagopalan 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 wholebody 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 interlinked. 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 hours per day of local regional monitor concentrations) and as an effect of the zero level of exposure while wearing the facemask for 2 hours/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 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., NO₂) 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PM_{2.5}	Fine particulate matter
DM	diabetes mellitus
HOMA-IR	homeostasis model assessment of insulin resistance
CV	cardiovascular
UMRVL	University of Michigan Research Vascular Laboratory
SDNN	standard deviation of normal-to-normal intervals
PBMC	peripheral blood mononuclear cells
GEE	generalized estimating equations

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Highlights

- Sub-acute exposure to low levels of ambient PM_{2.5} for approximately 5 days was associated with an increase in HOMA-IR, suggestive of a worsening of metabolic insulin sensitivity.
- The decrease in heart rate variability related to sub-acute PM_{2.5} exposures was associated with the reduction in metabolic insulin sensitivity, suggesting that autonomic imbalance may be an important underlying biological mechanism.
- Sub-acute PM_{2.5} exposure at these low levels did not promote systemic inflammation, an increase in blood pressure or alter vascular function.
- These findings offer support for the biological plausibility that PM_{2.5} could potentiate the development of diabetes mellitus over chronic periods of exposure consistent with observations from epidemiological studies.

Study day	"Pre-exposure block" sub-acute period																"Exposure-block" sub-acute period					"Post-exposure block" sub-acute period				
	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 start at midnight
 L=Local residence monitor levels used (closer of Dexter or Tecumseh sites to their house)

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

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)

Figure 1. Outline of the study design

Overview of the study flow and of the sub-acute exposure estimate methods.

The integrated sub-acute exposures start at 2 pm on each of the 3 visit days (1 hour before health outcomes were measured at 3 pm) as described above (L or C) retrospectively until midnight 4 days previous (day #1, #12, #19) for a total of 4 days and 14 hours (110 hours).

We had subjects wear the N95 facemask during car travel to the exposure site (1 hour before and 1 hour after daily exposures); hence a uniform zero level PM mass exposure was estimated for these 2 hours each day during the exposure-block. In the GEE, the health outcomes measured at each of the 3 time points were evaluated in relation to their temporally corresponding sub-acute PM_{2.5} exposure ending 1 hour prior to measurement.

