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Ambient Air Pollution and Pathophysiological Responses of the Cardiometabolic System

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Abbreviations

ABI	Ankle-brachial index
AF	Atrial fibrillation
ANS	Autonomic nervous system
AVB	Atrioventricular block
BBB	Bundle branch block
BMI	Body mass index
CAC	Coronary artery calcification
CAD	Coronary artery disease
CIMT	Carotid intima-media thickness
CVD	Cardiovascular disease
ECG	Electrocardiogram
GIS	Geographic information system
HDL	High-density lipoprotein
HR	Heart rate
HOMA-IR	Homeostasis model assessment of insulin resistance
HOMA-B	Homeostasis model assessment of β-cell function
IQR	Interquartile range
IR	Insulin resistance
IS	Insulin sensitivity
LUR	Land-use regression
MI	Myocardial infarction
NAAQS	National Ambient Air Quality Standards
NO_2	Nitrogen dioxide
O_3	Ozone
OR	Odds ratio
PAD	Peripheral artery disease
PM_{10}	Particulate matter with an aerodynamic diameter ≤ 10 μm
$PM_{2.5}$	Particulate matter with an aerodynamic diameter ≤ 2.5 μm
$PM_{2.5abs}$	$PM_{2.5}$ absorbance
PM_{coarse}	Particulate matter with an aerodynamic diameter > 2.5 μm and ≤ 10 μm
PNC	Particle number concentration
QTc	Heart-rate-corrected QT interval
SES	Socioeconomic status
UFP	Ultrafine particles

Publication list

This thesis consists of the following publications:

Zhang S, Wolf K, Breitner S, Kronenberg F, Stafoggia M, Peters A, Schneider A. Long-term effects of air pollution on ankle-brachial index. *Environment International*. 2018;118:17-25.

Zhang S, Breitner S, Cascio WE, Devlin RB, Neas LM, Diaz-Sanchez D, Kraus WE, Schwartz J, Hauser ER, Peters A, Schneider A. Short-term effects of fine particulate matter and ozone on the cardiac conduction system in patients undergoing cardiac catheterization. *Particle and Fibre Toxicology*. 2018;15(1):38.

Zhang S, Mwiberi S, Pickford R, Breitner S, Huth C, Koenig W, Rathmann W, Herder C, Roden M, Cyrys J, Peters A, Wolf K and Schneider A. Longitudinal associations between ambient air pollution and insulin sensitivity: results from the KORA cohort study. *The Lancet Planetary Health*, 2021; 5(1): e39-e49.

Summary

Ambient air pollution has been identified as a major risk factor for cardiometabolic diseases. However, biological mechanisms underlying the health impacts of air pollution are still not fully understood. Epidemiological studies have shown that subclinical atherosclerosis, heart arrhythmia, and impaired insulin sensitivity (IS) are involved in the pathogenesis of cardiometabolic diseases. Investigating associations of air pollution with these pathophysiological responses in population-based studies promotes understanding the mechanisms of air pollution-related health effects, and helps identify the susceptible individuals for targeted interventions.

This thesis aimed to assess long-term effects of residential air pollution exposure on ankle-brachial index (ABI), an index for the detection of peripheral atherosclerosis (ABI≤0.9) and arterial stiffness (ABI>1.4), and biomarkers of IS in participants from the German KORA study. In addition, as part of the CATHGEN study in North Carolina, United States, we examined short-term effects of fine particulate matter ($PM_{2.5}$) and ozone (O_3) on cardiac electrical impulse conduction measured by PR, QRS, and QT intervals in electrocardiograms (ECG) among patients undergoing cardiac catheterization.

Our study on ABI demonstrated that long-term exposure to particulate matter (PM) with an aerodynamic diameter less than 10 μm (PM_{10}), $PM_{2.5}$, and nitrogen dioxide (NO_2) were associated with higher risks of both low and high ABI. Positive associations with the prevalence of high ABI were also observed for PM with an aerodynamic diameter between 2.5 μm and 10 μm (PM_{coarse}) and $PM_{2.5}$ absorbance (a proxy of elemental carbon related to traffic exhaust). Using repeated measurements of biomarkers, we observed increases in homeostasis model assessment of insulin resistance (HOMA-IR), homeostasis model assessment of β-cell function (HOMA-B), and fasting insulin associated with elevated PM, NO_2, and O_3, indicating the air pollution-related decrease in IS. Consistent with these results,

air pollution exposure was positively associated with the annual rate of change in HOMA-IR, HOMA-B, and fasting insulin. Our CATHGEN analyses suggested positive associations of $PM_{2.5}$ and O_3 with the PR interval at a lag of 3–4 days, and a lengthening of the QRS interval four days after exposure to O_3. Both immediate (lag0) and delayed (lag3–lag4) effects of air pollution were found for the lengthening of heart rate-corrected QT interval. Generally, older adults and individuals who were physically inactive or of lower socioeconomic status were more susceptible to the air pollution effects.

This thesis extends the literature on pathophysiological responses of the cardiometabolic system associated with exposure to ambient air pollution. Specifically, it showed that a higher risk of atherosclerosis and stiffness in peripheral arteries, a higher degree and faster progression of impaired IS, as well as delays in atrioventricular conduction, ventricular depolarization and repolarization were associated with air pollution exposure. These findings substantiate the biological mechanisms linking ambient air pollution to the development of cardiometabolic disease, and provide guidance for the targeted interventions to mitigate the adverse health effects of air pollution, especially in the susceptible populations.

Zusammenfassung

Luftverschmutzung wurde als Risikofaktor für kardiometabolische Erkrankungen identifiziert. Die biologischen Mechanismen, die den gesundheitlichen Auswirkungen der Luftverschmutzung zugrunde liegen, sind jedoch noch nicht vollständig verstanden. Epidemiologische Studien haben gezeigt, dass subklinische Atherosklerose, Herzrhythmusstörungen und Störungen der Insulinsensitivität (IS) an der Pathogenese kardiometabolischer Erkrankungen beteiligt sind. Die Untersuchung der Zusammenhänge von Luftverschmutzung mit diesen subklinischen Phänotypen in bevölkerungsbezogenen Studien untermauert das Verständnis der Mechanismen luftverschmutzungsbedingter Gesundheitsschäden und hilft, anfällige Personen für gezielte Präventionsmaßnahmen zu identifizieren.

Ziel dieser Arbeit war es, die langfristigen Auswirkungen der Belastung durch Außenluftschadstoffe in Wohngebieten bei Teilnehmern der deutschen KORA-Studie zu untersuchen. Dabei wurden folgende Parameter betrachtet: Knöchel-Brachial-Index (ABI), ein Index zum Nachweis von peripherer Atherosklerose (ABI $\leqslant$0.9) und arterieller Steifheit (ABI>1.4), Biomarker für IS, gemessen anhand des HOMA-Index als Indikator für Insulinresistenz (HOMA-IR) und des Nüchterninsulins. Darüber hinaus untersuchten wir im Rahmen der CATHGEN-Studie in North Carolina, USA, Kurzzeiteffekte von Feinstaub ($PM_{2.5}$) und Ozon (O_3) bei Patienten, die sich einer Herzkatheterisierung unterzogen hatten, auf die elektrische Herzimpulsleitung, gemessen durch PR-, QRS- und QT-Intervalle in Oberflächenelektrokardiogrammen (EKG).

Unsere ABI-Studie hat zeigte, dass eine Langzeitbelastung mit Partikelmasse (PM) mit einem aerodynamischen Durchmesser von weniger als 10 µm (PM_{10}), $PM_{2.5}$ und Stickstoffdioxid (NO_2) mit einem höheren Risiko für niedrige und hohe ABI Werte verbunden ist. Positive Assoziationen mit der Prävalenz eines hohen ABI wurden auch für PM mit einem aerodynamischen Durchmesser zwischen 2.5 µm und 10 µm (PM_{coarse}) und der $PM_{2.5}$-Absorption (einem Proxy für elementaren Kohlenstoff in Bezug auf Verkehrsabgase) beobachtet.

Unter Verwendung wiederholter Messungen von Biomarkern beobachteten wir einen Anstieg der HOMA-IR und des Nüchterninsulins im Zusammenhang mit erhöhten PM-, NO_2- und O_3-Werten, was auf eine luftverschmutzungsbedingte Abnahme der IS hindeutet. In Übereinstimmung mit diesen Ergebnissen war die Exposition gegenüber Luftverschmutzung war positive mit der jährlichen Änderungsrate von HOMA-IR und Nüchterninsulin verbunden. Unsere CATHGEN-Analysen deuteten auf positive Assoziationen von $PM_{2.5}$ und O_3 mit dem PR-Intervall mit einer Verzögerung von 3 bis 4 Tagen, und einer Verlängerung des QRS-Intervalls vier Tage nach O_3-Exposition hin. Für die Verlängerung des herzfrequenzkorrigierten QT-Intervalls wurden sowohl unmittelbare (lag0) als auch verzögerte (lag3–lag4) Auswirkungen der Luftverschmutzung festgestellt. Im Allgemeinen waren ältere Erwachsene und Personen, die körperlich inaktiv waren oder einen niedrigeren sozioökonomischen Status hatten, anfälliger für die Auswirkungen der Luftverschmutzung.

Diese Arbeit erweitert die Literatur zu pathophysiologischen Reaktionen des kardiometabolischen Systems, die mit der Exposition gegenüber Luftverschmutzung verbunden sind. Insbesondere zeigte sich ein höheres Risiko für Arteriosklerose und Steifheit in peripheren Arterien, ein höheres Ausmaß und ein schnelleres Fortschreiten von beeinträchtigte IS sowie Verzögerungen bei der atrioventrikulären Überleitung, der ventrikulären Depolarisation und der Repolarisation im Zusammenhang mit einer Exposition gegenüber Außenluftschadstoffen. Diese Ergebnisse untermauern die biologischen Mechanismen, die die Luftverschmutzung mit der Entwicklung von kardiometabolischen Erkrankungen in Verbindung bringen, und geben Hinweise für gezielte Maßnahmen zur Minderung der gesundheitlichen Auswirkungen von Luftverschmutzung, insbesondere auf besonders empfindliche Bevölkerungsgruppen.

1 Background

1.1 Air pollution and cardiometabolic disease

Cardiovascular disease (CVD) and type 2 diabetes are two leading causes of premature death and disability. The estimated number of deaths from CVD and type 2 diabetes globally in 2017 were 17.8 million and 1.0 million, increasing by 21.1% and 43.0%, respectively, since 2007 (1). Ambient air pollution has been identified as a major contributor to the global burden of disease. Long-term exposure to ambient particulate matter with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) accounted for 4.2 million deaths worldwide in 2015, ranking fifth among all investigated risk factors, and accounted for 15.9% of mortality from ischemic heart disease and cerebrovascular disease (2,3). Furthermore, around 3.2 million incident diabetes cases and more than 0.2 million deaths from diabetes were attributable to ambient $PM_{2.5}$ in 2016 (4).

Epidemiological and experimental studies have proposed several biological mechanisms by which air pollution may affect the cardiometabolic system, which are summarized in Figure 1 (5-7). Inhaled fine particles deposit in pulmonary alveoli and lead to the formation of reactive oxygen species, resulting in local oxidative stress and inflammation. The release of pro-inflammatory mediators causes systemic inflammation, which can contribute to the development of endothelial dysfunction, atherosclerosis, and insulin resistance (IR). Another pathway involves air pollution-induced alteration in the autonomic nervous system (ANS) via stimulating the airway receptors. The imbalance of sympathetic and parasympathetic nervous system might affect the heart rhythm and blood pressure within a short timeframe. Ultrafine and soluble constituents of particulate matter (PM) can also directly translocate into the systemic circulation and cause pro-inflammatory responses.

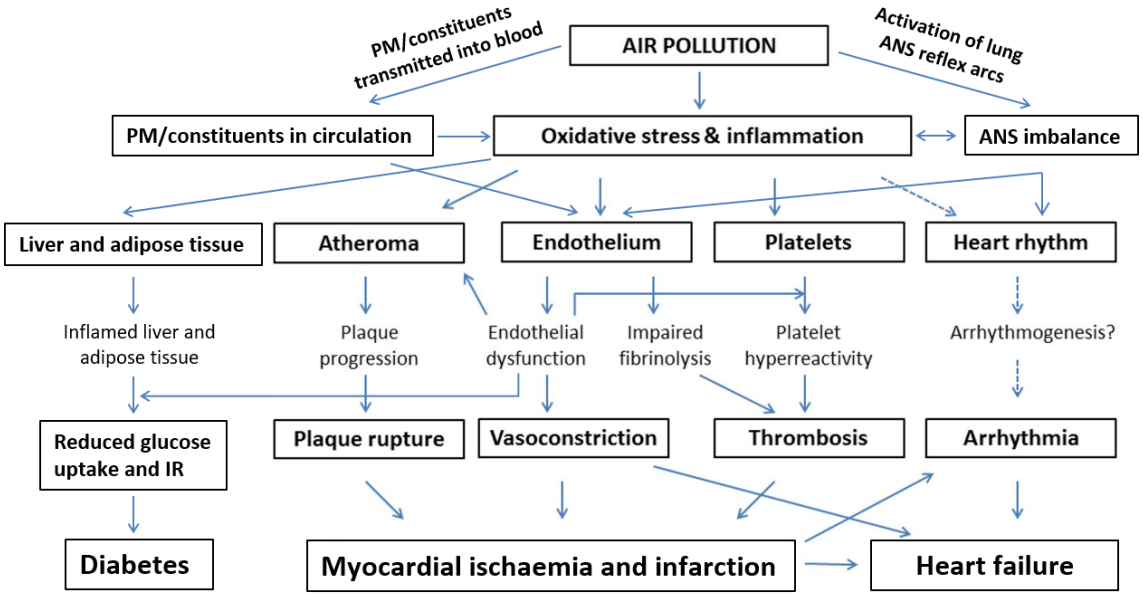

Figure 1. Hypothesized biological mechanisms of air pollution-mediated cardiometabolic diseases. Adapted from "*Expert position paper on air pollution and cardiovascular disease*". (Newby, D. E., et al. 2014, *European Heart Journal*, 36(2), 83-93. Copyright [2014] by European Society of Cardiology).

1.2 Pathophysiological responses of the cardiometabolic system

Subclinical atherosclerosis, heart arrhythmia, and IR are suggested to be important pathophysiological responses linked to cardiometabolic events (8-10). Investigating associations between air pollution and these pathophysiological responses in population-based studies promotes the understanding of the biological mechanisms of air pollution-mediated cardiometabolic diseases beyond the cellular/molecular level. Besides, it helps identify the susceptible subpopulations in order to deliver targeted interventions before the clinical manifestation of diseases.

1.2.1 Subclinical atherosclerosis and arterial stiffness

Atherosclerosis is characterized by asymmetric thickening of the innermost layer of arteries due to the formation of plaques (8). Rupture of the plaques is a major cause of thrombosis precipitating ischemic CVD (11,12). Thus, subclinical atherosclerosis plays a key role in the risk assessment of CVD. A variety of non-invasive methods

have been offered for measuring atherosclerosis, among which the measurements of carotid intima-media thickness (CIMT), coronary artery calcification (CAC), and ankle-brachial index (ABI) are commonly used in epidemiological studies (13-15).

ABI is the ratio of systolic blood pressure at the ankle to that at the brachial artery. An ABI lower than 0.9 indicates stenosis between the aorta and distal arteries of lower extremities. It is a widely used clinical tool for the diagnosis of peripheral artery disease (PAD) with high sensitivity and specificity (16). A low ABI is associated with higher risks of cardiovascular events and all-cause mortality, as well as greater functional impairment because of ischemic leg symptoms (13,17). In addition to subclinical atherosclerosis, abnormally high ABI is a marker of arterial stiffness, which results from calcification in the medial layer of the arterial wall (18,19). An ABI > 1.4 has been demonstrated to be an independent risk factor for CVD and mortality (13,20).

Epidemiological studies have reported associations of long-term air pollution exposure with CIMT and CAC (21-27). However, the atherosclerotic effects of air pollution are heterogeneous across different vascular beds, and evidence of the effect on ABI is still limited and inconsistent (28,29). A study in the German Ruhr Area showed that living within 50 m of a major road was associated with a decrease in ABI and a higher prevalence of ABI < 0.9, while no or even reverse associations were observed elsewhere (30-32). Moreover, since most previous studies focused on low ABI, little is known about the long-term air pollution effect on abnormally high ABI, i.e. arterial stiffness of lower extremities. Despite the association between atherosclerosis and arterial stiffness, the air pollution effects on low and high ABI could be distinct (32,33). Therefore, examining the full spectrum of ABI in the same population can provide a better understanding of the association of air pollution with the structural and functional changes in arteries.

1.2.2 Cardiac conduction disorders

The cardiac conduction system initiates and conducts electrical impulses, subsequently stimulating the contraction of the atria and ventricles. The cardiac

electrical activity can be recorded by an electrocardiogram (ECG), which facilitates the diagnosis of conduction disorders. For instance, the electrocardiographic PR interval reflects the impulse conduction from the sinus node through the atrioventricular node and His-Purkinje system. A PR interval exceeding 200 milliseconds indicates the presence of first-degree atrioventricular block (34). The QRS interval and heart rate-corrected QT interval (QTc) in the ECG are measures of ventricular depolarization and repolarization, respectively (35). Prolonged PR, QRS, and QTc have been associated with increased risks of cardiac and all-cause mortality (34,36-38).

Air pollution effects on the cardiac conduction system could be mediated through ANS dysfunction and/or inflammation. Compared with the lifelong process of atherosclerosis, the electrical conduction responses can occur within hours to days (39-47). The U.S. Veterans Affairs Normative Aging Study reported increased QTc within 10 hours after exposure to traffic-related pollutants (black carbon, nitrogen dioxide [NO_2], and carbon monoxide) (41). Similar results were observed in a panel study on healthy young adults in China, showing positive associations with exposure in the preceding 1–5 days (47). To date, short-term effects of air pollution on the PR and QRS intervals are still not well understood. Liao et al. (44) found prolonged PR duration associated with $PM_{2.5}$ exposure at a lag of 1.5–2 hours. In randomized crossover studies, an increase in the QRS interval was observed among individuals without a prominent antioxidant gene (*GSTM1*) after controlled exposure to ultrafine particles (UFPs) in the chamber, while a decrease in QRS was associated with controlled exposure to ozone (O_3) (46,48).

Patients with preexisting CVD are potentially more susceptible to the adverse health effects of air pollution (49). Besides, medical conditions and the usage of certain medication might have an impact on cardiac rhythms such as prolonging cardiac repolarization (50). Therefore, it is of greater clinical significance to investigate short-term air pollution effects on the cardiac conduction system among patients who are at an elevated risk of cardiovascular events.

1.2.3 Insulin resistance

Insulin is a hormone secreted by β-cells of pancreatic islets and acts as an important regulator in glucose homeostasis, via stimulating the uptake of glucose into metabolic tissues such as skeletal muscle and liver. IR may first lead to an increase in insulin secretion to compensate for reduced insulin signaling and to maintain normal glucose tolerance (51). The increased workload and stress result in a decline in β-cell function, promoting the progression to impaired glucose tolerance and impaired fasting glucose, which in turn contribute to the development of type 2 diabetes (52). In a case-control study of postmenopausal women in the U.S., the diabetic risk was 3.40 (95%CI: 2.95–3.92) for an increment of 1.93 unit in homeostasis model assessment of IR (HOMA-IR), a surrogate measure for quantifying IR (53). Furthermore, IR is associated with a higher risk of CVD events in individuals with or without diabetes (54,55).

Epidemiological studies have observed an association between pro-inflammatory biomarkers and IR independent of obesity, suggesting the potential role of subclinical inflammation in the pathogenesis of IR (56,57). Pro-inflammatory cytokines produced by adipocytes and immune cells activate Jun N-terminal kinase (JNK) and inhibitor of κB kinase β (IKKβ), which promote IR through pathways such as phosphorylation in insulin receptor substrate proteins and transcriptional activation of nuclear factor-κB (NF-κB) (58,59). An experimental study in humans found that the modulation of inflammatory and insulin signaling pathways preceded endotoxemia-induced IR (60). In accordance with aforementioned findings, anti-inflammatory medication has been demonstrated to improve glucose homeostasis (61).

Cumulative studies have examined long-term air pollution effects on IR and glucose homeostasis, aiming to elucidate a potential pathway linking air pollution to type 2 diabetes (62-70). Nevertheless, evidence from previous studies is mixed. In a German study, Wolf et al. (65) observed substantial increases in HOMA-IR and fasting insulin associated with elevated PM and NO_2, whereas only a slight increase in fasting glucose. Positive associations between air pollution and HOMA-IR were

also reported in adolescents of two German birth cohorts and in Mexican Americans who were at a greater risk of type 2 diabetes (64,66). However, in a large cross-sectional study of 15,477 participants from 33 communities in northeastern China, exposure to various air pollutants (PM, NO_2, sulfur dioxide, and O_3) were consistently associated with increased fasting glucose, while associations with HOMA-IR and fasting insulin were merely found for NO_2 (70). Given the conflicting evidence, further investigations on air pollution and IR are still needed, especially in a longitudinal setting, which additionally allows the assessment of air pollution effects on the progression of IR over time.

1.3 Specific Aims

The three main objectives of this thesis were to investigate:

(1) Associations between long-term exposure to air pollution and abnormal ankle-brachial index.

(2) Short-term effects of air pollution on the cardiac electrical conduction among patients undergoing cardiac catheterization.

(3) Long-term effects of air pollution on the level and rate of change of insulin sensitivity over time.

2 Contributing manuscripts

This cumulative thesis comprises three manuscripts.

The first manuscript, addressing specific aim (1), entitled "*Long-term effects of air pollution on ankle-brachial index*" (71) investigated the associations between long-term exposure to air pollution and abnormal ABI. In this cross-sectional study, we analyzed data of 4,544 participants from two population-based surveys, and observed positive associations between annual air pollution concentrations and the prevalence of both low and high ABI. I was responsible for conceptualization, data analyses, and writing the manuscript.

The second manuscript, addressing specific aim (2), entitled "*Short-term effects of*

fine particulate matter and ozone on the cardiac conduction system in patients undergoing cardiac catheterization" (72) presented the short-term associations of $PM_{2.5}$ and O_3 with cardiac electrical conduction. Using ECGs recorded repeatedly on 5,332 patients who underwent cardiac catheterization, we found delays in atrioventricular conduction, ventricular depolarization and repolarization associated with short-term air pollution exposure. I was responsible for data analyses and writing the manuscript.

The third manuscript, addressing specific aim (3), entitled "*Longitudinal associations between ambient air pollution and insulin sensitivity: results from the KORA cohort study*" (73) reported the long-term effects of air pollution on IS-related biomarkers. This longitudinal study collected data of 3,297 participants from a baseline survey and its two follow-up examinations. In this study, air pollutant concentrations were positively associated with the level and the rate of change of HOMA-IR, homeostasis model assessment of β-cell function (HOMA-B), and fasting insulin. I was responsible for conceptualization, data analyses, and writing the manuscript.

3 Methods

3.1 Study population

3.1.1 Cooperative Health Research in the Region of Augsburg (KORA)

The assessment of long-term cardiometabolic effects of air pollution was based on data collected in the Cooperative Health Research in the Region of Augsburg (KORA) study. Within the framework of the KORA study, four cross-sectional health surveys (S1 to S4) were conducted at five-year intervals, and each of them consisted of a random sample selected independently in the city of Augsburg, Germany and the two adjacent rural counties, Augsburg and Aichach-Friedberg (74). An overview of the KORA study is presented in Figure 2. One follow-up examination of S3 (F3) and two follow-up examinations of S4 (F4 and FF4) were performed between 2004 and 2014. Each baseline and follow-up examination comprised a self-administered

questionnaire, a personal interview, physical examinations, and a collection of various biological samples, gathering information on demographics, socioeconomic status, lifestyle, medical history, and physiological parameters.

The study of long-term exposure to air pollution and abnormal ABI was a cross-sectional study using data of altogether 4,544 participants from F3 (2004–2005) and F4 (2006–2008). The longitudinal study on IS analyzed 3,297 participants of the S4 survey, including baseline and follow-up observations from S4, F4, and FF4.

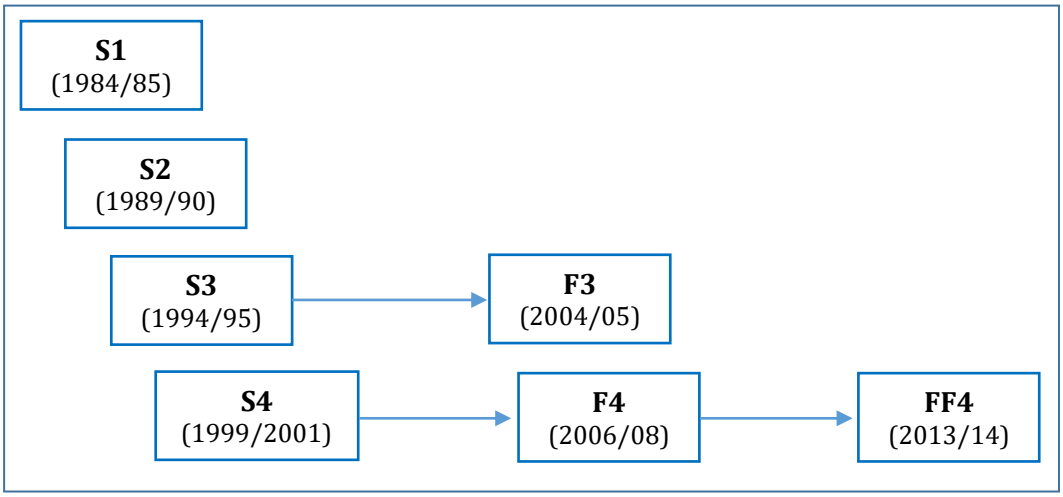

Figure 2. Overview of the KORA study. The overview consists of the baseline surveys and follow-up examinations, but does not include the General Health Follow-up by telephone interview.

3.1.2 Catheterization Genetics (CATHGEN) study

The assessment of short-term effects of air pollution was based on data collected in the Catheterization Genetics (CATHGEN) Study. The CATHGEN study comprises 9,334 patients undergoing cardiac catheterization for the diagnosis or treatment of coronary heart disease at Duke University Hospital in North Carolina, United States (NC, U.S.) between 2000 and 2010. Information on individual demographics, anthropometry, life-style factors, and medical history was obtained from medical records and a questionnaire administered at the time of catheterization.

3.2 Outcome measurement

3.2.1 Ankle-brachial index

A Doppler probe was employed to measure systolic blood pressure in the posterior tibial artery of each ankle and the brachial artery of the right arm (75). ABI was calculated for both left and right sides as the ratio of systolic blood pressure at the ankle to that at the right arm. Based on the presence of abnormal ABI, we classified the participants into three categories: (1) having normal ABI if $0.9 < ABI \leq 1.4$ in both legs; (2) having low ABI if $ABI \leq 0.9$ in at least one leg; (3) having high ABI if $ABI > 1.4$ in one leg and $0.9 < ABI \leq 1.4$ in the contralateral leg, or $ABI > 1.4$ in both legs.

3.2.2 Electrocardiogram

12-lead ECGs were performed at the time of catheterization and in follow-up visits, and were analyzed by the Philips TraceMaster ECG system (Andover, MA, USA) to determine the PR, QRS, QT intervals and heart rate (HR). The HR-correction of the QT interval in the main analyses used the Bazett formula. The exclusion criteria were: (1) participants residing outside NC, U.S. at catheterization; (2) participants with bundle branch block (QRS > 120 millisecond [ms]); (3) ECGs with the diagnosis of atrial fibrillation, atrial flutter, multifocal atrial tachycardia, or paced rhythms; (4) ECGs with non-physiological parameter values (PR < 100 ms or > 400 ms, QRS < 50 ms, QTc < 350 ms or > 600 ms, HR < 20 beats per minute [bpm] or > 180 bpm).

3.2.3 Biomarkers of insulin sensitivity

Blood concentrations of fasting insulin and fasting glucose were measured at baseline and two follow-ups between 1999 and 2014 (76-78). HOMA-IR was calculated as HOMA-IR = (fasting insulin [μIU/mL]) × (fasting glucose [mmol/L]) / 22.5. HOMA-B was calculated as 20 × fasting insulin (μIU/mL) / (fasting glucose [mmol/L] – 3.5). A higher HOMA-IR indicates reduced insulin sensitivity, and a lower HOMA-B indicates decreased fasting insulin secretion. We excluded the observations (n = 348) on glucose-lowering medication (Anatomical Therapeutic

Chemical code = A10) to ensure the steady state of fasting insulin and glucose concentrations during blood sample collection (79).

3.3 Exposure assessment

3.3.1 Annual average concentrations of air pollution in Augsburg (KORA study)

Following the protocols of the European Study of Cohorts for Air Pollution Effects (ESCAPE), we monitored PM and NO_2 at 20 and 40 sites, respectively, in the region of Augsburg and Munich (80,81). The measurements were conducted in three two-week campaigns between October 2008 and July 2009, covering the cold, warm, and intermediate seasons. We built land-use regression (LUR) models incorporating annual average concentrations of pollutants at monitoring sites and corresponding geographic information as potential predictors. The fitted models were then applied to the home addresses of participants in our study of ABI to estimate individual long-term exposure levels.

A similar process was carried out with measurements in Augsburg and its two adjacent counties between March 2014 and April 2015. The new LUR modeling extended the previous one by developing LUR models for particle number concentrations (PNC, an indicator for UFPs]) and O_3, as well as by including further predictor variables to refine and update the models for PM and NO_2 (82). The estimated annual average concentrations were assigned to participants of the study on subclinical inflammation and IS.

3.3.2 Daily air pollution concentrations and temperature in North Carolina (CATHGEN study)

For the study period (2000–2012), daily average concentrations of $PM_{2.5}$ and daily 8-hour maximum concentrations of O_3 in NC were predicted using neural network-based hybrid models (83,84). Daily average air temperature in NC was estimated by a three-stage modeling approach (85). The modeling of air pollutants and temperature were all at a spatial resolution of 1 km. CATHGEN participants'

residential addresses were geocoded and matched with air pollution and temperature data based on the spatial location and date.

3.4 Statistical methods

3.4.1 Long-term associations of air pollution with abnormal ABI

Cross-sectional associations between long-term exposure to air pollution and abnormal ABI were assessed by a multinomial logistic regression model, with adjustment for age, sex, time trend, an indicator for study, years of education, neighborhood socioeconomic status, smoking status, and smoking pack-years. We also conducted quantile regression to explore the air pollution effects on different percentiles of the ABI distribution using the lower ABI value of the two sides as the outcome variable.

3.4.2 Short-term associations of air pollution with cardiac electrical conduction

We examined the effects of $PM_{2.5}$ and O_3 on cardiac electrical conduction at single-day lags from lag0 (same day of ECGs) to lag4 (four days prior to ECGs), and at cumulative lag04 (5-day moving average) by generalized additive mixed model with random participant intercepts. In the model, we incorporated a penalized spline of time with four degrees of freedom per year and an indicator for season to control for the long-term and seasonal trend, as well as an indicator for day of the week. We further adjusted for air temperature using natural splines and individual characteristics at each measurement time point, including age, sex, race, area-level educational attainment, body mass index (BMI), smoking status, and the living area. For pollutant-outcome pairs showing delayed associations at lag4, we investigated the lagged effects up to 14 days using distributed-lag models.

3.4.3 Long-term associations of air pollution with insulin sensitivity

The longitudinal associations of air pollution with IS were examined in two stages. First, we applied mixed-effects models with random intercepts for participants to assess air pollution effects on the repeated measurements of biomarkers. Second, for each participant with a biomarker measured at two or three visits, we built a

linear regression model of biomarker levels regressed against the years since baseline. We took the slope coefficient of the regression model as the annual rate of change in this biomarker. We investigated associations between air pollutants and the annual rate of change in IS-related biomarkers by quantile regression models.

In all studies, we explored the effect modification on associations between air pollution and biomarkers by demographics, socioeconomic status, and lifestyles.

4 Results

4.1 Long-term effects on abnormal ABI

This analysis on ABI addresses the first specific aim of this thesis: To investigate associations between long-term exposure to air pollution and abnormal ABI.

Long-term exposure to PM with an aerodynamic diameter less than 10 μm (PM_{10}), $PM_{2.5}$, and NO_2 were associated with a higher prevalence of both low and high ABI. For an increment of an interquartile range (IQR, 7.6 $\mu g/m^3$) in PM_{10}, the odds ratios (ORs) of having low ABI and high ABI were 1.82 (95% confidence interval [CI]: 1.11, 2.97) and 1.63 (1.07, 2.50), respectively. Positive associations with the prevalence of high ABI were also observed for PM with an aerodynamic diameter between 2.5 μm and 10 μm (PM_{coarse}) and $PM_{2.5}$ absorbance ($PM_{2.5abs}$, as a proxy of elemental carbon levels related to traffic exhaust). In addition to the air pollutants, traffic exposure was assessed by traffic intensity on the nearest major road and traffic load within 100 m of the residence. Both traffic indicators were positively but non-significantly associated with having abnormal ABI. The associations of PM and NO_2 with the prevalence of low ABI were stronger among hypertensive or physically inactive participants.

In quantile regression, elevated air pollution concentrations were associated with a decrease in ABI values lower than 0.98 (i.e. the 5th and 10th percentiles), indicating an increased risk for low ABI. Among participants with ABI values higher than 1.28 (i.e. the 90th and 95th percentiles), elevated air pollution concentrations were

associated with an increase in ABI, which represents an increased risk for high ABI. The $PM_{2.5}$-associated increase in ABI was already present at a comparatively low ABI value of 1.14.

4.2 Short-term effects on cardiac electrical conduction

This analysis on cardiac electrical conduction addresses the second specific aim of this thesis: To investigate short-term effects of air pollution on the cardiac electrical conduction among patients undergoing cardiac catheterization.

We observed a lengthening of the PR interval 3–4 days after exposure to elevated $PM_{2.5}$ and O_3, as well as a lengthening of the QRS interval four days after exposure to elevated O_3. An increment of 19.4 ppb (IQR) in O_3 was associated with an increase in PR by 0.29% (0.05%, 0.53%) and in QRS by 0.21% (0.04%, 0.37%) at lag4. Both immediate (lag0) and delayed (lag3–lag4) effects of $PM_{2.5}$ were found for the lengthening of QTc. For O_3, the positive association with QTc was significant at lag0 and lag1. HR was positively associated with $PM_{2.5}$ and O_3, with the strongest associations at lag1. In distributed-lag models, the effects of $PM_{2.5}$ and O_3 on PR and the effect of O_3 on QRS peaked at lag4–lag5 and persisted until lag7. However, the effect of $PM_{2.5}$ on QTc peaked at lag0 and then decreased, and no effect was observed beyond four days. Stronger associations between air pollution and QRS and QTc intervals were shown in patients with low educational attainment or obesity, or living in rural areas.

4.3 Long-term effects on insulin sensitivity

This analysis using repeatedly measured biomarkers of IS addresses the third specific aim of this thesis: To investigate long-term effects of air pollution on the level and the rate of change of IS over time.

We observed increases in HOMA-IR, HOMA-B, and fasting insulin linearly associated with elevated PM, $PM_{2.5abs}$, NO_2, and to a lesser extent, O_3. For instance, an increment of 1.4 $\mu g/m^3$ (IQR) in $PM_{2.5}$ was associated with an increase in HOMA-IR by 3.1% (0.9%, 5.3%), an increase in HOMA-B by 2.7% (0.6%, 4.7%), and an increase in

fasting insulin by 3.0% (1.0%, 5.0%). We did not find evidence of a long-term association between air pollution and fasting glucose. In addition, higher susceptibility to air pollution effects was observed in participants who were over 60 years, male, not employed, or led a sedentary lifestyle.

Consistent with the results of repeated measurements, air pollution exposure was positively associated with the annual rate of change in HOMA-IR, HOMA-B, and fasting insulin. For example, $PM_{2.5abs}$ was associated with the annual rate of change in HOMA-IR at the 10th to 70th percentiles of the distribution of rate values (i.e. annual rate of change in HOMA-IR ≤ 0.10 unit/year), and associated with the annual rate of change in HOMA-B at the 20th to 90th percentiles (i.e. annual rate of change in HOMA-B ≥ -5.34 unit/year). For an increment of 0.3×10^5 (IQR) in $PM_{2.5abs}$, the median annual rate of change in HOMA-IR and HOMA-B increased by 0.010 (0.001, 0.019) unit/year and 0.574 (0.185, 0.963) unit/year, respectively. The air pollution effects on the rate of change in biomarkers were stronger among older adults, males, and participants with prediabetes or diabetes.

5 Discussion

5.1 Air pollution and peripheral atherosclerosis and arterial stiffness

In our study of air pollution and ABI, higher risks of peripheral atherosclerosis and arterial stiffness were associated with long-term exposure to PM and traffic-related air pollutants. These results were consistent with previous studies that reported positive associations of air pollution with the severity of atherosclerosis and stiffness in central arteries (27,86,87). The non-monotonic relationship between air pollution and ABI was evident in the quantile regression. The associations at two ends of the distribution of ABI indicated that participants who were prone to developing either atherosclerosis (low ABI) or stiffness (high ABI) were more susceptible to the adverse effects of air pollution.

So far, long-term air pollution effects on the full spectrum of ABI have not been fully investigated. Rivera et al. (32) performed a cross-sectional analysis using data from 2,780 participants of the Spanish REGICOR cohort, and observed that elevated residential 10-year average NO_2, traffic intensity, and traffic load were associated with a higher prevalence of ABI > 1.3, but not with ABI < 0.9. In addition to the variance in exposure and participant health status (e.g. lower exposure levels and higher prevalence of low ABI in KORA), the differences in effect estimation between REGICOR and KORA might be partly due to the definition of the outcome, as the associations between traffic indicators and high ABI were more comparable when the same definition was used.

5.2 Air pollution and cardiac electrical conduction

Our study of the short-term air pollution effects demonstrated that exposure to $PM_{2.5}$ and O_3 were associated with delays in atrioventricular conduction, ventricular depolarization and repolarization among patients undergoing cardiac catheterization, even when the exposure levels were below the current U.S. National Ambient Air Quality Standards (NAAQS). In spite of the relatively small effect estimates, air pollution exposure may add to the effects of preexisting factors that affect cardiac conduction (e.g. left ventricular hypertrophy, ischemia, certain medications, etc.), and drive the interval duration across critical thresholds.

Using data from 106 adults without severe cardiac problems, the Air Pollution and Cardiac Risk and its Time Course (APACR) study found a $PM_{2.5}$-associated lengthening of the PR and QTc intervals at a lag of 1.5–2 hours and 3–3.5 hours, respectively (44,88). However, the magnitude of the effects was weaker than that in our study, and no significant changes were observed in the QRS interval. Since the $PM_{2.5}$ levels were comparable in these two studies, we hypothesized that the health status of participants and the time course of exposure could affect the air pollution effects on cardiac conduction.

5.3 Air pollution and insulin sensitivity

Our longitudinal analyses of IS and glucose homeostasis showed the long-term effect of air pollution on reduced IS, but not on fasting glucose. This finding is supported by the theory that impaired IS could first lead to the compensatory insulin hypersecretion to maintain glucose homeostasis (51). Several cross-sectional studies have examined the association of long-term air pollution exposure with IS and the results are mixed. Similar to our findings, significant effects of PM and NO_2 on HOMA-IR were observed among adults and adolescents in Germany (65,89). However, in the 33 Communities Chinese Health Study (33 CCHS), an increase in glucose was associated with PM, NO_2, and O_3, whereas effects on HOMA-IR and fasting insulin were only significant for NO_2 (90). Given the much higher concentrations of air pollution in the 33 CCHS, differences in exposure intensity and chemical composition could be one explanation for the inconsistent results.

Furthermore, the positive associations of air pollution with the annual rate of change in HOMA-IR, HOMA-B, and fasting insulin suggested a faster deterioration of IS in relation with elevated air pollutant concentrations among participants with deteriorated IS over time (annual rate of change above zero). In the subgroup with improved IS over time, which might be due to lifestyle interventions, our results indicated that exposure to elevated air pollution could slow down the process of improvement. Our finding was consistent with a cohort study among overweight and obese Latino children living in Los Angeles, U.S., showing a faster decline in IS associated with long-term exposure to $PM_{2.5}$ and NO_2 (91).

5.4 Biological mechanisms

Systemic inflammation is a plausible mechanism underlying the associations between long-term air pollution exposure and physiological responses in the cardiometabolic system. Particles with soluble constituents and gases that deposit in the lung and translocate into the circulation can lead to an inflammatory response. Experimental studies have shown that inflammatory cells are involved in the formation of atherosclerotic plaques, and inflammatory mediators may promote

atheroma progression and reduce plaque stability (92,93). Inflammatory cytokines can also contribute to the development of IR by activating intracellular pathways such as IKKβ/NF-κB and JNK (58,59).

With respect to the short-term effects on cardiac electrical conduction, we observed immediate associations on the concurrent day and delayed associations persisting until up to seven days after exposure, suggesting that both autonomic dysfunction and systemic inflammation could be potential mechanisms of air pollution effects on the cardiac conduction system. The process might involve the alterations in the autonomic tone that directly impact the innervation of cardiomyocytes, as well as the effects of inflammation on cardiomyocytes ion currents via cytokine- and sympathetic-induced modulation (94,95).

5.5 Susceptible subpopulations

Results of this thesis generally suggested stronger air pollution effects on the cardiometabolic system among participants who were older than 60 years, male, physically inactive, not employed, had comorbidities, had lower education, or were living in rural areas. With regard to cost-benefit considerations, potential interventions (e.g. air quality alerts) could be targeted to these susceptible subpopulations. In addition, the underlying health status also seems to play a role in the susceptibility. For instance, participants with ABI below 0.98 or above 1.28 were more susceptible to air pollution effects on the development and progression of atherosclerosis or arterial stiffness, respectively, and accounted for altogether 20% of the study population in KORA F3 and F4. It is noteworthy that greater risks were not only observed in individuals with more pronounced pathological changes, but also in still relatively healthy subpopulations, i.e. in the early stage of developing IR. This finding provides guidance on more targeted interventions to mitigate adverse health effects of air pollution. As shown in this thesis, prevention strategies for air pollution-associated atherosclerosis or arterial stiffness could be focused on individuals with either low or high ABI, whereas the prevention of air pollution

effects on type 2 diabetes should already start in participants in the early stage of IR, i.e. in a still relatively healthy period of life.

6 Conclusions

In conclusion, by assessing the associations of air pollution with ABI, ECG parameters, and IS-related biomarkers, this thesis demonstrates that long-term exposure to air pollution was associated with a higher prevalence of peripheral atherosclerosis and arterial stiffness, as well as decreased IS and more pronounced deterioration of IS over time. Furthermore, in a potentially susceptible study population, short-term air pollution exposure was associated with delays in atrioventricular conduction, ventricular depolarization and repolarization. This thesis extends the literature on biological mechanisms linking air pollution to the development of cardiometabolic disease, and provides guidance for the targeted interventions to mitigate the adverse health effects of air pollution. We acknowledge that the observational data in this thesis limited our ability to make causal inferences, and the estimated residential exposure without allowing for the mobility of participants might have resulted in non-differential exposure misclassification and biased the effects towards the null. Therefore, further studies are needed to confirm our findings and investigate the causal relationship between air pollution and pathophysiological responses of the cardiometabolic system.

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Manuscript I

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Long-term effects of air pollution on ankle-brachial index

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ABSTRACT

Background: Ankle-brachial index (ABI) has been linked to the risk of cardiovascular events. However, the association between long-term exposure to air pollution and abnormal ABI has not been fully investigated.

Methods: This cross-sectional study involved 4544 participants from the KORA Study (2004–2008) in the region of Augsburg, Germany. Participants' residential annual mean concentrations of particulate matter (PM) and nitrogen dioxide (NO_2) were predicted with land-use regression models, and the traffic information was collected from geographic information systems. We applied multinomial logistic regression models to assess the effects of air pollution on the prevalence of low and high ABI, and quantile regression models to explore the non-monotonic relationship between air pollution and ABI. We also examined effect modification by individual characteristics.