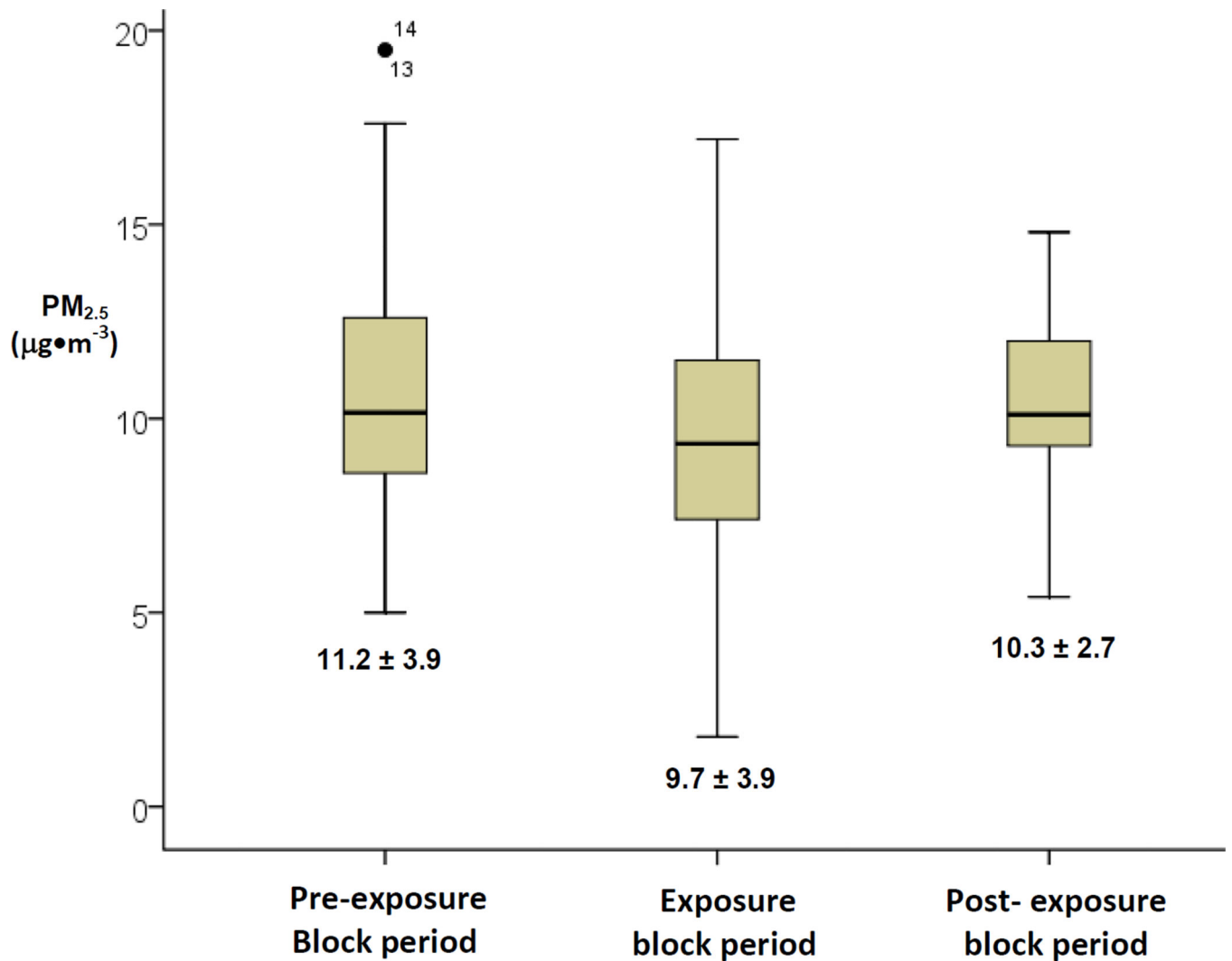


Figure 2. Time-averaged fine particulate matter levels during 5-day periods prior to each outcome measurement

Box and whiskers represent the inter-quartile ranges and 2 standard deviations of PM_{2.5} (µg•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 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 [†]
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 @ HR 75 (%)	13.8 ± 13.5	14.1 ± 11.2	14.6 ± 10.7	0.83
Pulse Wave Velocity (m•sec ⁻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) (msec)	57.3 ± 34.6	65.1 ± 29.2	61.1 ± 29.7	0.30
High Frequency Peak (msec ²)	1217 ± 2274	1544 ± 3762	1225 ± 2014	0.73
Low Frequency Peak (msec 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)*	84.0 ± 8.0, n=24	74.3 ± 6.3, n=23	78.5 ± 7.1, n=23	0.001
Insulin (μU•ml ⁻¹)*	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)*	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

* 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)

† P value represents significance of differences between the three measures determined using a general linear model repeated measures analysis of variance.

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

Table 2

OUTCOME	β^{\dagger}	95% CI	p	β^*	95% CI	p
SDNN (msec)	-11.60	-24.0 to 0.9	0.070	-13.1	-25.3 to -0.9	0.035
Glucose (mg•dl ⁻¹)	4.6	-1.0 to 10.3	0.109	5.4	0.5 to 10.3	0.029
Insulin (μ U•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 μ g•m⁻³ increase in PM_{2.5} level:

[†] β estimate from the GEE (unadjusted)

* β estimate from the GEE adjusted for age + body mass index (SNDD); age (glucose); body mass index (insulin, HOMA-IR). The parameters included in adjusted models were pre-selected as stated in methods 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.

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 includes all values.

95%CI, 95% confidence interval; SDNN, Standard Deviation of Normal-to-Normal Beats