Results: Long-term exposure to PM with an aerodynamic diameter ≤ 10 μm (PM_{10}) and ≤ 2.5 μm ($PM_{2.5}$) was significantly associated with a higher prevalence of low ABI, with the respective odds ratios (ORs) of 1.82 (95%CI: 1.11–2.97) and 1.59 (95%CI: 1.01–2.51) for a 5th to 95th percentile increment in pollutants. Positive associations with the prevalence of high ABI were observed for PM (e.g., PM_{10}: OR = 1.63, 95%CI: 1.07–2.50) and NO_2 (OR = 1.84, 95%CI: 1.15–2.94). Quantile regression analyses revealed similar non-monotonic results. The effects of air pollution on having abnormal ABI were stronger in physically inactive, hypertensive, or non-diabetic participants.

Conclusions: Long-term exposure to PM and NO_2 was associated with a higher prevalence of both low and high ABI, indicating the adverse effects of air pollution on atherosclerosis and arterial stiffness in the lower extremities.

1. Introduction

The ankle-brachial index (ABI) is the ratio of systolic blood pressure at the ankle to that at the brachial artery. A low ABI indicates the presence of atherosclerosis and is used clinically in the diagnosis of peripheral artery disease (PAD) (Heald et al., 2006). A high ABI implies the incompressibility of vessels due to arterial stiffness in the lower extremities (Aboyans et al., 2012). Epidemiological studies have found that both low and high ABI are associated with increased risk of cardiovascular disease and mortality (Resnick et al., 2004; O'Hare et al., 2006; Lamina et al., 2006; Ankle Brachial Index Collaboration, 2008). In addition, individuals with low ABI show greater deterioration of physical function (McDermott et al., 2004).

As a major underlying pathology of cardiovascular disease, the prevalence and progression of atherosclerosis have been linked to air pollution, mainly by using carotid intima-media thickness (CIMT), coronary arterial or aortic calcification as indicators (Adar et al., 2013; Kaufman et al., 2016; Kälsch et al., 2014). The atherosclerotic effects of air pollution on different vascular beds are heterogeneous (Diez Roux et al., 2008; Wang et al., 2016). So far, only a few studies investigated the chronic effects of air pollution on low ABI, and yielded inconsistent results. A study from Germany found that living within 50 m of a main road was associated with a higher prevalence of low ABI (Hoffmann et al., 2009). However, the North-American Multi-Ethnic Study of Atherosclerosis (MESA) reported lower prevalence of low ABI among participants exposed to elevated particulate matter (PM) (Diez Roux et al., 2008).

Despite the association between arterial stiffness and atherosclerosis

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(van Popele et al., 2001), the air pollution effects on low and high ABI could be distinct. A study from Spain observed significantly positive associations of residential nitrogen dioxide (NO_2), traffic load, and traffic intensity with the prevalence of high ABI, while no associations with low ABI (Rivera et al., 2013). Arterial stiffness was found to be associated with acute exposure to air pollution in observational and experimental studies (Mehta et al., 2014; Schneider et al., 2008; Lundback et al., 2009); yet evidence of the long-term effects of air pollution is limited, especially on the stiffness in lower extremity arteries (O'Neill et al., 2011; Lenters et al., 2010).

Given the different pathologies of atherosclerosis and arterial stiffness, we hypothesized a non-monotonic relationship between air pollution and ABI, in which long-term exposure to air pollution would increase the prevalence of both low and high ABI. In the framework of the KORA Cohort (Cooperative Health Research in the Region of Augsburg), we conducted this cross-sectional study to test our hypothesis for air pollution measures including PM with an aerodynamic diameter ≤ 10 μm (PM_{10}), 2.5–10 μm (PM_{coarse}), ≤ 2.5 μm ($PM_{2.5}$), $PM_{2.5}$ absorbance ($PM_{2.5abs}$) as a proxy of elemental carbon levels related to traffic exhaust, NO_2, traffic intensity on the nearest major road, and traffic load within 100 m of the residence.

2. Methods

2.1. Study population

The data for this cross-sectional study were taken from KORA F3 (2004–2005) and F4 (2006–2008), which are population-based surveys among registered German residents in Augsburg and its two adjacent counties (Southern Germany) (Holle et al., 2005). The KORA Study was approved by the ethics committee and all participants gave written informed consent.

2.2. Outcome measurement

Systolic blood pressure was measured in supine position after resting for at least 15 min. The measurements were taken twice in the posterior tibial artery of each ankle and the brachial artery of the right arm using a Doppler probe for pulse detection. We inflated the cuff to about 30 mmHg above the usual systolic blood pressure of the participant and then deflated it by 2–3 mmHg per second. The blood pressure at which the Doppler probe redetected the pulse was recorded as the systolic blood pressure of the limb. The order of measurements was right arm, right leg and left leg, and repeated measurements were in the same order. If the two values of one limb differed by > 10 mmHg, a third measurement was taken. We calculated the ABI of each side separately as the ratio of average systolic blood pressure at the ankle to that at the right arm. We defined participants having normal ABI as participants with $0.9 < ABI \leq 1.4$ in both legs. Participants with $ABI \leq 0.9$ in one or both legs were defined as having low ABI; participants with $ABI > 1.4$ in one leg and normal ABI in the contralateral leg, or with $ABI > 1.4$ in both legs were defined as having high ABI (Aboyans et al., 2012; Rooke et al., 2011; Allison et al., 2008). For participants with ABI values only in one leg, the available ABI value was classified into low ($ABI \leq 0.9$), normal ($0.9 < ABI \leq 1.4$) and high ($ABI > 1.4$).

2.3. Exposure assessment

We estimated the annual average concentration of air pollutants within the European Study of Cohorts for Air Pollutant Effects (ESCAPE) based on standardized protocols (Eeftens et al., 2012; Beelen et al., 2013). In brief, PM and nitrogen oxides were monitored at 20 and 40 sites, respectively, in the region of Augsburg and Munich. Three two-week measurements were taken in different seasons between October 2008 and July 2009, and the monitored values were used to calculate annual mean concentration for each site. Meanwhile, geographic variables from the geographic information system (GIS) were collected to build land-use regression (LUR) models to estimate the individual outdoor pollutant concentrations, including PM_{10}, PM_{coarse}, $PM_{2.5}$, $PM_{2.5abs}$ and NO_2 at each participant's home address. The model explained variance (R^2) for the investigated pollutants ranged from 78% ($PM_{2.5}$) to 91% ($PM_{2.5abs}$), and the leave-one-out cross validation R^2 ranged from 62% ($PM_{2.5}$) to 82% ($PM_{2.5abs}$). Residential background NO_2 levels were predicted using a similar method except that the LUR model was developed with only background monitoring data and GIS predictors. Traffic intensity on the nearest major road (> 5000 vehicles/day) and traffic load within 100 m of the residence (sum of traffic intensity multiplied by length of major roads in a 100 m buffer), were also analyzed in this study.

To control the effect of long-term road traffic noise, annual average Day-Night Sound Level (dB(A) Leq) was estimated for each participant's residential address using the model developed by ACCON GmbH (Pitchika et al., 2017).

2.4. Potential confounding and mediating factors

Trained medical staff administered a standardized face-to-face interview to collect information on sociodemographic characteristics (age, sex, marital status, years of education, current occupation, and income), lifestyle variables (smoking status, smoking pack years, alcohol consumption, and physical activity), self-reported medical history (hypertension, diabetes, myocardial infarction, angina pectoris, and stroke), and medication intake (antihypertensive drugs, antidiabetic drugs, anticoagulants, antiplatelet drugs, and statins). In addition, physical examinations and laboratory tests were conducted to obtain anthropometric data (height, weight, waist and hip circumference), systolic and diastolic blood pressure, blood lipid levels, and glomerular filtration rate (Meisinger et al., 2002). We also assessed neighborhood socioeconomic status (SES) by the percentage of households with low income (< 1250 €) in $(5\,km)^2$ grid cells based on participants' home addresses.

We defined smoking pack years as the number of packs of cigarettes (20 cigarettes per pack) smoked per day multiplied by the number of years the participant had smoked, which is an indicator of the lifelong cumulative exposure to tobacco smoke. Body mass index (BMI) was calculated as weight divided by height squared. Waist-hip ratio was calculated as waist circumference divided by hip circumference. We categorized physical activity based on the time spent on physical exercise and converted it into low level (no or almost no physical exercise), medium level (about one hour per week), and high level (more than two hours per week). Hypertension was defined by blood pressure ≥ 140/90 mmHg or taking antihypertensive medication in people reporting a previous diagnosis of hypertension. Participants who reported doctor-diagnosed diabetes or taking antidiabetic medication were defined as having diabetes.

2.5. Statistical analysis

We applied multinomial logistic regression to investigate the association between air pollution and abnormal ABI. The minimum model was adjusted for age, sex, time trend, and a dummy variable for study. The time trend was modeled as a penalized spline of day of year with three degrees of freedom. Other covariates were chosen by minimizing Bayesian Information Criterion. The main model additionally adjusted for years of education, neighborhood SES, smoking pack years, and smoking status. The extended model further adjusted for diabetes and hypertension. When analyzing the effects of traffic indicators, background NO_2 level was additionally controlled for. Results are presented as odds ratio (OR) of low ABI and high ABI with reference to normal ABI for increments from 5th to 95th percentiles in exposure with a 95% confidence interval (95%CI).

We also conducted quantile regression to explore the non-monotonic relationship between air pollution and ABI. The effects of air pollution were examined on different percentiles of the ABI distribution (using the lower ABI value of two sides) from the 5th to 95th quantiles with a 5% increment. The confounders being adjusted for in the quantile regression models were identical to those in the main multinomial logistic regression model.

To examine modification effects of individual characteristics, we incorporated an interaction term between the exposure and the potential modifier into the main model. Potential effect modifiers included age (≥60 years vs. < 60 years), sex (male vs. female), physical activity (low vs. medium or high), overweight (BMI ≥ 25 kg/m^2 vs. < 25 kg/m^2), hypertension (yes vs. no), and diabetes (yes vs. no).

2.6. Sensitivity analysis

We applied two-pollutant models for PM and NO_2 by adding a second exposure variable (air pollution or noise) with a Spearman's correlation coefficient < 0.7. We also explored the interaction effects between PM and NO_2 by adding an interaction term of PM and dichotomized NO_2 or vice versa, using the median concentration as the cut-off value. To reduce exposure misclassification due to change of residence, a subgroup of participants who had lived at the same address for at least five years before the F3 or F4 survey were analyzed. Given the different age structures in F3 and F4, we excluded participants in F3 who were below the minimum age of F4 (51 years) to guarantee a non-differential age structure. Moreover, we tested if the associations were sensitive to further adjustment for significant effect modifiers, which were not included in models as confounders or mediators.

In addition, we assessed the impact of changing the definition of having high ABI on the associations between air pollution and the prevalence of abnormal ABI, considering the mixed definitions in previous studies (Ankle Brachial Index Collaboration, 2008; Rivera et al., 2013; Allison et al., 2008; Hendriks et al., 2016). Thus, we applied an upper cut-off point of either 1.3 or 1.4, in combination with an alteration in the ABI values using the lower ABI of two sides. We also used the higher ABI of two sides in the quantile regression.

All analyses were conducted with R version 3.4.1 using the 'mgcv' and 'quantreg' packages. The significance level alpha was set at 0.05.

3. Results

3.1. Participant characteristics and exposure concentrations

Among altogether 4775 participants with ABI measurement, we excluded 75 individuals because the residential information was not available. Further 156 individuals were excluded due to incomplete data on main covariates, leaving 4544 participants for analyses. The main participant characteristics are summarized in Table 1. The prevalence of low and high ABI were 4.6% and 6.0%, respectively. There were two participants with low ABI in one leg and high ABI in the contralateral leg, who were classified as having low ABI based on our criteria. Differences between ABI subgroups were significant for all presented individual characteristics (p < 0.001). Participants with low ABI were likely to be of lower socioeconomic status, have a higher proportion of current or former smokers and more smoking pack years, do less physical exercise, and have higher prevalence of hypertension and diabetes. There were more male and overweight individuals in the high ABI group, and participants with abnormal ABI were older than the ones with normal ABI. Participants exposed to a higher level of air pollution tended to be older, of lower neighborhood SES, have higher prevalence of smokers and more smoking pack years, and have higher prevalence of hypertension (Supplemental Table S1).

Annual mean levels of air pollution and noise are presented in Table 2. Regulated air pollutants were well below the European Union limits of 40 μg/m^3 for PM_{10} and NO_2, and 25 μg/m^3 for $PM_{2.5}$, but PM_{10} and $PM_{2.5}$ exceeded the World Health Organization guidelines of 20 μg/m^3 and 10 μg/m^3, respectively. The correlation between exposure variables was weak or moderate ($r_s \leq 0.7$), except for PM_{coarse} being highly correlated with PM_{10}, $PM_{2.5abs}$, and NO_2. The Spearman correlation coefficients between air pollution and age, years of education, neighborhood SES, and smoking pack years were all low, except for NO_2 and background NO_2 with neighborhood SES (Supplemental Table S2).

3.2. Air pollution and ABI

In our minimum models, PM and NO_2 were positively associated with having low ABI; the associations with having high ABI were positive, but only significant for PM_{coarse} (Table 3). After adjusting for further confounders in the main model, we observed significant positive associations with the prevalence of low ABI for PM_{10} and $PM_{2.5}$, and a borderline significant association for NO_2. The prevalence of high ABI was significantly associated with PM_{10}, PM_{coarse}, $PM_{2.5abs}$, and NO_2, and borderline significantly associated with $PM_{2.5}$. The positive associations with having low and high ABI were weak and non-significant for traffic indicators. Further adjustment for diabetes and hypertension (extended model) did not substantially affect the estimates of air pollution.

We further explored the covariates that drove the changes in air pollution effects between the minimum and main models (Supplemental Fig. S1). The effects of PM_{10}, PM_{coarse}, $PM_{2.5abs}$, and NO_2 changed significantly after controlling for neighborhood SES, showing a decrease on having low ABI, and an increase on having high ABI. The further adjustment for smoking status and smoking pack years slightly reduced the effects on having low ABI.

3.3. Quantile regression

The directions of associations with air pollution were generally opposite for the low and the high ends of the ABI distribution (Fig. 1; Supplemental Fig. S2). In specific, elevated air pollution was associated with a decrease in ABI lower than 0.98 (i.e., the 5th and 10th percentiles), which indicated an increased risk for having low ABI, and the association was statistically significant for $PM_{2.5abs}$. Among participants with ABI larger than 1.28 (i.e., the 90th and 95th percentiles), elevated air pollution was associated with an increase in ABI, representing an increased risk for having high ABI. Furthermore, the positive association between $PM_{2.5}$ and ABI already occurred in individuals with comparatively low ABI of around 1.14 (i.e., the 50th percentile).

3.4. Effect modification by individual characteristics

The effects of PM and NO_2 on the prevalence of low ABI were stronger among participants doing little or no physical activity, or having hypertension. Non-diabetic participants were more susceptible to the effects of PM and NO_2 on the prevalence of high ABI (Fig. 2). No significant or consistent patterns were observed for other potential effect modifiers (Supplemental Fig. S3).

3.5. Sensitivity analyses

The two-pollutant models showed in general robust results (Fig. 3). For the prevalence of low ABI, we observed a slightly attenuated effect of PM_{10} when adjusted for $PM_{2.5}$ and vice versa, and attenuated effects of $PM_{2.5abs}$ and NO_2 when adjusted for PM_{10} and $PM_{2.5}$. For the prevalence of high ABI, the effect of PM_{10} was decreased by the inclusion of $PM_{2.5abs}$ and NO_2, and the effect of $PM_{2.5}$ were decreased when adjusted for the other PM metrics and NO_2. Besides, we did not find significant interaction between PM and NO_2 (Supplemental Table S3).

The associations between air pollution and the prevalence of high ABI were sensitive to the definition of high ABI (Supplemental Table S4). Changing the upper cut-off value to 1.3 decreased the effects of PM

Table 1
Descriptive statistics of participant characteristics.

	Mean ± SD/N (%)			
	All (n = 4544)	Low ABI (n = 209)	Normal ABI (n = 4064)	High ABI (n = 271)
ABI	1.13 ± 0.15	0.76 ± 0.11	1.14 ± 0.09	1.41 ± 0.23
Age (years)	60.1 ± 11.8	67.9 ± 9.7	59.3 ± 11.8	66.3 ± 9.3
Sex (male)	2204 (48.5)	119 (56.9)	1874 (46.1)	211 (77.9)
BMI (kg/m^2)[a]	28.0 ± 4.6	29.1 ± 5.1	27.8 ± 4.6	29.3 ± 4.4
Years of education	11.4 ± 2.6	10.4 ± 2.1	11.4 ± 2.6	11.4 ± 2.6
Percentage of households with low income in $(5\,km)^2$ grid cell (%)	27.9 ± 18.4	33.4 ± 17.5	27.7 ± 18.4	26.4 ± 18.2
Smoking pack years	11.5 ± 19.6	29.0 ± 29.5	10.8 ± 18.6	9.1 ± 19.2
Smoking status				
Current smoker	748 (16.5)	61 (29.2)	680 (16.7)	7 (2.6)
Former smoker	1725 (38.0)	99 (47.4)	1502 (37.0)	124 (45.8)
Never smoker	2071 (45.6)	49 (23.4)	1882 (46.3)	140 (51.7)
Physical activity				
Low	1532 (33.7)	117 (56.0)	1324 (32.6)	91 (33.6)
Medium	1977 (43.5)	63 (30.1)	1809 (44.5)	105 (38.7)
High	1035 (22.8)	29 (13.9)	931 (22.9)	75 (27.7)
Hypertension (yes)	2318 (51.0)	163 (78.0)	1997 (49.1)	158 (58.3)
Diabetes (yes)	397 (8.7)	60 (28.7)	293 (7.2)	44 (16.2)
Overweight (yes)	3312 (72.9)	161 (77.0)	2916 (71.8)	235 (86.7)

[a] Data on BMI were available for 4527 participants; $N_{Low\ ABI} = 206$; $N_{High\ ABI} = 269$.

and NO_2, leaving only a borderline significant association for PM_{coarse}. When defining high ABI with the lower ABI of two sides and a cut-off value of 1.4, we observed attenuated effect estimates, except for the increased point estimates of $PM_{2.5abs}$ and traffic indicators; similar patterns were shown when we further changed the cut-off value to 1.3. The associations between air pollution and the prevalence of low ABI remained stable when changing the definition of high ABI.

Participants living for at least five years at the same residential address showed slightly stronger associations for PM_{10} and having low ABI, as well as for PM_{coarse}, $PM_{2.5abs}$, and having high ABI (Supplemental Table S5). Similar results were observed for participants over 50 years old. Excluding participants with low ABI in one leg and high ABI in the contralateral leg or further adjustment for physical activity did not affect the results substantially. We could still see the non-monotonic relationship between air pollution and ABI when using the higher ABI of two sides in the quantile regression model (Supplemental Fig. S4).

4. Discussion

In this cross-sectional study, the prevalence of low and high ABI were positively associated with residential long-term exposure to PM and NO_2, but were not significantly associated with traffic intensity or traffic load. The results were robust to further control for comorbidity. The findings in quantile regression models also supported the non-monotonic relationship between air pollution and ABI. We observed stronger effects of PM and NO_2 on the prevalence of low ABI in participants doing little or no physical activities or having hypertension, and stronger effects on high ABI in non-diabetic participants.

Our result of the association between PM and having low ABI reflected the effects of air pollution on peripheral atherosclerosis. Previous studies also observed an increased prevalence of low ABI associated with living in proximity to main roads, but not with annual average $PM_{2.5}$, NO_2, traffic intensity, or traffic load (Wang et al., 2016; Hoffmann et al., 2009; Rivera et al., 2013). Our findings are supported by the positive associations between air pollution and the prevalence and progression of atherosclerosis in other vascular beds. For instance, cross-sectional analyses in the German HNR Study reported significant effects of $PM_{2.5}$ on thoracic aortic calcification and CIMT (Kälsch et al., 2014; Bauer et al., 2010). In the MESA Study, an increased level of $PM_{2.5}$ was associated with accelerated progression in CIMT (Adar et al., 2013).

Several mechanisms contributing to the effect of air pollution on atherosclerosis have been proposed and mainly involve systemic inflammation and oxidative stress. In animal experiments, Sun et al. (2005) demonstrated that exposure to concentrated ambient $PM_{2.5}$ exacerbated plaque progression and affected vascular constriction function in high-fat chow $ApoE^{-/-}$ mice, with elevated vascular inflammation and protein nitration. Human studies have also shown that air pollution is associated with increased serum biomarkers of

Table 2
Descriptive statistics and Spearman correlation coefficients of exposure for the study population.

	Mean (SD)	5%–95%	Correlation coefficients							
			PM_{10}	PM_{coarse}	$PM_{2.5}$	$PM_{2.5abs}$	NO_2	Traffic intensity	Traffic load	Background NO_2
PM_{10} ($\mu g/m^3$)	20.3 (2.3)	16.5–24.1	–							
PM_{coarse} ($\mu g/m^3$)	6.2 (1.0)	4.9–8.2	0.75	–						
$PM_{2.5}$ ($\mu g/m^3$)	13.5 (0.8)	12.4–15.2	0.43	0.30	–					
$PM_{2.5abs}$ ($10^{-5}/m$)	1.7 (0.2)	1.5–2.0	0.66	0.83	0.47	–				
NO_2 ($\mu g/m^3$)	18.6 (3.7)	13.7–25.4	0.67	0.78	0.44	0.66	–			
Traffic intensity on nearest road (vehicles/day)	1539 (3273)	500–7841	0.09	0.14	0.16	0.16	0.21	–		
Traffic load of main roads in 100 m buffer (10^6 vehicles*m/day)	0.5 (1.2)	0–3.1	0.21	0.28	0.33	0.37	0.42	0.35	–	
Background NO_2 ($\mu g/m^3$)	18.4 (3.4)	14.0–24.6	0.30	0.28	0.21	0.21	0.60	0.12	0.29	–
Noise (dB(A))	54.6 (6.5)	44.7–67.1	0.28	0.36	0.38	0.45	0.41	0.40	0.49	0.29

Table 3
ORs (95%CI) of having low and high ABI corresponding to an increase in exposure from the 5th to the 95th percentile.

	Air pollution	Minimum model[a]	Main model[b]	Extended model[c]
Low ABI	PM_{10}	2.27 (1.43, 3.61)**	1.82 (1.11, 2.97)*	1.79 (1.09, 2.94)*
	PM_{coarse}	1.89 (1.24, 2.90)**	1.39 (0.87, 2.22)	1.36 (0.85, 2.19)
	$PM_{2.5}$	1.80 (1.15, 2.81)**	1.59 (1.01, 2.51)*	1.50 (0.94, 2.38)†
	$PM_{2.5abs}$	1.83 (1.20, 2.80)**	1.43 (0.91, 2.23)	1.41 (0.90, 2.22)
	NO_2	2.17 (1.44, 3.28)**	1.59 (0.94, 2.68)†	1.55 (0.91, 2.64)
	Traffic intensity	1.15 (0.89, 1.50)	1.16 (0.88, 1.52)	1.15 (0.87, 1.51)
	Traffic load	1.15 (0.82, 1.62)	1.14 (0.79, 1.62)	1.10 (0.77, 1.57)
High ABI	PM_{10}	1.44 (0.95, 2.17)†	1.63 (1.07, 2.50)*	1.65 (1.08, 2.53)*
	PM_{coarse}	1.54 (1.04, 2.29)*	1.92 (1.26, 2.92)**	1.94 (1.27, 2.95)**
	$PM_{2.5}$	1.39 (0.91, 2.10)	1.44 (0.94, 2.20)†	1.44 (0.94, 2.20)†
	$PM_{2.5abs}$	1.45 (0.98, 2.14)†	1.73 (1.14, 2.61)**	1.75 (1.16, 2.65)**
	NO_2	1.21 (0.81, 1.79)	1.84 (1.15, 2.94)*	1.87 (1.16, 3.01)**
	Traffic intensity	1.19 (0.92, 1.54)	1.20 (0.93, 1.55)	1.21 (0.94, 1.56)
	Traffic load	1.07 (0.76, 1.50)	1.12 (0.79, 1.58)	1.12 (0.80, 1.58)

An increase from the 5th to the 95th percentile was 7.6 μg/m^3 for PM_{10}, 3.4 μg/m^3 for PM_{coarse}, 2.8 μg/m^3 for $PM_{2.5}$, 5.2 × 10^{-6}/m for $PM_{2.5abs}$, 11.7 μg/m^3 for NO_2, 7341 vehicles/day for traffic intensity on the nearest road, and 3.1 × 10^6 vehicles m/day for traffic load in a 100 m buffer.

[a] The minimum model was adjusted for age, sex, day of year, and study.

[b] The main model was adjusted for age, sex, day of year, study, years of education, neighborhood SES, smoking status, and smoking pack years.

[c] Main model further adjusted for diabetes and hypertension.

† p-Value < 0.1.

* p-Value < 0.05.

** p-Value < 0.01.

inflammation and oxidative stress, such as cytokines, C-reactive protein, and reactive oxygen species (Hajat et al., 2015; Risom et al., 2005; Rückerl et al., 2014). These mediators can promote the formation of foam cells and fibrous plaque in arteries by inducing endothelial dysfunction and leucocyte transmigration (Freitas Lima et al., 2015).

Our results showing significant effects of air pollution on the prevalence of high ABI indicated an association between air pollution and arterial stiffness in the lower extremities. Arterial stiffness is due to calcification in the medial layer of the arterial wall and is often seen in patients with diabetes or end-stage renal disease (Al-Aly, 2007; Schwaiger et al., 2006). It has been linked to acute exposure to $PM_{2.5}$ in the elderly and diabetic individuals (Mehta et al., 2014; Schneider et al., 2008). In a double-blind experimental study, acute exposure to diesel exhaust was also shown to have immediate effects on arterial stiffness in healthy men (Lundback et al., 2009). However, the chronic effect of air pollution on arterial stiffness is less prominent. In the Atherosclerosis Risk in Young Adults Study, Lenters et al. (2010) reported significant effects on arterial stiffness for long-term exposure to NO_2 and SO_2, but not for $PM_{2.5}$, black smoke, or traffic indicators. Furthermore, the MESA Study found no association for particle

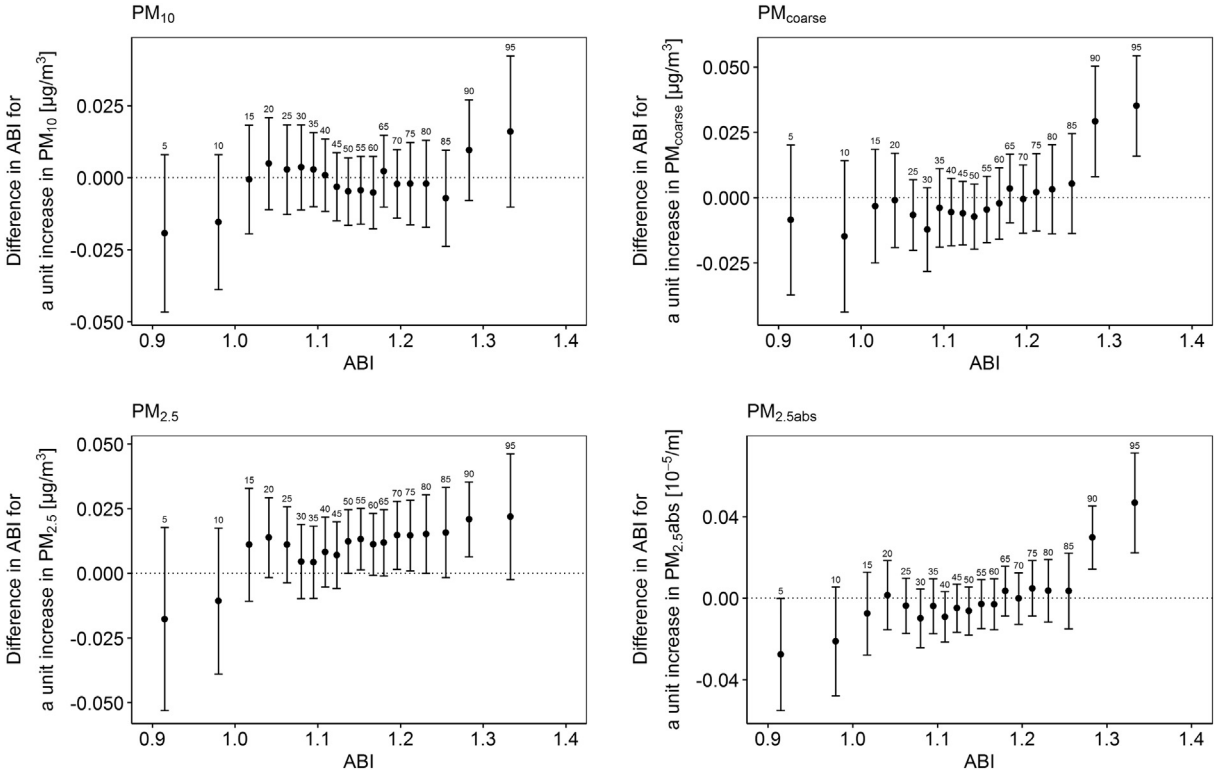

Fig. 1. Absolute difference in ABI at the 5th to 95th percentiles associated with an increase from the 5th to the 95th percentile in PM_{10}, PM_{coarse}, $PM_{2.5}$, and $PM_{2.5abs}$.

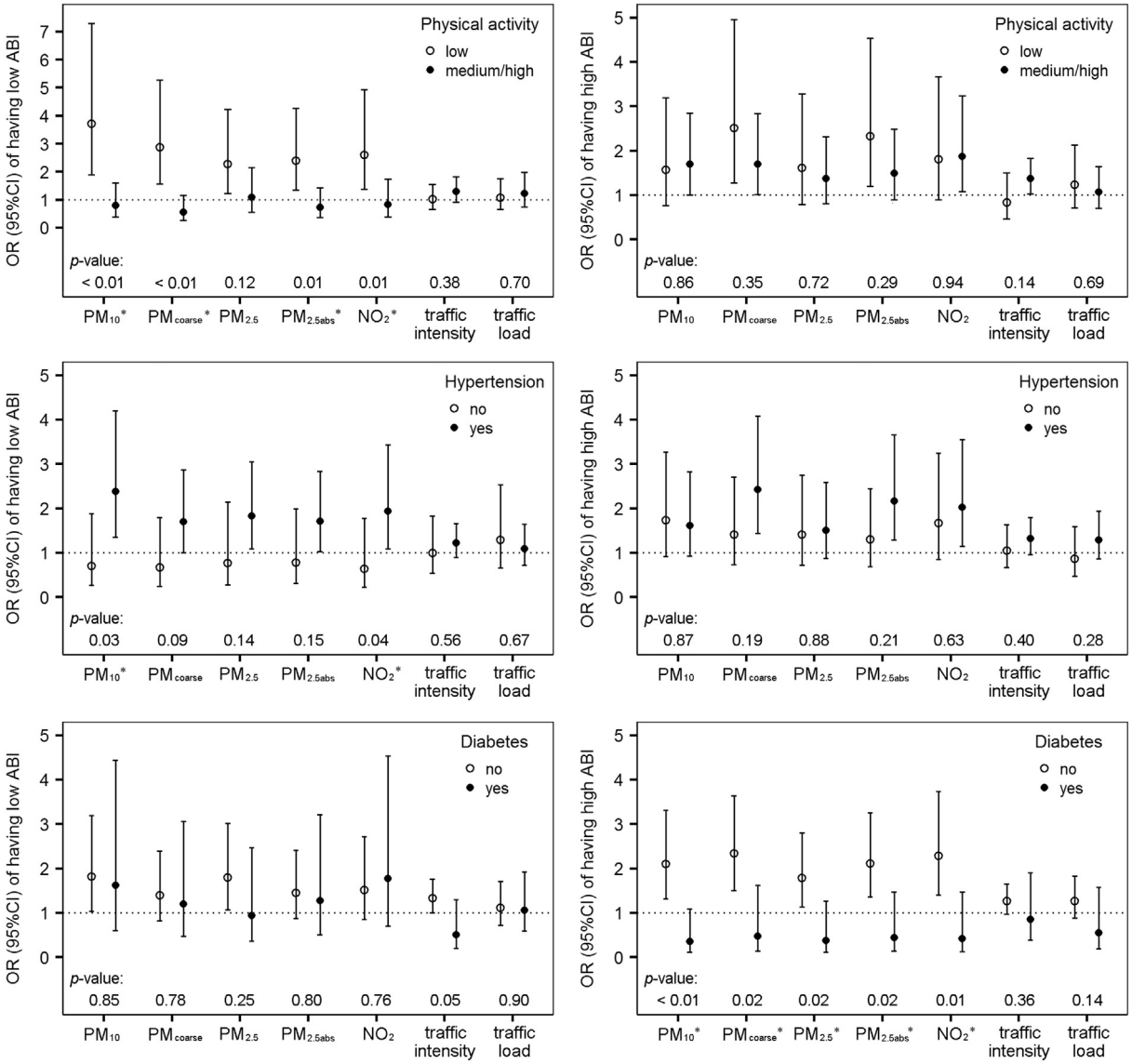

Fig. 2. Effect modification by physical activity, hypertension, and diabetes on the associations of air pollution with low ABI (left panel) and high ABI (right panel). *p-Value for the interaction term < 0.05.

exposure (O'Neill et al., 2011).

The measures used in previous studies indicated mostly central arterial stiffness, whereas our study provided evidence of the chronic effects of air pollution on stiffness in the artery of lower extremities. Consistent with our results, the Girona Heart Register (REGICOR) Study found an increased prevalence of high ABI associated with residential 10-year average NO_2 (Rivera et al., 2013). However, the effects of traffic intensity and traffic load were also significant in the REGICOR Study while not in our study. One potential explanation might be the difference in defining high ABI. We noticed that the effect estimates of having high ABI changed substantially for most pollutants when using different definitions of high ABI, reflecting the bias caused by the outcome misclassification. Using the ABI values of both sides is a more appropriate method in identifying high ABI, which could reduce the risk of misclassification in participants with high ABI in one leg and normal ABI in the contralateral leg. When we applied the same definition as in the REGICOR Study (categorizing the lower ABI with a cut-off value of 1.3), more consistent results were found for traffic indicators but not for NO_2. The comparatively low contrast of NO_2 in Augsburg (5th to 95th percentile: 11.7 μg/m^3 in Augsburg vs. 25 μg/m^3 in Girona) may have contributed to limited statistical power to detect significant effects.

Our study showed that physically inactive or hypertensive participants were at greater risk from effects of air pollution on atherosclerosis. Similar effect modification by hypertension was also reported on the associations of air pollution with coronary artery calcification and inflammatory markers (Kaufman et al., 2016; Dubowsky et al., 2006). Although physically inactive lifestyle is associated with systemic inflammation and subclinical atherosclerosis (Bertoni et al., 2008; Hamer et al., 2012), there exists no clear evidence of the interaction effect between physical activity and air pollution (Zhang et al., 2018; Andersen et al., 2015). Thus, the effect modification by physical activity should be interpreted with caution as it might be partly attributable to differential misclassification of air pollution exposure. Regarding the prevalence of high ABI, we observed stronger air pollution effects among non-diabetic participants. This finding was not expected because it has been shown that diabetes increases the susceptibility of air pollution-induced impairment of vascular reactivity (O'Neill et al., 2005). Further investigation is still needed to confirm this effect modification in other populations and clarify the mechanisms.

The two-pollutant models revealed independent effects of PM_{10} and $PM_{2.5}$ on atherosclerosis, as well as independent effects of PM_{coarse},

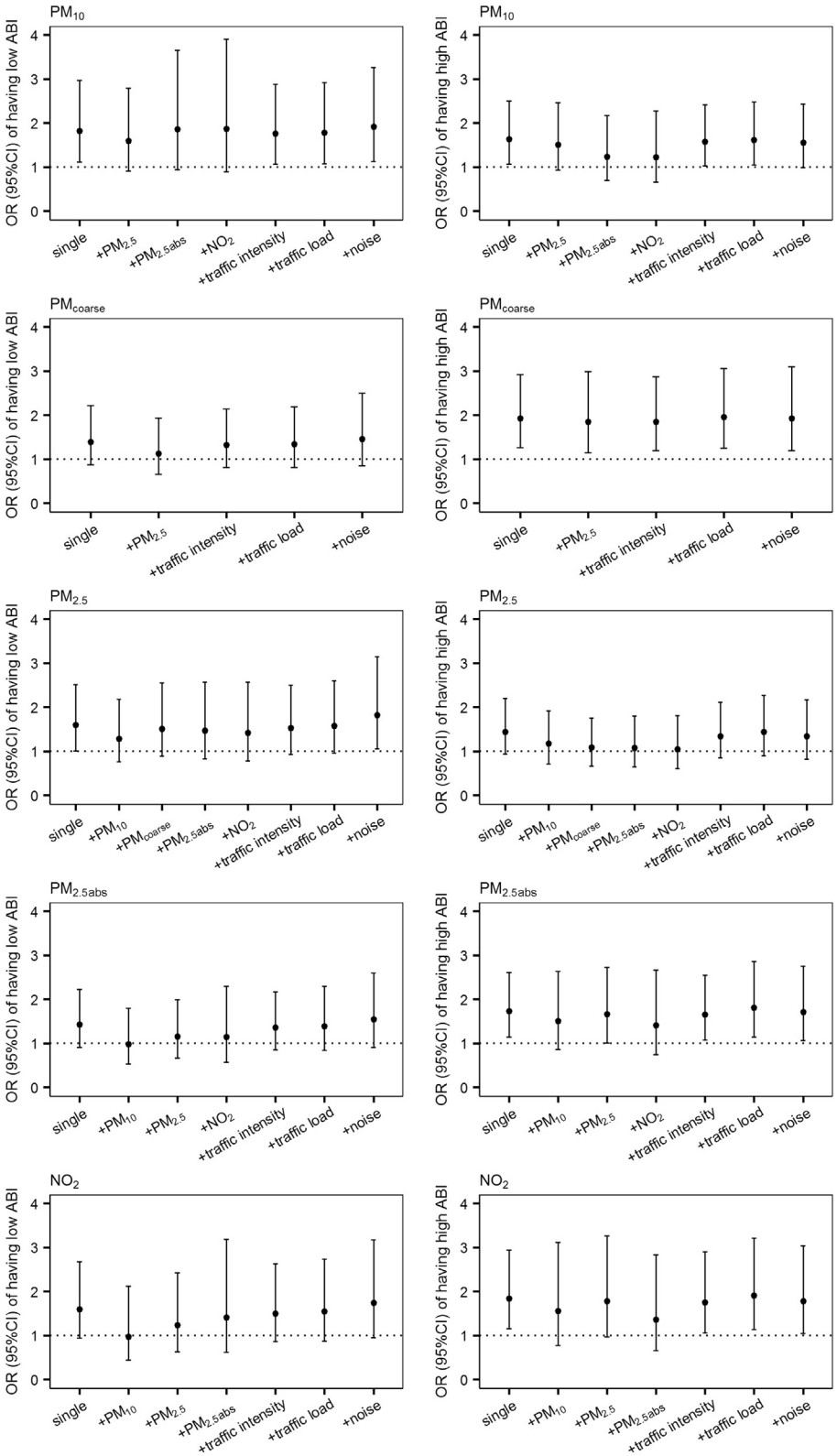

Fig. 3. ORs (95%CIs) of having low ABI (left panels) and high ABI (right panels) in single and two-pollutant models for PM and NO_2.

$PM_{2.5abs}$, and NO_2 on arterial stiffness in the lower extremities. Given the strong correlation between $PM_{2.5abs}$, NO_2, and PM_{coarse}, we did not build two-pollutant models for $PM_{2.5abs}$ and NO_2 with co-adjustment for PM_{coarse}. Therefore, the effects of $PM_{2.5abs}$ and NO_2 could not be separated from PM_{coarse} and vice versa. The positive associations of $PM_{2.5abs}$ and NO_2 with the prevalence of low ABI were attenuated to the null by the inclusion of PM_{10} and $PM_{2.5}$. This finding indicated that the effects of $PM_{2.5abs}$ and NO_2 on atherosclerosis could be substantially explained by PM. The same pattern was also observed for PM_{10} and $PM_{2.5}$ with the prevalence of high ABI by $PM_{2.5abs}$ and NO_2.

The effects of PM_{coarse}, $PM_{2.5abs}$, and NO_2, which are the more spatially heterogeneously distributed pollutants, on ABI were

significantly affected by the inclusion of neighborhood SES. This finding might be due to the moderate to strong correlation between air pollution and the neighborhood SES indicator. Studies have shown that individuals living in deprived areas tended to be exposed to higher levels of air pollution and have poorer health outcomes (Havard et al., 2009; Hajat et al., 2013; Diez Roux and Mair, 2010). Thus, it is necessary to account for the potential confounding effect of neighborhood SES in analyses on air pollution and health. We did not find substantial impact of neighborhood SES on the effect estimates of traffic indicators. This could result from the further adjustment of background NO_2, which was strongly correlated with neighborhood SES.

The strengths of this study include the large sample size and a comprehensive investigation of various air pollution indicators. Repeated ABI measurements by trained nurses according to a highly standardized protocol also enhanced the reliability of data. Besides, concerning the distinct interpretations of low and high ABI, the multinomial logistic regression and quantile regression analyses allowed for a better understanding of the nonlinear relationship between air pollution and ABI.

One limitation of our study is the lack of data on ABI progression to make causal inference. Future follow-up surveys might provide data to fill this gap. Secondly, the exposure assessment in this study was based on the LUR models using pollutant concentrations monitored in 2008 and 2009. This approach relied on the hypothesis that the spatial variation stayed stable over time. Although previous studies supported this assumption (Cesaroni et al., 2012; Eeftens et al., 2011), there could also exist non-differential exposure misclassification that would bias the effect estimates towards null. Furthermore, the limited contrast in exposure across the study area may reduce the statistical power to detect significant associations, and cause null findings for some pollutants.

5. Conclusions

In summary, long-term residential exposure to PM and NO_2 was associated with a higher prevalence of both low and high ABI. This study provides evidence for the effects of air pollution on atherosclerosis and stiffness in lower extremity arteries.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.05.025.

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SUPPLEMENTAL MATERIAL

Full Title: Long-term effects of air pollution on ankle-brachial index

Authors: Siqi Zhang, Kathrin Wolf, Susanne Breitner, Florian Kronenberg, Massimo Stafoggia, Annette Peters, Alexandra Schneider

Contents

Abbreviations

ABI = ankle-brachial index

BMI = body mass index

CI = confidence interval

CIMT = carotid intima-media thickness

GIS = geographic information system

LUR = land use regression

NO_2 = nitrogen dioxide

OR = odds ratio

PAD = peripheral artery disease

PM = particulate matter

PM_{10} = particulate matter with an aerodynamic diameter ≤ 10 μm

PM_{coarse} = particulate matter with an aerodynamic diameter > 2.5 μm and ≤ 10 μm

$PM_{2.5}$ = particulate matter with an aerodynamic diameter ≤ 2.5 μm

SES = socioeconomic status

Table S1 Descriptive statistics of participant characteristics by quartile of annual concentration of PM_{10}.

	Mean ± SD / N (%)				
	Q1 (14.8–18.5 µg/m^3)	Q2 (18.6–20.5 µg/m^3)	Q3 (20.6–21.8 µg/m^3)	Q4 (21.9–30.7 µg/m^3)	*p* for trend
	n = 1014	n = 1254	n = 1139	n = 1137	
ABI	1.15 ± 0.15	1.13 ± 0.14	1.14 ± 0.16	1.13 ± 0.16	0.04
ABI categories					< 0.001
low	30 (3.0)	56 (4.5)	54 (4.7)	69 (6.1)	
normal	926 (91.3)	1,138 (90.7)	1,008 (88.5)	992 (87.2)	
high	58 (5.7)	60 (4.8)	77 (6.8)	76 (6.7)	
Age (years)	58.6 ± 12.5	60.0 ± 11.5	60.8 ± 11.4	60.6 ± 11.8	< 0.001
Sex (male)	492 (48.5)	626 (49.9)	540 (47.4)	546 (48.0)	0.52
BMI (kg/m^2)[a]	28.0 ± 4.6	27.8 ± 4.6	27.9 ± 4.5	28.2 ± 4.8	0.32
Years of education	11.3 ± 2.7	11.4 ± 2.6	11.3 ± 2.6	11.3 ± 2.4	0.77
Percentage of households with low income in (5km)² grid cell (%)	18.6 ± 16.6	29.0 ± 18	30.0 ± 17.8	32.8 ± 17.9	< 0.001
Smoking pack years	9.9 ± 17.6	12.0 ± 19.5	11.3 ± 19.5	12.7 ± 21.5	0.006
Smoking status					0.03
current smoker	151 (14.9)	224 (17.9)	183 (16.1)	190 (16.7)	
former smoker	357 (35.2)	475 (37.9)	443 (38.9)	450 (39.6)	
never smoker	506 (49.9)	555 (44.3)	513 (45.0)	497 (43.7)	
Physical activity					0.63
low	349 (34.4)	393 (31.3)	379 (33.3)	411 (36.1)	
medium	459 (45.3)	549 (43.8)	500 (43.9)	469 (41.2)	
high	206 (20.3)	312 (24.9)	260 (22.8)	257 (22.6)	
Hypertension (yes)	470 (46.4)	646 (51.5)	592 (52.0)	610 (53.6)	0.001
Diabetes (yes)	79 (7.8)	112 (8.9)	95 (8.3)	111 (9.8)	0.17
Overweight (yes)	743 (73.3)	900 (71.8)	831 (73.0)	838 (73.7)	0.64

[a] Data on BMI were available for 4527 participants; $N_{Low\ ABI} = 206$; $N_{High\ ABI} = 269$.

Table S2 Spearman correlation coefficients between continuous confounders and air pollution.

	PM_{10}	PM_{coarse}	$PM_{2.5}$	$PM_{2.5abs}$	NO_2	Traffic intensity	Traffic load	Background NO_2
Age (years)	0.06	0.05	0.02	0.03	0.10	0.03	0.03	0.09
Years of education	0.02	0.04	0.02	0.02	0.06	-0.05	-0.01	0.03
Neighborhood SES	0.26	0.37	0.08	0.24	0.61	0.08	0.32	0.66
Smoking pack years	0.05	0.07	0.05	0.06	0.10	0.00	0.08	0.12

Table S3 ORs (95%CIs) of having low ABI and high ABI for PM and NO_2 in models with an interaction term.

		OR (95%CI) for PM [a]			OR (95%CI) for NO_2 [b]		
		NO_2 < median	NO_2 ≥ median	*p*-value [c]	PM < median	PM ≥ median	*p*-value [c]
Low ABI							
	PM_{10}	2.10 (0.84, 5.25)	1.81 (0.89, 3.68)	0.81	0.64 (0.20, 2.11)	1.60 (0.83, 3.06)	0.14
	$PM_{2.5}$	3.11 (1.14, 8.50)	1.29 (0.74, 2.26)	0.13	1.97 (0.74, 5.27)	1.24 (0.65, 2.35)	0.38
	$PM_{2.5abs}$	1.23 (0.41, 3.68)	1.45 (0.83, 2.53)	0.80	0.66 (0.21, 2.10)	1.57 (0.81, 3.01)	0.16
High ABI							
	PM_{10}	1.16 (0.59, 2.26)	2.13 (1.08, 4.21)	0.21	1.42 (0.54, 3.77)	1.52 (0.84, 2.74)	0.90
	$PM_{2.5}$	1.15 (0.49, 2.67)	1.53 (0.91, 2.59)	0.57	2.36 (1.02, 5.47)	1.63 (0.92, 2.89)	0.42
	$PM_{2.5abs}$	1.95 (0.83, 4.57)	1.56 (0.91, 2.69)	0.67	2.44 (0.94, 6.32)	1.22 (0.66, 2.26)	0.19

[a] NO_2 acted as the effect modifier; the interaction term was between PM and low/high levels of NO_2.

[b] PM acted as the effect modifier; the interaction term was between NO_2 and low/high levels of PM.

[c] p-Value for the interaction term between PM and NO_2.

Table S4 ORs (95%CIs) of having low and high ABI using different definitions of high ABI.

		Using ABI of both sides		Using lower ABI	
		Cut-off value = 1.4[a]	Cut-off value = 1.3[b]	Cut-off value = 1.4[c]	Cut-off value = 1.3[d]
Low ABI					
	PM_{10}	1.82 (1.11, 2.97)*	1.79 (1.09, 2.94)*	1.76 (1.08, 2.88)*	1.76 (1.07, 2.87)*
	PM_{coarse}	1.39 (0.87, 2.22)	1.39 (0.87, 2.22)	1.35 (0.84, 2.15)	1.36 (0.85, 2.18)
	$PM_{2.5}$	1.59 (1.01, 2.51)*	1.61 (1.02, 2.54)*	1.56 (0.99, 2.46)†	1.58 (1.00, 2.50)*
	$PM_{2.5abs}$	1.43 (0.91, 2.23)	1.42 (0.91, 2.22)	1.41 (0.90, 2.20)	1.42 (0.91, 2.22)
	NO_2	1.59 (0.94, 2.68)†	1.57 (0.93, 2.64)†	1.54 (0.91, 2.59)	1.55 (0.92, 2.62)†
	Traffic intensity	1.16 (0.88, 1.52)	1.17 (0.89, 1.54)	1.15 (0.88, 1.51)	1.17 (0.89, 1.53)
	Traffic load	1.14 (0.79, 1.62)	1.11 (0.78, 1.59)	1.14 (0.80, 1.63)	1.16 (0.81, 1.66)
High ABI					
	PM_{10}	1.63 (1.07, 2.50)*	1.14 (0.87, 1.49)	1.20 (0.64, 2.27)	1.05 (0.72, 1.53)
	PM_{coarse}	1.92 (1.26, 2.92)**	1.29 (0.98, 1.70)†	1.75 (0.93, 3.30)†	1.43 (0.97, 2.09)†
	$PM_{2.5}$	1.44 (0.94, 2.20)†	1.20 (0.92, 1.58)	1.34 (0.71, 2.55)	1.29 (0.88, 1.89)
	$PM_{2.5abs}$	1.73 (1.14, 2.61)**	1.19 (0.91, 1.55)	2.20 (1.19, 4.07)*	1.54 (1.07, 2.23)*
	NO_2	1.84 (1.15, 2.94)*	1.19 (0.88, 1.63)	1.62 (0.80, 3.31)	1.37 (0.89, 2.10)
	Traffic intensity	1.20 (0.93, 1.55)	1.14 (0.96, 1.36)	1.30 (0.89, 1.89)	1.26 (1.01, 1.58)*
	Traffic load	1.12 (0.79, 1.58)	0.95 (0.75, 1.20)	1.54 (0.95, 2.50)†	1.40 (1.04, 1.87)*

[a] Definition of high ABI: have ABI > 1.4 in both legs or ABI >1.4 in one leg and a normal ABI (0.9 < ABI ≤ 1.4) in the other leg. Number of participants in each group: N_{normal} = 4064; $N_{Low\ ABI}$ = 209; $N_{High\ ABI}$ = 271.

[b] Definition of high ABI: have ABI > 1.3 in both legs or ABI > 1.3 in one leg and a normal ABI (0.9 < ABI ≤ 1.3) in the other leg. N_{normal} = 3530; $N_{Low\ ABI}$ = 209; $N_{High\ ABI}$ = 805.

[c] Definition of high ABI: the lower ABI of both sides was higher than 1.4. N_{normal} = 4222; $N_{Low\ ABI}$ = 209; $N_{High\ ABI}$ = 113.

[d] Definition of high ABI: the lower ABI of both sides was higher than 1.3. N_{normal} = 3982; $N_{Low\ ABI}$ = 209; $N_{High\ ABI}$ = 353.

† p-Value < 0.1; * p-Value < 0.05; ** p-Value < 0.01.

Table S5 ORs (95%CIs) of having low and high ABI in subgroup analyses and the extended model with adjustment for effect modifiers.

		Live at the same address for ≥ 5 years [a]	Age > 50 years [b]	Exclude participants with both low and high ABI [c]	Main model + physical activity [d]
Low ABI					
	PM_{10}	1.98 (1.19, 3.29)**	2.10 (1.26, 3.48)**	1.82 (1.11, 2.99)*	1.78 (1.09, 2.92)*
	PM_{coarse}	1.45 (0.90, 2.34)	1.44 (0.89, 2.34)	1.38 (0.86, 2.21)	1.35 (0.84, 2.17)
	$PM_{2.5}$	1.48 (0.92, 2.38)	1.64 (1.03, 2.62)*	1.55 (0.98, 2.46)†	1.58 (1.00, 2.50)†
	$PM_{2.5abs}$	1.48 (0.94, 2.34)†	1.47 (0.93, 2.32)†	1.43 (0.92, 2.24)	1.38 (0.88, 2.15)
	NO_2	1.62 (0.95, 2.78)†	1.68 (0.99, 2.87)†	1.57 (0.93, 2.66)†	1.55 (0.92, 2.62)
	Traffic intensity	1.07 (0.80, 1.45)	1.14 (0.86, 1.51)	1.14 (0.87, 1.50)	1.15 (0.88, 1.51)
	Traffic load	1.04 (0.71, 1.53)	1.15 (0.80, 1.66)	1.13 (0.79, 1.61)	1.11 (0.78, 1.59)
High ABI					
	PM_{10}	1.68 (1.08, 2.60)*	1.69 (1.09, 2.63)*	1.63 (1.06, 2.49)*	1.64 (1.07, 2.51)*
	PM_{coarse}	2.03 (1.32, 3.14)**	2.10 (1.36, 3.23)**	1.92 (1.26, 2.92)**	1.95 (1.28, 2.97)**
	$PM_{2.5}$	1.47 (0.94, 2.29)†	1.47 (0.95, 2.29)†	1.45 (0.95, 2.21)†	1.45 (0.95, 2.22)†
	$PM_{2.5abs}$	1.94 (1.26, 2.97)**	1.90 (1.24, 2.91)**	1.72 (1.14, 2.60)**	1.76 (1.17, 2.66)**
	NO_2	1.82 (1.11, 2.97)*	1.94 (1.19, 3.15)**	1.84 (1.15, 2.94)*	1.85 (1.16, 2.97)*
	Traffic intensity	1.22 (0.93, 1.59)	1.19 (0.91, 1.56)	1.20 (0.93, 1.55)	1.21 (0.93, 1.56)
	Traffic load	1.11 (0.77, 1.60)	1.11 (0.77, 1.59)	1.12 (0.79, 1.58)	1.14 (0.80, 1.60)

[a] Analyzing a subgroup of participants who lived at the same address for at least five years. Number of participants in this subgroup N = 4099; $N_{Low\ ABI}$ = 197; $N_{High\ ABI}$ = 253.

[b] Analyzing a subgroup of participants with age over 50 years. N = 3556; $N_{Low\ ABI}$ = 199; $N_{High\ ABI}$ = 255.

[c] Excluding participants with low ABI in one leg and high ABI in the contralateral leg. N = 4542; $N_{Low\ ABI}$ = 207; $N_{High\ ABI}$ = 271.

[d] Main model further adjusted for physical activity.

† p-Value < 0.1; * p-Value < 0.05; ** p-Value < 0.01.

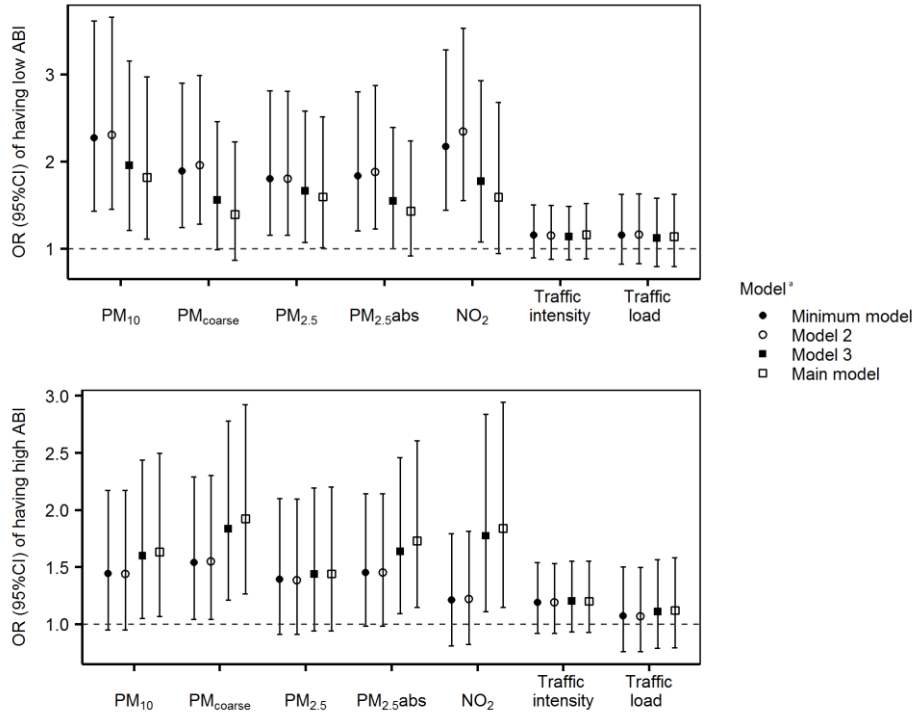

Fig. S1 ORs (95%CIs) of having low (upper panel) and high ABI (lower panel) for an increment in air pollution in different models.

An increment was 7.6 μg/m^3 for PM_{10}, 3.4 μg/m^3 for PM_{coarse}, 2.8 μg/m^3 for $PM_{2.5}$, 5.2×10^{-6}/m for $PM_{2.5abs}$, 11.7 μg/m^3 for NO_2, 7,341 vehicles/day for traffic intensity on the nearest road, and 3.1×10^6 vehicles·m/day for traffic load in a 100 m buffer.

[a] (1) The minimum model was adjusted for age, sex, the day of year, and study. (2) Model 2 was the minimum model with further adjustment for years of education. (3) Model 3 was model 2 with further adjustment for neighborhood SES. (4) The main model was model 3 with further adjustment for smoking status and smoking pack years.

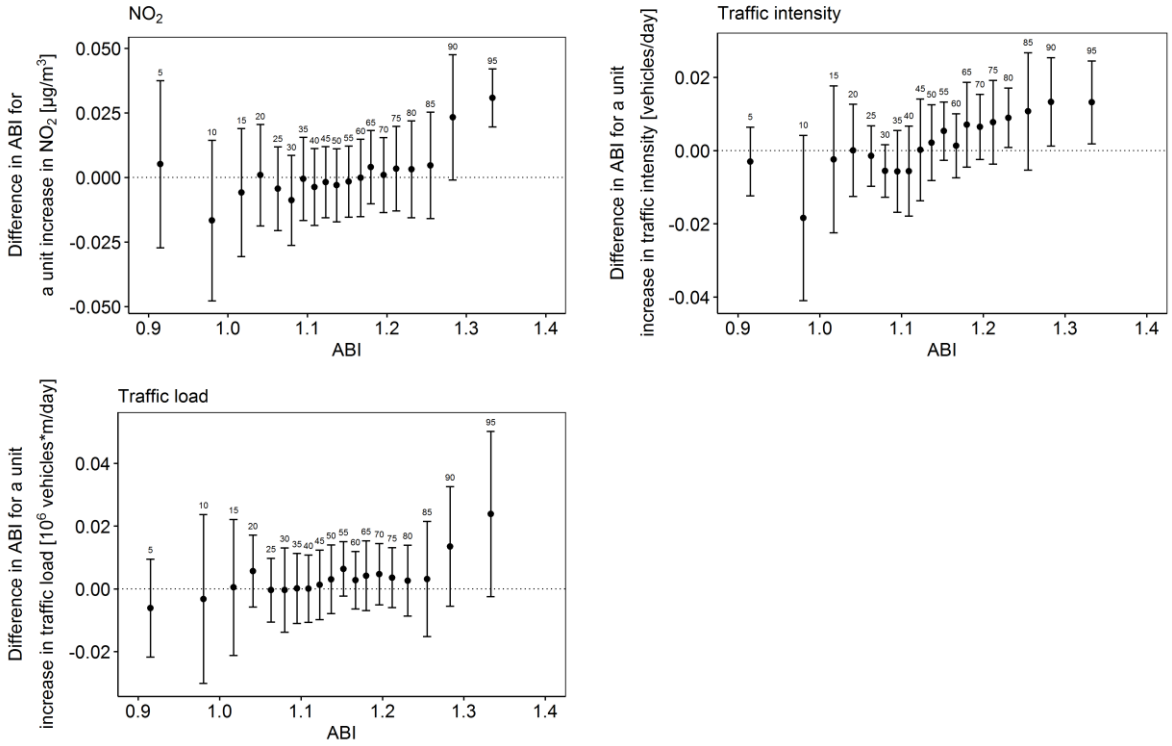

Fig. S2 Absolute difference in ABI at the 5th to 95th percentiles associated with an increase from the 5th to the 95th percentile in NO_2, traffic intensity, and traffic load.

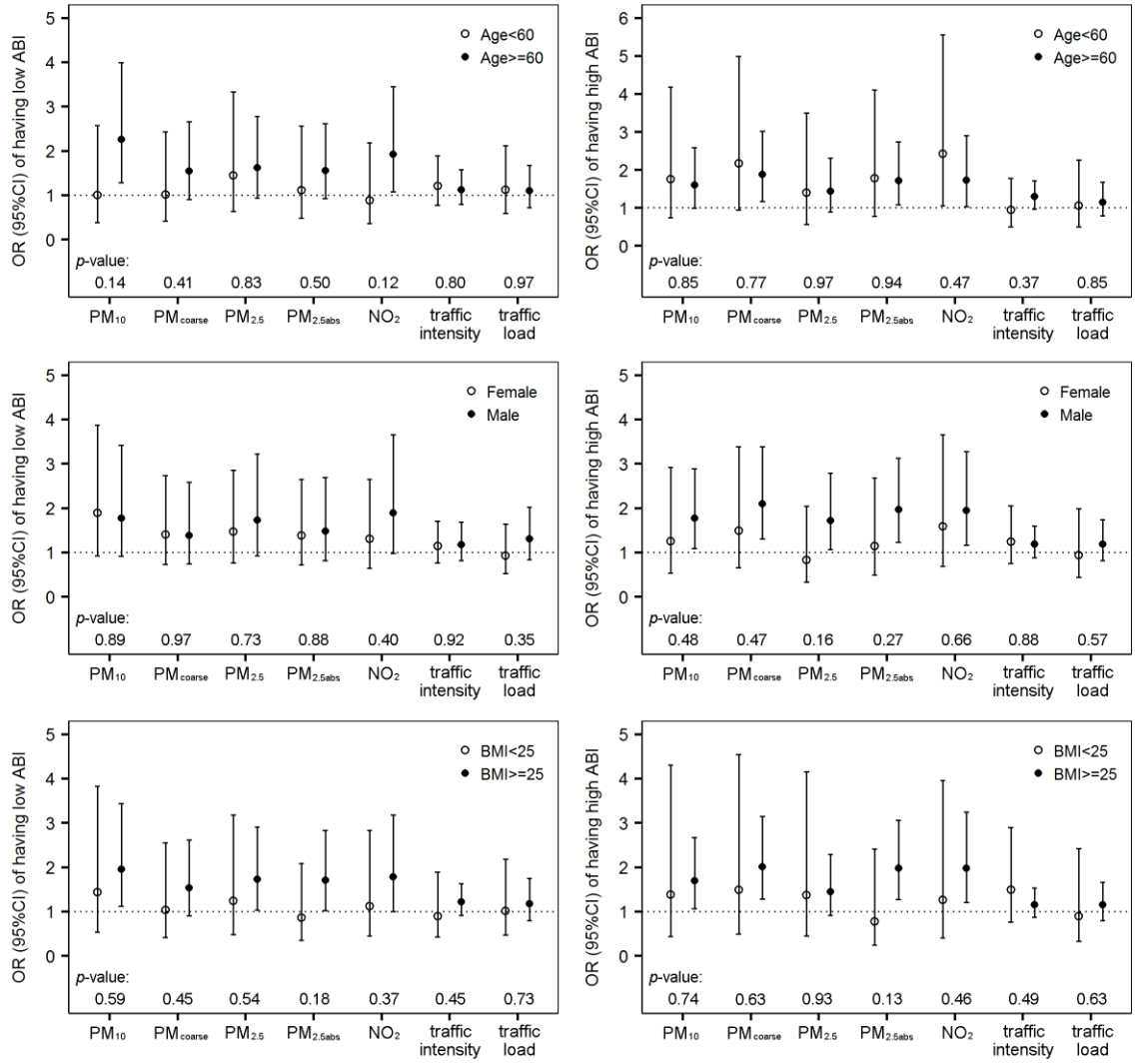

Fig. S3 Effect modification by age, sex, and overweight on the associations of air pollution with low ABI (left panels) and high ABI (right panels).

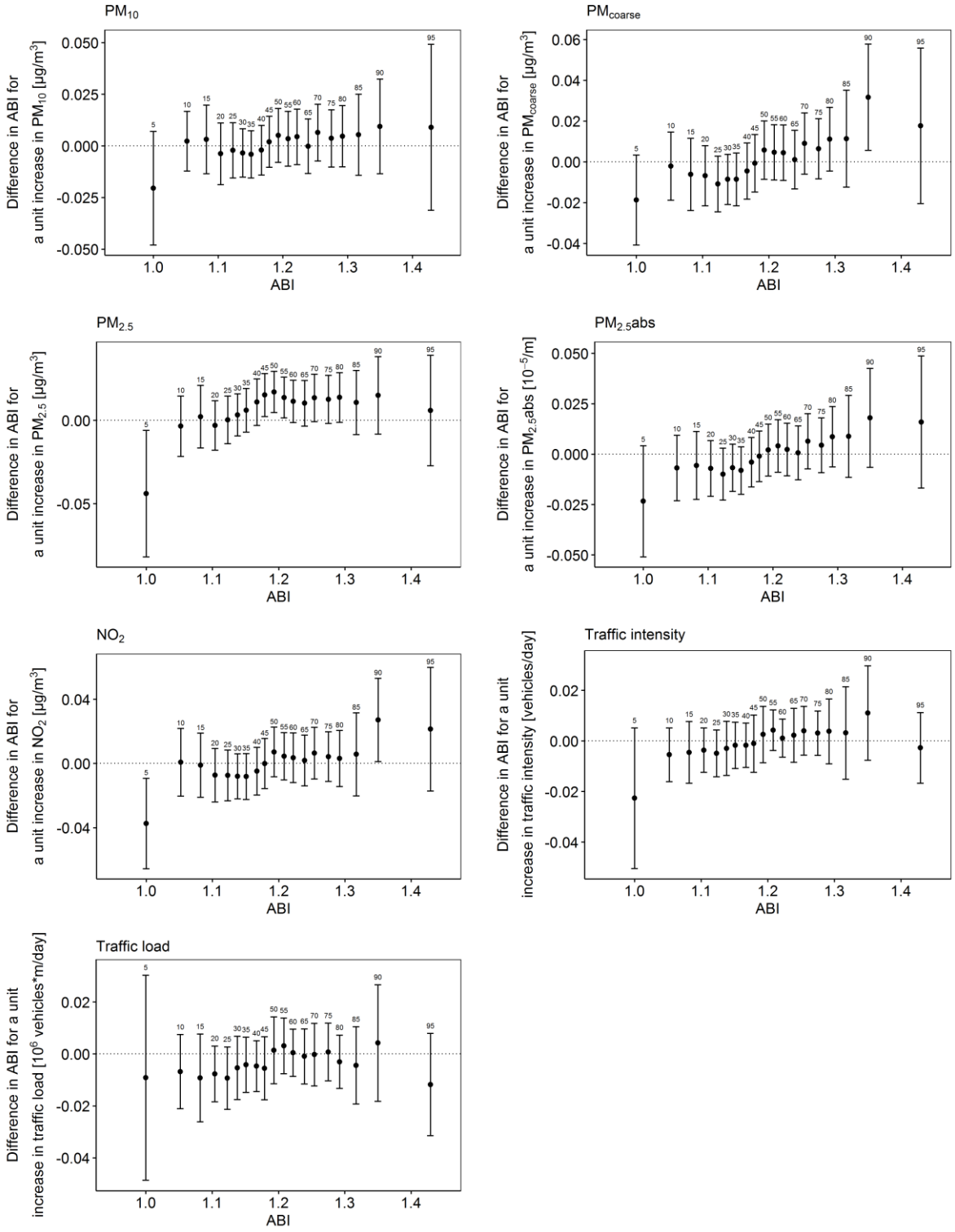

Fig. S4 Absolute difference in higher ABI of two sides at the 5th to 95th percentiles associated with an increase from the 5th to the 95th percentile in air pollution.

Manuscript II

Title:	Short-term Effects of Fine Particulate Matter and Ozone on the Cardiac Conduction System in Patients Undergoing Cardiac Catheterization
Authors:	Siqi Zhang, Susanne Breitner, Wayne E Cascio, Robert B Devlin, Lucas M Neas, David Diaz-Sanchez, William E Kraus, Joel Schwartz, Elizabeth R Hauser, Annette Peters, Alexandra Schneider
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Particle and Fibre Toxicology

RESEARCH Open Access

Short-term effects of fine particulate matter and ozone on the cardiac conduction system in patients undergoing cardiac catheterization

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Abstract

Background: Air pollution-induced changes in cardiac electrophysiological properties could be a pathway linking air pollution and cardiovascular events. The evidence of air pollution effects on the cardiac conduction system is incomplete yet. We investigated short-term effects of particulate matter ≤ 2.5 μm in aerodynamic diameter ($PM_{2.5}$) and ozone (O_3) on cardiac electrical impulse propagation and repolarization as recorded in surface electrocardiograms (ECG).

Methods: We analyzed repeated 12-lead ECG measurements performed on 5,332 patients between 2001 and 2012. The participants came from the Duke CATHGEN Study who underwent cardiac catheterization and resided in North Carolina, United States (NC, U.S.). Daily concentrations of $PM_{2.5}$ and O_3 at each participant's home address were predicted with a hybrid air quality exposure model. We used generalized additive mixed models to investigate the associations of $PM_{2.5}$ and O_3 with the PR interval, QRS interval, heart rate-corrected QT interval (QTc), and heart rate (HR). The temporal lag structures of the associations were examined using distributed-lag models.

Results: Elevated $PM_{2.5}$ and O_3 were associated with four-day lagged lengthening of the PR and QRS intervals, and with one-day lagged increases in HR. We observed immediate effects on the lengthening of the QTc interval for both $PM_{2.5}$ and O_3, as well as delayed effects for $PM_{2.5}$ (lagged by 3 – 4 days). The associations of $PM_{2.5}$ and O_3 with the PR interval and the association of O_3 with the QRS interval persisted until up to seven days after exposure.

Conclusions: In patients undergoing cardiac catheterization, short-term exposure to air pollution was associated with increased HR and delays in atrioventricular conduction, ventricular depolarization and repolarization.

Keywords: Air pollution, Electrocardiogram, PR interval, QT interval, QRS interval

Background

The associations between ambient air pollution and cardiovascular morbidity and mortality are well established [1–3]. One potential pathway of the linkage might be through the air pollution-induced changes in cardiac electrophysiological properties. The cardiac conduction system initiates and conducts electrical impulses as recorded in the electrocardiogram (ECG). Cardiac conduction abnormalities, such as first-degree atrioventricular block (first-degree AVB) or prolonged ventricular repolarization, are associated with increased incidence and prevalence of atrial fibrillation (AF), total mortality, and sudden cardiac death [4, 5].

The acute effects of air pollution on the cardiac conduction system could be mediated by physiological mechanisms including autonomic imbalance and systemic inflammation, which trigger both immediate and delayed responses over a period ranging from hours to days [6, 7]. Epidemiological studies have reported associations of a lengthening of the heart rate-corrected

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QT interval (QTc), a measure of ventricular repolarization, with particulate matter in the elderly and patients having diabetes or preexisting ischemic heart disease [6, 8–11]. However, such associations were not observed in a panel study of cardiac rehabilitation patients [7]. In addition to the inconsistent results of particulate matter, evidence of ambient ozone (O_3) effects on the QTc interval is still limited [12, 13], and the impacts of air pollution on atrioventricular conduction and ventricular depolarization have not been fully investigated [9, 14].

Hypothesizing that air pollution exposure would be associated with cardiac conduction delay, we performed this study to investigate the short-term effects of $PM_{2.5}$ and O_3 on the electrocardiographic intervals reflecting impulse propagation and repolarization in high-risk patients from a cardiovascular cohort.

Methods

Study population

The data used in this study were obtained from the Catheterization Genetics (CATHGEN) Study, a cohort of 9,334 patients who underwent cardiac catheterization at Duke University Medical Center from 2001 through 2010. More details of the CATHGEN Study can be found elsewhere [15].

Our analyses were restricted to 6,209 individuals who had ECG measurements and resided in North Carolina, United States (NC, U.S.) at catheterization. From the CATHGEN database, we collected information on participant demographic characteristics (age, sex, and race), body mass index (BMI), smoking status, and the history of myocardial infarction (MI). The Coronary Artery Disease Prognostic index (CAD index) was assessed during the catheterization procedure. The CAD index is an indicator of the severity of coronary artery disease (CAD) based upon cardiovascular outcomes. A CAD index > 23 represents at least one ≥ 75% occlusion in one major epicardial coronary artery [16]. Data on area-level educational attainment and urban/rural status were obtained from the 2000 U.S. Census based on each patient's home address at catheterization. Area-level educational attainment refers to the percentage of adults (≥ 25 years old) in the block group with less than a high school education; it was categorized into low (≥ 25%) and high (< 25%) levels.

ECG measurement

During the study period (2001–2012), 71,194 12-lead ECGs were performed at the time of catheterization and in follow-up examinations, and analyzed automatically using the Philips TraceMaster ECG system (Andover, MA). ECG parameters of interest were the PR interval (ms), QRS interval (ms), QT interval (ms), and heart rate (HR, beats/min). The PR interval is measured from the beginning of the P wave to the beginning of the QRS complex, reflecting the electrical impulse conduction from the sinus node through the atrioventricular node and His-Purkinje system. The QRS interval is the time from the onset of the Q wave to the end of the S wave, which represents ventricular depolarization. The QT interval is defined as the duration from the beginning of the Q wave to the end of the T wave. The QT interval is dependent on HR; after HR-correction, the QTc interval is a measure of ventricular repolarization. The QT correction for HR was performed using the Bazett formula in our main analyses [17].

We first excluded 13,632 ECGs with the diagnosis of atrial fibrillation, atrial flutter, multifocal atrial tachycardia, or paced rhythms. For participants with multiple ECGs on the same day or ECGs on consecutive days, we only included the first of the day and the first on consecutive days to reduce the potential impact of intervening medical treatment. To reduce bias caused by artifacts, we excluded ECGs with non-physiological parameter values in the following ranges: (1) PR interval < 100 ms or > 400 ms, (2) QRS interval < 50 ms or > 170 ms, (3) QTc < 350 ms or > 600 ms, (4) HR < 20 beats per minute (bpm) or > 180 bpm. We further excluded participants with bundle branch block (BBB, QRS interval > 120 ms), leaving 28,741 eligible ECGs on 5,376 participants.

Exposure assessment

Daily concentrations of $PM_{2.5}$ (daily average in $\mu g/m^3$) and O_3 (daily 8-h maximum in ppb) for NC were predicted at a 1 km × 1 km spatial grid resolution from 2000 to 2012. Predictions were made using a neural network-based hybrid model, incorporating input variables such as chemical transport model outputs, satellite-based aerosol optical depth data, absorbing aerosol index, land-use terms, and meteorological variables. The ten-fold cross-validation indicated good model performances with coefficients of determination of 0.86 and 0.68 for $PM_{2.5}$ and O_3, respectively. Detailed descriptions and predictive performance of the model were reported elsewhere [18, 19].

Daily air temperature in NC was also predicted at a 1 km × 1 km grid resolution for the study period. The modeling process involved satellite-derived daily surface temperature, daily air temperature from NC weather stations, normalized difference vegetation index, and predictors of air temperature (percent of urban areas, elevation, and distance to water body). A three-stage modeling approach was used, allowing the prediction in grid cells without weather monitors or grid cells/days without data on satellite surface temperature [20].

The latitude and longitude of each participant's residential address were geocoded by the Children's

Environmental Health Initiative in the Duke Nicholas School of the Environment (https://cehi.rice.edu/). For individuals who moved during the study period, we used the address most closely linked with the date on which the ECG was performed. The geocoded addresses were matched with air pollution and temperature data based on the spatial location and date. Daily air pollutant concentrations and air temperature on the same day and 1–14 days prior to the ECG measurement were assigned to each ECG.

Statistical analysis

Short-term effects of $PM_{2.5}$ and O_3 on ECG parameters were investigated using generalized additive mixed models with random intercepts for patients. The ECG parameters were log-transformed in our regression models to increase the conformity to a normal distribution of residuals. To control for systematic variation over time, we included a penalized spline for long-term time trend with four degrees of freedom per year, and two categorical variables for season (spring: March–May; summer: June–August; autumn: September–November; winter: December–February) and day of the week. Air temperature was adjusted for by modeling low and high temperatures separately [21]. For days with average temperature on the previous four days (lag1–4) lower than the median annual temperature, we introduced a natural spline with two degrees of freedom for lag1–4 temperature. Similarly, for days with average temperature on the current and previous day (lag0–1) higher than the median annual temperature, we introduced a natural spline for lag0–1 temperature with three degrees of freedom. Besides, we controlled for individual characteristics at each measurement time point, including age (continuous), sex (male or female), race (European-Americans, African-Americans, or others), area-level educational attainment (low or high), BMI (continuous), smoking status (never smoker, or current/former smoker), and living area (rural or urban). The adjusted confounders were identical across models for the various air pollutants and ECG parameters. We investigated the effects of air pollution on the concurrent day (lag0), for single-day lags from one to four days (lag0–lag4), and for a multi-day lag of five days (lag04).

For pollutant-outcome pairs showing significant delayed associations four days after exposure, we examined lagged effects up to 14 days using distributed-lag models [22]. We therefore built a cross-basis matrix with a third degree polynomial function of lags, which was then incorporated into the generalized additive mixed model adjusted for the same confounders as in the main model.

To explore effect modification and identify the subgroups that might be more susceptible to the effects of $PM_{2.5}$ and O_3, we incorporated interaction terms between air pollution and individual characteristics in the model. The examined potential modifiers included sex, age (< 60 years vs. ≥ 60 years), area-level educational attainment, obesity (BMI < 30 kg/m^2 vs. ≥ 30 kg/m^2), smoking status, urban/rural status, CAD index (CAD index ≤ 23 vs. > 23), and history of MI.

In sensitivity analyses, we excluded ECGs with single-day (lag0–lag4) exposure levels of $PM_{2.5}$ above 35 $\mu g/m^3$ or O_3 above 70 ppb to examine the effects of air pollution below the current U.S. National Ambient Air Quality Standards (NAAQS) [23]. As the electrophysiological parameters are potentially dependent on the HR, we further adjusted for the HR in models for the PR interval, QRS interval, and the raw QT interval without HR-correction. In addition, we used Fridericia formula in QT correction [24], and investigated air pollution effects on corrected JT interval (JTc), which was defined by subtracting the QRS from QTc. The JTc interval is also an indicator to measure the duration of ventricular repolarization and is reported to reduce the impact of wide QRS complex on the QTc interval [25]. To examine the influence of BBB on associations between air pollution and ECG parameters, we performed analyses using 33,117 eligible ECG measurements on 5,819 participants regardless of the presence of BBB. We tested the robustness of the results by building two-pollutant models with $PM_{2.5}$ and O_3 of the same lag, restricting the analyses to participants with two or more ECG measurements, changing the degree of freedom for the trend spline, excluding season as a categorical variable, and applying generalized additive mixed models with linear spatial correlation structure given that the dependency between repeated ECG measurements might decrease with increasing time interval. Furthermore, we added long-term air pollution exposure (365-day moving average of air pollution of 0–364 days prior to each ECG measurement) to our models and replaced the daily mean concentration with the deviation between daily mean and long-term average. In this way, we sought to investigate the acute effect of temporal variation of pollutants with the control for spatial variation. The linearity of the exposure-response relationships was examined by including a spline for air pollution variables in models.

The effect estimates are reported as percent changes of the geometric mean (GM) of outcomes and 95% confidence intervals (95% CI) corresponding to an interquartile range (IQR) increase in $PM_{2.5}$ and O_3. We performed the analyses with the software R (version 3.5.1), using the 'gamm4', 'mgcv', and 'dlnm' packages. The significance level was set at 0.05.

Results

Participant characteristics and exposure concentrations

After further exclusion of 44 patients without complete data on ECG parameters of interest, air pollution concentrations, or main covariates, we analyzed a final sample of 28,578 ECGs on 5,332 participants (See Additional file 1: Figure S1). Among them, 4,009 participants had two or more eligible ECG recordings during the study period. The mean age and BMI at enrollment were 59.8 years and 30.1 kg/m^2, respectively (Table 1). 60.7% of the participants were male, over half were never smokers, and the majority were European-American (72.3%). More individuals lived in rural areas and areas with a high level of educational attainment. Compared to excluded individuals, the participants included in our main analyses tended to be younger and more likely to live in urban areas, have a higher proportion of African-Americans and a higher level of educational attainment (See Additional file 1: Table S1).

Table 2 shows the descriptive statistics of ECG parameters in all ECG recordings. The correlations between ECG parameters were weak or negligible. During the study period, the average concentrations of $PM_{2.5}$ and O_3 in geocoded areas with participants were 11.2 μg/m^3 and 40.5 ppb, respectively (Table 3). Most daily $PM_{2.5}$ and O_3 levels (99.9% for $PM_{2.5}$ and 98.7% for O_3) were below the current NAAQS (daily average concentration of 35 μg/m^3 for $PM_{2.5}$ and daily maximum 8-hour concentration of 70 ppb for O_3). $PM_{2.5}$ and O_3 were moderately correlated with a Spearman correlation coefficient of 0.49.

Table 1 Descriptive statistics of the study population at baseline (*n*=5332)

	Mean ± SD / N (%)
Age (years)	59.8 ± 11.7
BMI (kg/m^2)	30.1 ± 7.2
Sex (male)	3237 (60.7)
Race	
European-Americans	3854 (72.3)
African-Americans	1188 (22.3)
Others	290 (5.4)
Smoking (never smoker)	2753 (51.6)
Education (high)	3231 (60.6)
Area (rural)	2953 (55.4)
CAD-index > 23 (yes)[a]	2418 (50.4)
History of MI (yes)	1449 (27.2)

SD standard deviation, *BMI* body mass index, *CAD* coronary artery disease, *MI* myocardial infarction
[a]Data on CAD-index were available for 4801 participants

Air pollution and ECG parameters

Increments in $PM_{2.5}$ and O_3 were significantly associated with the lengthening of the PR interval lagged three or four days, and with the concurrent as well as lagged lengthening of the QTc interval (Table 4). Positive associations with the QRS interval were significant for O_3 at lag4 and marginally significant for $PM_{2.5}$ at lag1 and lag4. We also observed significant increases in the HR associated with elevated $PM_{2.5}$ and a marginally significant increase for O_3, with the strongest single-day effects at lag1.

We used polynomial distributed-lag models for PR, QRS, and QTc intervals as they showed delayed responses to air pollution. Estimates of the distributed-lag models indicated that the effects of $PM_{2.5}$ and O_3 on the PR interval and the effect of O_3 on the QRS interval persisted until seven days after exposure. For the QTc interval, we did not find lagged effects of $PM_{2.5}$ beyond four days (Fig. 1).

Effect modification

We observed stronger effects of O_3 on the QRS and QTc intervals in patients living in rural areas, and stronger air pollution effects on the QTc interval in patients with low educational attainment or obesity. We did not find significant or consistent effect modification by other examined potential modifiers (See Additional file 1: Figure S2).

Sensitivity analyses

Analyses of exposure below the NAAQS showed slightly attenuated associations between air pollution and ECG parameters; the effects of air pollution on the PR interval, QTc interval, and HR remained significant (See Additional file 1: Table S2). Associations of air pollution with the PR, QRS, and QT intervals were not sensitive to the adjustment for HR (See Additional file 1: Figure S3). We observed reduced effects of air pollution on the QTc interval calculated using the Fridericia formula compared to using the Bazett formula at lag0–lag2 (See Additional file 1: Figure S4). However, the associations between air pollution and ventricular repolarization were generally consistent across different indicators. Including participants with BBB reduced the air pollution effects on the QRS and QTc intervals and did not significantly affect the effects on the PR interval and HR (See Additional file 1: Figure S5).

We did not observe substantial changes in effect estimates in two-pollutant models, except for the attenuated effect of $PM_{2.5}$ on the PR interval at lag4 when adjusted for O_3 and vice versa (See Additional file 1: Figure S6). The associations of $PM_{2.5}$ and O_3 with ECG parameters were robust to excluding participants with only one ECG measurements, changing the degree of freedom of trend spline, excluding season, controlling for long-term

Table 2 Descriptive statistics and Spearman correlation coefficients of ECG parameters (n=28578)

	Mean ± SD		Min	25%	Median	75%	Max	Correlation coefficients		
	Geometric	Arithmetic						PR	QRS	QTc
PR (ms)	170 ± 1	173 ± 31	100[a]	152	168	188	400[a]	--		
QRS (ms)	91 ± 1	91 ± 12	53	83	90	99	120[a]	0.18	--	
QTc (ms)	434 ± 1	435 ± 33	350[a]	412	433	456	587	-0.02	0.24	--
HR (bpm)	73 ± 1	74 ± 17	31	62	72	85	160	-0.28	-0.09	0.32

SD standard deviation, *Min* minimum, *25%* the 25th percentile, *75%* the 75th percentile, *Max* maximum, *QTc* heart rate-corrected QT interval, *HR* heart rate, *bpm* beats per minute
[a]The minimum and maximum values were set by the exclusion criteria

exposure to air pollution, or applying spatial correlation structure in mixed-effects models. The linear exposure-response relationships between air pollution and ECG parameters held true when air pollution variables were included in models as splines (results not shown).

Discussion

In high-risk patients undergoing cardiac catheterization, we observed associations of increments in $PM_{2.5}$ and O_3 with the lengthening of the PR, QRS, and QTc intervals and increased HR. The effects of $PM_{2.5}$ and O_3 on the PR interval and the effect of O_3 on the QRS interval persisted until up to one week in distributed lag models. These findings supported our hypothesis that short-term exposure to air pollution was associated with atrioventricular and intraventricular conduction delay.

An increased PR interval could relate to parasympathetic activation, sympathetic withdrawal, or the block of inward calcium current through membrane channels. A lengthening of the PR interval, even below the diagnostic threshold for first-degree AVB (PR interval > 200 ms), is associated with increased incidence of AF, pacemaker implantation, and all-cause mortality [5]. Few prior studies investigated the effect of air pollution on the PR interval. The Air Pollution and Cardiac Risk and its Time Course (APACR) Study found a 0.09% increase in the PR interval for each 10 μg/m^3 increment in $PM_{2.5}$ [14]. Since individuals with cardiovascular disease are potentially more sensitive to air pollution effects, the smaller effect estimate compared to our study (0.25%) could be due to the healthier participants in the APACR Study. The distinct lag times of associations (1.5–2 hours in the APACR Study and 3–4 days in our study) might also partly explain the difference. In addition to $PM_{2.5}$, our study provided evidence for an association between O_3 and the PR interval, which to our knowledge has not been reported previously.

The associations between air pollution and the QRS interval in our study indicated the effects of air pollution

Table 3 Descriptive statistics of air pollution and temperature in geocoded areas with participants during the study period

	Mean ± SD	Min	25%	Median	75%	Max	IQR
$PM_{2.5}$ (μg/m^3)	11.2 ± 5.5	0.9	7.3	10.1	14.2	54.5	7.0
O_3 (ppb)	40.5 ± 12.8	8.6	30.4	39.5	49.8	97.6	19.4
Air temperature (°C)	16.8 ± 8.1	-4.7	10.2	17.8	24.0	31.0	13.8

SD standard deviation, *Min* minimum, *25%* the 25th percentile, *75%* the 75th percentile, *Max* maximum, *IQR* interquartile range, $PM_{2.5}$ particulate matter ≤ 2.5 μm in aerodynamic diameter, O_3 ozone, *ppb* parts per billion

Table 4 Percent change (95% CI) of the geometric mean of ECG parameters per interquartile range increase in pollutants

ECG parameter	Lag (day)	$PM_{2.5}$ % Change (95% CI)	O_3 % Change (95% CI)
PR	0	-0.07 (-0.23, 0.08)	-0.01 (-0.24, 0.23)
	1	-0.07 (-0.23, 0.09)	0.03 (-0.21, 0.27)
	2	-0.07 (-0.23, 0.09)	-0.12 (-0.36, 0.12)
	3	0.17 (0.01, 0.33)*	0.02 (-0.22, 0.26)
	4	0.18 (0.03, 0.34)*	0.29 (0.05, 0.53)*
	04	0.07 (-0.18, 0.32)	0.09 (-0.26, 0.43)
QRS	0	0.11 (0.00, 0.22)	-0.04 (-0.21, 0.12)
	1	0.03 (-0.08, 0.14)	0.00 (-0.17, 0.16)
	2	0.01 (-0.10, 0.12)	-0.05 (-0.22, 0.11)
	3	0.04 (-0.07, 0.15)	0.04 (-0.13, 0.21)
	4	0.11 (0.00, 0.21)	0.21 (0.04, 0.37)*
	04	0.15 (-0.03, 0.32)	0.06 (-0.18, 0.30)
QTc	0	0.11 (0.02, 0.19)*	0.17 (0.04, 0.30)**
	1	0.05 (-0.04, 0.14)	0.18 (0.04, 0.31)**
	2	0.05 (-0.04, 0.14)	0.07 (-0.06, 0.21)
	3	0.11 (0.03, 0.20)*	0.02 (-0.11, 0.15)
	4	0.13 (0.05, 0.22)**	0.04 (-0.09, 0.17)
	04	0.23 (0.09, 0.36)**	0.20 (0.01, 0.39)*
HR	0	0.22 (-0.05, 0.49)	0.23 (-0.17, 0.63)
	1	0.47 (0.20, 0.75)**	0.40 (0.00, 0.81)
	2	0.28 (0.01, 0.56)*	0.28 (-0.13, 0.69)
	3	-0.11 (-0.38, 0.16)	-0.21 (-0.62, 0.20)
	4	0.04 (-0.23, 0.30)	-0.12 (-0.53, 0.29)
	04	0.44 (0.01, 0.86)*	0.24 (-0.34, 0.83)

CI confidence interval, *ECG* electrocardiogram, $PM_{2.5}$ particulate matter ≤ 2.5 μm in aerodynamic diameter, O_3 ozone; *QTc* heart rate-corrected QT interval, *HR* heart rate
*p-Value <0.05; **p-Value <0.01

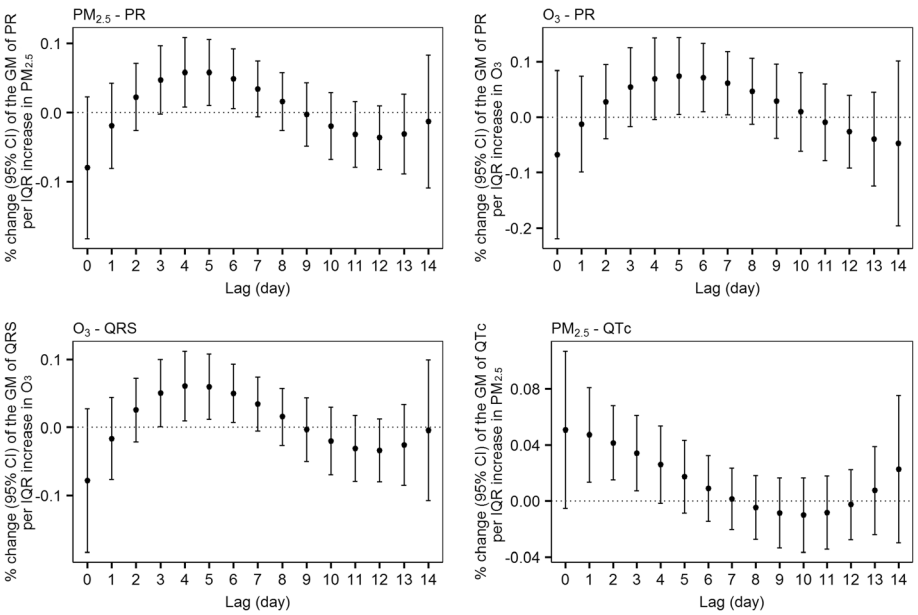

Fig. 1 Percent change (95% CI) of the geometric mean of ECG parameters per interquartile range increase in $PM_{2.5}$ and O_3 in distributed-lag models. *CI* confidence interval, *ECG* electrocardiogram, $PM_{2.5}$ particulate matter ≤ 2.5 μm in aerodynamic diameter, O_3 ozone *QTc* heart rate-corrected QT interval, *GM* geometric mean, *IQR* interquartile range

on ventricular depolarization among individuals without bundle branch block. An increase in the QRS interval is an independent predictor of cardiovascular mortality [26]. Yet, the evidence of air pollution effects on the QRS interval is still limited. Consistent with our results, a higher prevalence of prolonged QRS interval was associated with long-term residential $PM_{2.5}$ exposure in the U.S. Multi-Ethnic Study of Atherosclerosis (MESA) Cohort. Besides, in a controlled exposure study among individuals with metabolic syndrome, the GSTM1 null participants showed an increased QRS interval after acute exposure to concentrated ambient ultrafine particles [27]. However, the QRS interval was not associated with $PM_{2.5}$ in the APACR Study [9], and an immediate decrease of 5.8% (95%CI: -10.5, -1.0) in the QRS interval after exposure to O_3 was observed in a crossover study among healthy volunteers [13]. The mechanism by which air pollution might lead to a change in QRS complex remains unclear and needs to be clarified by further epidemiological and experimental studies. Some theoretical explanations could be the impact of air pollution on the inward sodium current and the extracellular resistance.

Our findings of both concurrent and delayed effects of air pollution on the lengthening of the QTc interval are supported by previous studies [6, 8, 13]. The potential pathway of the immediate associations could be the direct impact of air pollution on the autonomic nervous system, and the delayed effects are possibly mediated by air pollution-induced inflammatory responses [6]. Ambient air pollutants trigger reactive oxygen species production, which in turn induces pulmonary and systemic inflammation. The concentrations of circulating inflammatory biomarkers, such as C-reactive protein (CRP), interleukin 6, and fibrinogen, are increased after acute air pollution exposure [6, 28]. Further, inflammation is a modulator of cardiomyocyte ion currents in the cardiac conduction system, through a pathway involving cytokine- and sympathetic-induced modulation [29]. Elevated levels of circulating inflammatory biomarkers have been proven to be associated with QTc prolongation [30–32].

The QTc interval calculated using the Bazett formula has been reported to be inferior to using the Fridericia formula in the prediction of mortality [33]. In our study, the associations between air pollution and the QTc interval calculated using the Fridericia formula were generally comparable to using the Bazett formula. Similar results were also found in the APACR Study [9]. In addition, since the QT interval encompasses the duration of ventricular depolarization as reflected by the QRS interval, the air pollution-induced lengthening of the QTc interval could be partly attributable to the effects on the QRS interval. When subtracting the QRS from the QTc, we still observed significant associations between air pollution and the JTc interval. The robust results provided strong evidence for the air pollution effects on ventricular repolarization.

The 1–2 days lagged associations between air pollution and HR suggested the effects of air pollution on the

autonomic nervous system [8, 34–36]. These associations could be potentially affected by the use of medication. For example, stronger effects of air pollution on HR and heart rate variability are observed among individuals not taking beta-blockers or calcium-channel blockers [8, 34]. On the other hand, taking medication indicates the presence of underlying clinical conditions, which might increase individual's susceptibility to air pollution. Therefore, other studies reported non-significant effect modification by medication or even stronger effects in individuals taking angiotensin-converting-enzyme inhibitor (ACE inhibitor) [37, 38]. The interaction between medication usage and clinical conditions potentially limits the interpretability of the non-significant effect modification by CAD in our study.

Although the effects of air pollution on the cardiac conduction system were relatively small in this study, it is still of public health significance because of its implications for the entire population. Using the World Health Organization air quality guideline for 24-hour mean of $PM_{2.5}$ (25 μg/m^3) as reference [39], exposure to the maximum $PM_{2.5}$ in this study (54.5 μg/m^3) would account for an increase of 2.4 ms in the QTc interval in exposed individuals. Moreover, cardiac conduction is affected by many other factors. For instance, preexisting medical conditions (left ventricular hypertrophy, ischemia, etc.) and certain medications can prolong cardiac repolarization [40]. Among patients with these conditions, further exposure to air pollution may add to the effects of other factors, and drive the QT interval across a critical threshold.

Strengths and limitations

A major strength of this study is the large sample size of the study population and the vast number of ECG recordings for analyses, which to the best of our knowledge is the largest cohort for analyzing air pollution effects on ECG parameters. The repeated measures study design provided substantial statistical power and enabled control for unmeasured individual-level confounders. Besides, we investigated the associations of $PM_{2.5}$ and O_3 with ECG parameters that have rarely been examined previously, such as the PR and QRS intervals.

One limitation of the study is the heterogeneity of time intervals caused by unscheduled follow-up visits. In the analyses, we applied mixed-effects models, which can reduce the impact of the unbalanced data structure. Second, we used daily residential exposure assessment instead of personal exposure. This may have resulted in non-differential exposure misclassification and bias the results towards the null [41]. Third, due to the unavailability of data, we were not able to control for medication intake, and the smoking status was roughly divided into current/former smoker or never smoker, which may have led to inaccuracy in assessing effect modification by pre-existing morbidities and residual confounding. Finally, our study was performed in high-risk patients receiving cardiac catheterization; thus, the results may not be generalizable to the general population. However, it enabled us to assess the association in a population subgroup at greater risk of cardiovascular events and potentially more susceptible to the adverse effects of air pollution.

Conclusions

In summary, short-term exposure to $PM_{2.5}$ and O_3 was associated with lengthening of the PR, QRS, and QTc intervals, and increasd heart rate in patients with cardiovascular disease. These findings provide evidence for the acute effects of air pollution on atrioventricular conduction and ventricular deporlarization and repolarization, which could potentially mediate the associations of air pollution with cardiac arrhythmias and cardiovascular mortality.

Additional file

Additional file 1: Table S1. Comparison of individual characteristics between participants included and excludes in main analyses. **Table S2.** Percent change (95% CI) of the geometric mean of ECG parameters per interquartile range increase in $PM_{2.5}$ and O_3 below the NAAQS. **Figure S1.** Flow chart of the exclusion procedure. **Figure S2.** Effect modification (percent change with 95% CI) by participant characteristics on the associations of air pollution with ECG parameters. **Figure S3.** Percent change (95% CI) of the geometric mean of the PR, QRS, and raw QT intervals per interquartile range increase in $PM_{2.5}$ and O_3 in models with adjustment for HR. **Figure S4**. Comparison of the air pollution effects (percent change with 95% CI) on different ventricular repolarization indicators. **Figure S5.** Percent change (95% CI) of the geometric mean of ECG parameters per interquartile range increase in $PM_{2.5}$ and O_3 among participants with QRS ≤ 120 ms and participants with QRS in the full range (50 ms ≤ QRS ≤ 170 ms). **Figure S6.** Percent change (95% CI) of the geometric mean of ECG parameters per interquartile range increase in $PM_{2.5}$ and O_3 in sensitivity analyses. (DOC 884 kb)

Abbreviations

AF: Atrial fibrillation; AVB: Atrioventricular block; BBB: Bundle branch block; BMI: Body mass index; BPM: Beats per minute; CAD: Coronary artery disease; CI: Confidence interval; CRP: C-reactive protein; ECG: Electrocardiogram; GM: Geometric mean; HR: Heart rate; IQR: Interquartile range; MI: Myocardial infarction; NAAQS: National Ambient Air Quality Standards; O_3: Ozone; $PM_{2.5}$: Particulate matter ≤ 2.5 μm in aerodynamic diameter; QTc: Heart rate-corrected QT interval; SD: Standard deviation

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Disclaimer
The research described in this article has been reviewed by the EPA and approved for publication. The contents of this article do not necessarily reflect the views of the Health Effects Institute (HEI), or its sponsors, nor do they necessarily reflect the view and policies of the EPA or motor vehicle and engine manufacturers. Further, the records included in the CATHGEN database reflect data that were compiled by medical staff at a private hospital facility. The EPA does not warrant or assume any legal liability or responsibility for the accuracy, completeness, or condition of any information, data, or process disclosed through these records.

Authors' contributions
SZ performed the statistical analyses and drafted the manuscript. AS, SB, AP, WC were substantially involved in the design of the study and interpretation of the data, and revised the manuscript critically. RD, LN, DDS, WK, and EH were involved in the acquisition of the CATHGEN data and reviewed the manuscript critically. JS was involved in the acquisition of the air pollution and temperature data and reviewed the manuscript critically. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The CATHGEN Study was approved by the Duke University Institutional Review Board; all participants signed informed consent prior to enrollment.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests

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ADDITIONAL FILE

Short-term Effects of Fine Particulate Matter and Ozone on the Cardiac Conduction System in Patients Undergoing Cardiac Catheterization

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Table S1. Comparison of individual characteristics between participants included and excludes in main analyses.

	Mean ± SD / N (%)		
	Included participants (n=5332)	Excluded participants (n=1784)	*p*-value
Age (years)	59.8 ± 11.7	62.8 ± 12.9	<0.001
BMI (kg/m^2)	30.1 ± 7.2	30.1 ± 7.5	0.90
Sex (male)	3237 (60.7)	1131 (63.4)	0.05
Race			< 0.001
European-Americans	3854 (72.3)	1369 (76.7)	
African-Americans	1188 (22.3)	263 (14.7)	
Others	290 (5.4)	152 (8.5)	
Smoking (never smoker)	2753 (51.6)	901 (50.5)	0.43
Education (high)	3231 (60.6)	961 (53.9)	< 0.001
Area (rural)	2953 (55.4)	1199 (67.2)	< 0.001
CAD-index > 23 (yes)[a]	2418 (50.4)	816 (49.5)	0.56
History of MI (yes)	1449 (27.2)	496 (27.8)	0.63

SD: standard deviation; BMI: body mass index; CAD: coronary artery disease; MI: myocardial infarction.

[a] Data on CAD-index were available for 5246 included participants and 1203 excluded participants.

Table S2. Percent change (95% CI) of the geometric mean of ECG parameters per interquartile range increase in PM2.5 and O3 below the NAAQS[¶].

ECG parameter	Lag (day)	$PM_{2.5}$	O_3
PR	0	-0.10 (-0.29, 0.08)	-0.01 (-0.29, 0.26)
	1	-0.08 (-0.27, 0.10)	0.06 (-0.22, 0.34)
	2	-0.02 (-0.20, 0.17)	-0.02 (-0.29, 0.26)
	3	0.18 (0.00, 0.37) *	0.09 (-0.18, 0.37)
	4	0.08 (-0.10, 0.27)	0.29 (0.01, 0.56)*
	04	0.03 (-0.26, 0.32)	0.18 (-0.23, 0.58)
QRS	0	0.11 (-0.02, 0.24)	-0.10 (-0.29, 0.09)
	1	0.02 (-0.11, 0.15)	-0.05 (-0.25, 0.14)
	2	-0.02 (-0.15, 0.11)	-0.09 (-0.28, 0.11)
	3	0.02 (-0.11, 0.15)	-0.02 (-0.21, 0.18)
	4	0.09 (-0.03, 0.22)	0.13 (-0.06, 0.33)
	04	0.11 (-0.09, 0.31)	-0.05 (-0.34, 0.23)
QTc	0	0.12 (0.02, 0.22) *	0.19 (0.04, 0.34)*
	1	0.06 (-0.04, 0.16)	0.22 (0.07, 0.38)**
	2	0.03 (-0.07, 0.13)	0.08 (-0.07, 0.23)
	3	0.07 (-0.03, 0.17)	-0.02 (-0.17, 0.13)
	4	0.11 (0.01, 0.21) *	-0.01 (-0.16, 0.14)
	04	0.19 (0.03, 0.35) *	0.20 (-0.02, 0.42)
HR	0	0.15 (-0.16, 0.46)	0.00 (-0.46, 0.46)
	1	0.41 (0.10, 0.73) **	0.28 (-0.19, 0.76)
	2	0.16 (-0.15, 0.47)	0.12 (-0.35, 0.60)
	3	-0.19 (-0.50, 0.11)	-0.36 (-0.82, 0.11)
	4	0.08 (-0.23, 0.39)	-0.13 (-0.59, 0.34)
	04	0.29 (-0.19, 0.78)	-0.04 (-0.72, 0.65)

CI: confidence interval; ECG: electrocardiogram; $PM_{2.5}$: particulate matter ≤ 2.5 μm in aerodynamic diameter; O_3: ozone; NAAQS: U.S. National Ambient Air Quality Standards; QTc: heart rate-corrected QT interval; HR: heart rate.

[¶] Number of ECGs with exposure below the NAAQS (35 $\mu g/m^3$ for $PM_{2.5}$ and 70 ppb for O_3) were 26255 on 5205 participants.

* p-Value <0.05; ** p-Value <0.01.

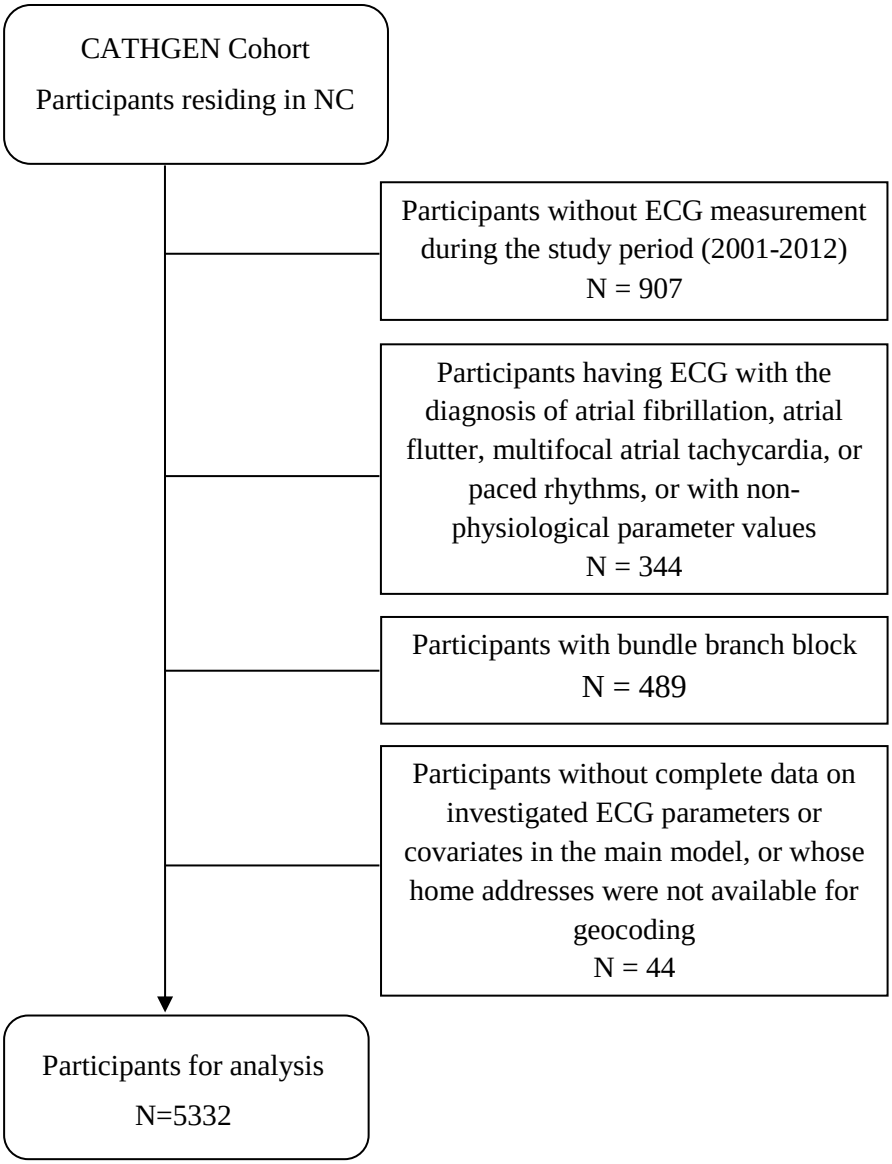

Figure S1. Flow chart of the exclusion procedure.

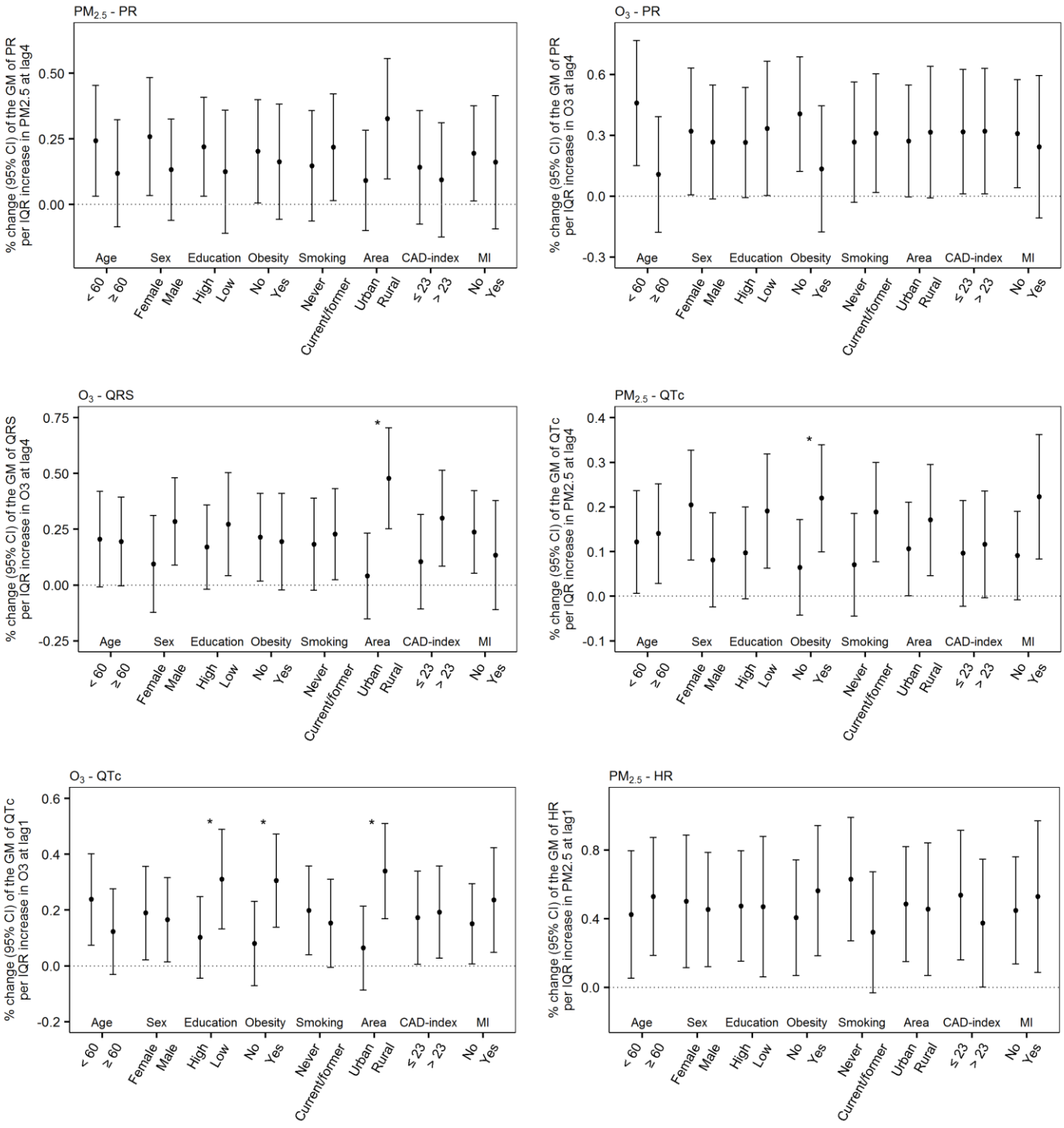

Figure S2. Effect modification (percent change with 95% CI) by participant characteristics on the associations of air pollution with ECG parameters.

* p-Value <0.05.

CI: confidence interval; ECG: Electrocardiogram; PM2.5: particulate matter ≤ 2.5 µm in aerodynamic diameter; O3: ozone; GM: geometric mean; IQR: interquartile range; QTc: heart rate-corrected QT interval; HR: heart rate; CAD: coronary artery disease.

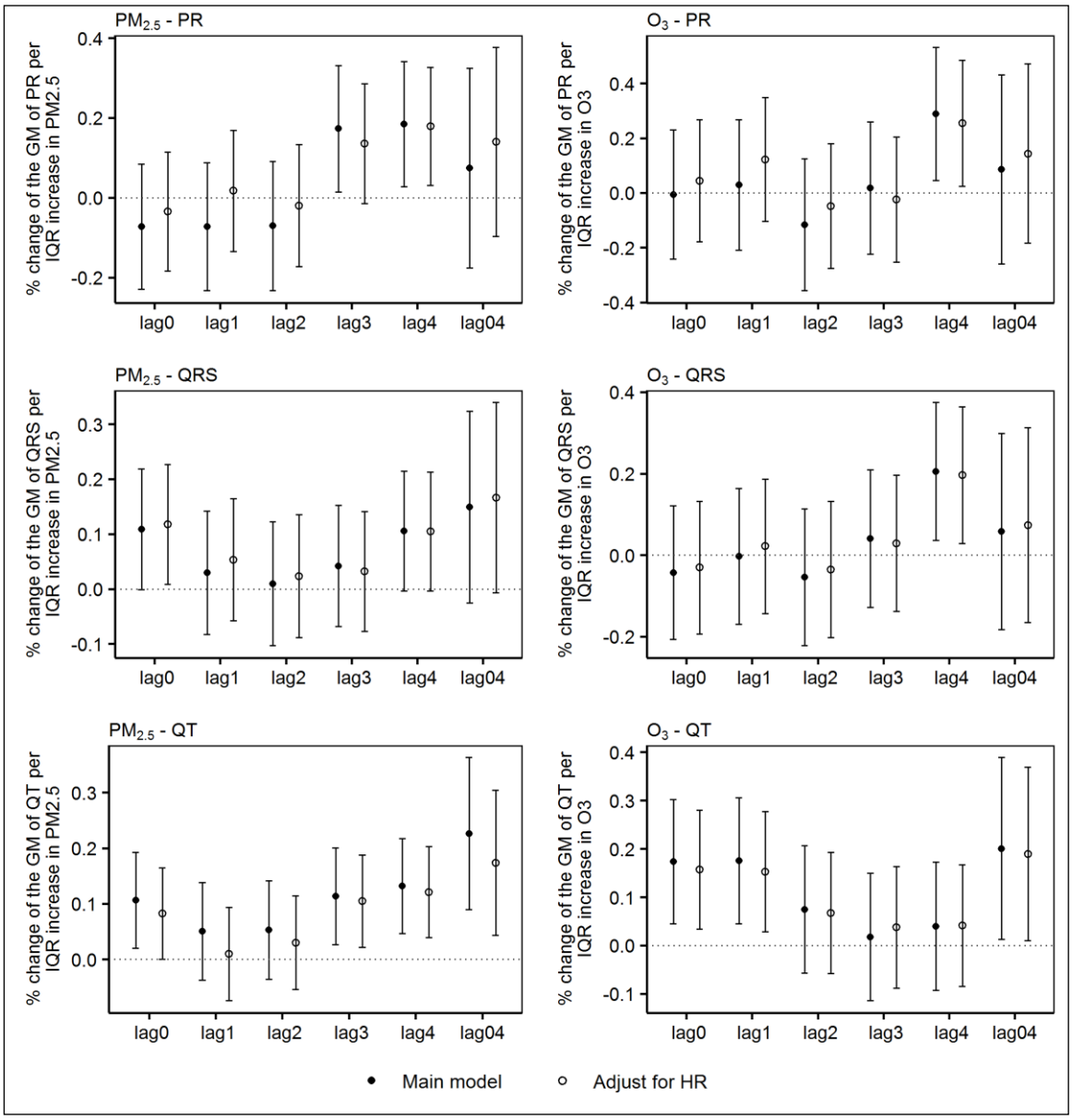

Figure S3. Percent change (95% CI) of the geometric mean of the PR, QRS, and raw QT intervals per interquartile range increase in PM2.5 and O3 in models with adjustment for HR.

CI: confidence interval; PM2.5: particulate matter ≤ 2.5 μm in aerodynamic diameter; O3: ozone; HR: heart rate; GM: geometric mean; IQR: interquartile range.

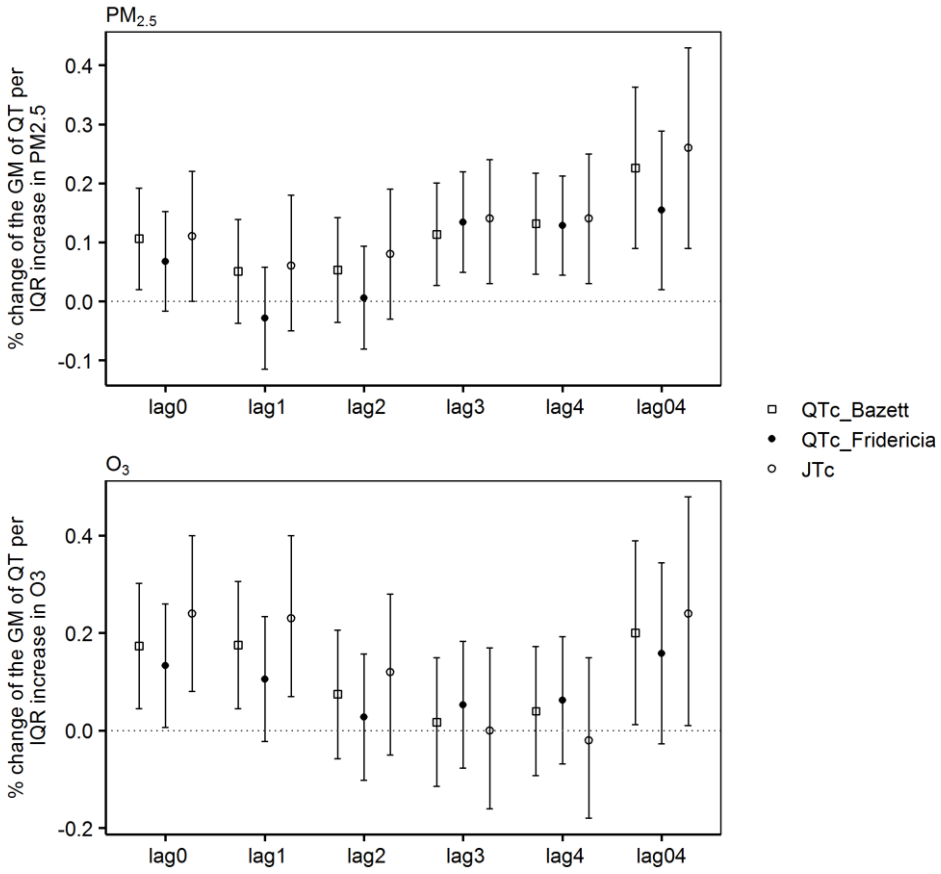

Figure S4. Comparison of the air pollution effects (percent change with 95% CI) on different ventricular repolarization indicators.

CI: confidence interval; PM2.5: particulate matter ≤ 2.5 μm in aerodynamic diameter; O3: ozone; GM: geometric mean; IQR: interquartile range; QTc_Bazett: heart rate-corrected QT interval calculated using the Bazett formula; QTc_Fridericia: heart rate-corrected QT interval calculated using the Fridericia formula; JTc: corrected JT interval calculated by subtracting QRS from QTc_Bazett.

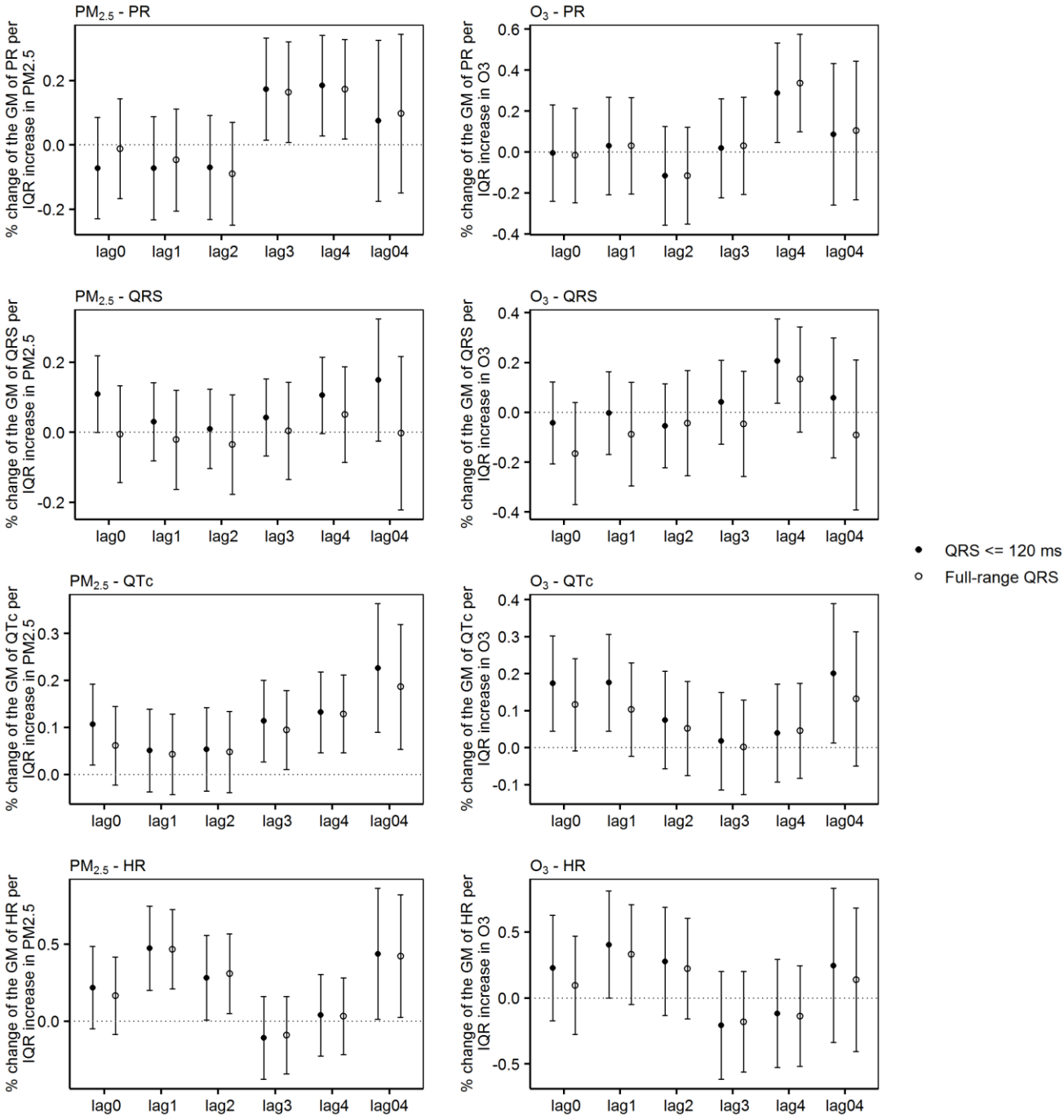

Figure S5. Percent change (95% CI) of the geometric mean of ECG parameters per interquartile range increase in PM2.5 and O3 among participants with QRS ≤ 120 ms and participants with QRS in the full range (50 ms ≤ QRS ≤ 170 ms).

CI: confidence interval; ECG: Electrocardiogram; PM2.5: particulate matter ≤ 2.5 µm in aerodynamic diameter; O3: ozone; HR: heart rate; GM: geometric mean; IQR: interquartile range.

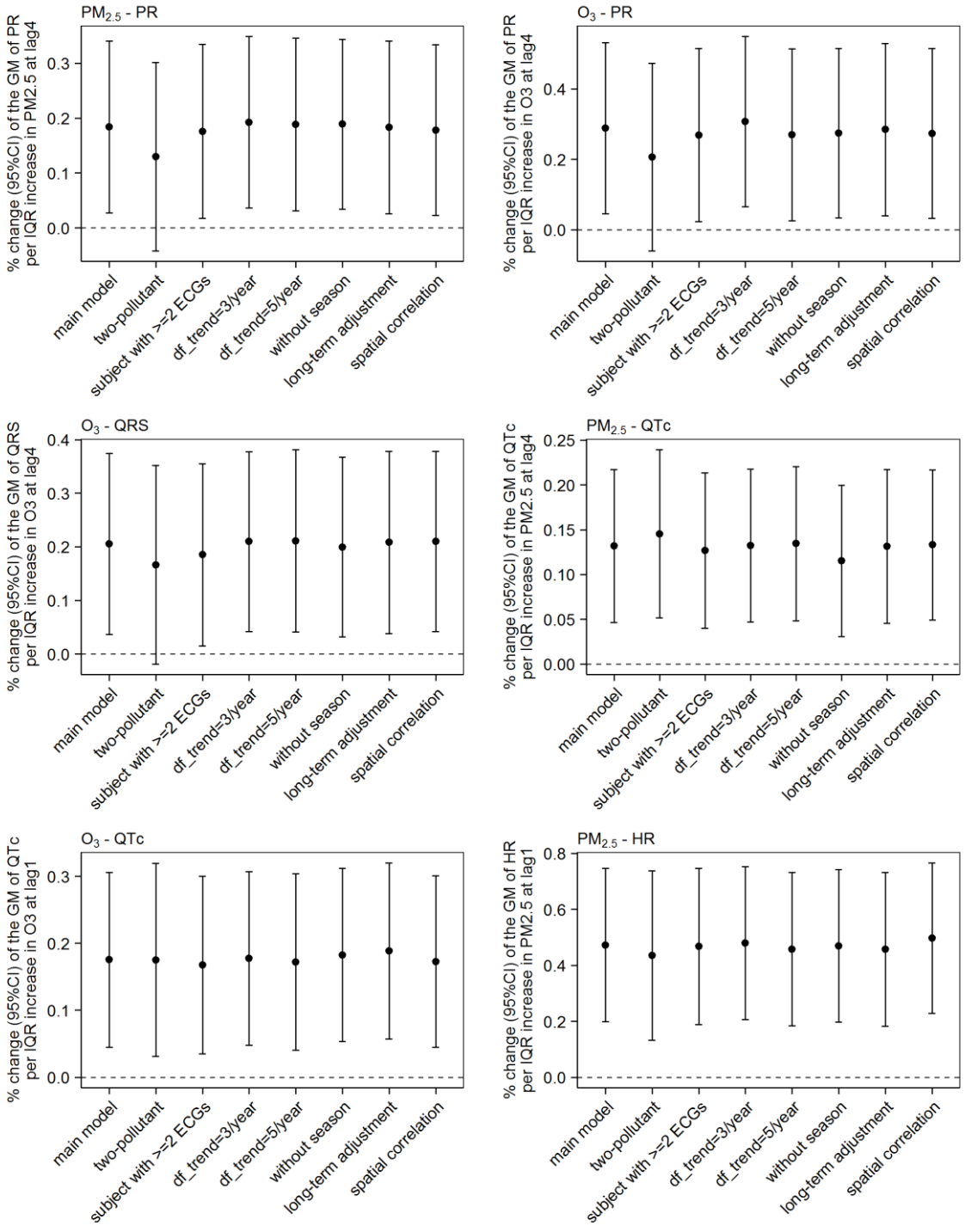

Figure S6. Percent change (95% CI) of the geometric mean of ECG parameters per interquartile range increase in PM2.5 and O3 in sensitivity analyses.

CI: confidence interval; ECG: Electrocardiogram; PM2.5: particulate matter ≤ 2.5 µm in aerodynamic diameter; O3: ozone; QTc: heart rate-corrected QT interval; HR: heart rate; GM: geometric mean; IQR: interquartile range.

The order of effect estimates in each panel: (1) Main single-pollutant model; (2) two-pollutant model; (3) restricted to participants with two or more ECG measurements; (4) degree of freedom of time trend = 3/year; (5) degree of freedom of time trend = 5/year; (6) regression models without adjustment for season; (7) regression model with adjustment for long-term exposure; (8) regression model using spatial correlation structure of ECGs.

Manuscript III

Title: Longitudinal Associations between Ambient Air Pollution and Insulin Sensitivity: Results from the KORA Cohort Study

Authors: Siqi Zhang, Sarah Mwiberi, Regina Pickford, Susanne Breitner, Cornelia Huth, Wolfgang Koenig, Wolfgang Rathmann, Christian Herder, Michael Roden, Josef Cyrys, Annette Peters, Kathrin Wolf, and Alexandra Schneider

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Longitudinal associations between ambient air pollution and insulin sensitivity: results from the KORA cohort study

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Summary

Background Impaired insulin sensitivity could be an intermediate step that links exposure to air pollution to the development of type 2 diabetes. However, longitudinal associations of air pollution with insulin sensitivity remain unclear. Our study investigated the associations of long-term air pollution exposure with the degree and rate of change of insulin sensitivity.

Methods In this longitudinal study, we analysed data from the Cooperative Health Research in the Region of Augsburg (KORA) cohort from Augsburg, Germany, which recruited participants aged 25–74 years in the survey between 1999 and 2001 (KORA S4), with two follow-up examinations in 2006–08 (KORA F4) and 2013–14 (KORA FF4). Serum concentrations of fasting insulin and glucose, and homoeostasis model assessment of insulin resistance (HOMA-IR, a surrogate measure of insulin sensitivity) and β-cell function (HOMA-B, a surrogate marker for fasting insulin secretion) were assessed at up to three visits between 1999 and 2014. Annual average air pollutant concentrations at the residence were estimated by land-use regression models. We examined the associations of air pollution with repeatedly assessed biomarker levels using mixed-effects models, and we assessed the associations with the annual rate of change in biomarkers using quantile regression models.

Findings Among 9620 observations from 4261 participants in the KORA cohort, we included 6008 (62·5%) observations from 3297 (77·4%) participants in our analyses. Per IQR increment in annual average air pollutant concentrations, HOMA-IR significantly increased by 2·5% (95% CI 0·3 to 4·7) for coarse particulate matter, by 3·1% (0·9 to 5·3) for $PM_{2.5}$, by 3·6% (1·0 to 6·3) for $PM_{2.5absorbance}$, and by 3·2% (0·6 to 5·8) for nitrogen dioxide, and borderline significantly increased by 2·2% (–0·1 to 4·5) for ozone, whereas it did not significantly increase for the whole range of ultrafine particles. Similar positive associations in slightly smaller magnitude were observed for HOMA-B and fasting insulin levels. In addition, air pollutant concentrations were positively associated with the annual rate of change in HOMA-IR, HOMA-B, and fasting insulin. Neither the level nor the rate of change of fasting glucose were associated with air pollution exposure.

Interpretation Our study indicates that long-term air pollution exposure could contribute to the development of insulin resistance, which is one of the key factors in the pathogenesis of type 2 diabetes.

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Introduction

Increasing prevalence of type 2 diabetes in the past decades has contributed to a rising global burden of mortality and disability.[1] Besides traditional risk factors, such as being overweight and having sedentary lifestyles, cumulative evidence is pointing to an association between ambient air pollution and a higher risk for type 2 diabetes.[2–5] It was estimated that in 2016, around 3·2 million incident diabetes cases and more than 0·2 million deaths from diabetes worldwide were attributable to exposure to $PM_{2.5}$.[6]

Although the association is established, the underlying mechanisms through which air pollution increases the risk for type 2 diabetes remain unclear. Insulin resistance is a key factor in the pathogenesis of type 2 diabetes.[7] Recent studies have shown that long-term air pollution exposure was associated with decreased insulin sensitivity among the general population of adults and youth,[8–10] patients with diabetes,[11] and individuals prone to type 2 diabetes,[12–14] suggesting impaired insulin sensitivity could be an important intermediate step linking air pollution to the development of type 2 diabetes. Although most existing evidence is from cross-sectional studies, the longitudinal association between air pollution and insulin sensitivity has not been fully investigated.[14,15]

In addition, decreasing insulin sensitivity over time has been identified as a predictor of incident hyperglycaemia and type 2 diabetes, independent of baseline metabolic measures in prospective studies.[16,17] However, the effects of air pollution on the change of insulin sensitivity over time have rarely been reported in population-based studies to date. A cohort study[14] on Latino children who were

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Research in context

Evidence before this study

We searched PubMed and Google Scholar for studies on air pollution and insulin sensitivity published before June 1, 2020, using a combination of search terms concerning air pollution ("air pollution" OR "air pollutant*" OR "particulate matter" OR "PM" OR "ultrafine particles" OR "PNC" OR "soot" OR "black carbon" OR "nitrogen dioxide" OR "NO_2" OR "ozone" OR "O_3") and insulin sensitivity ("insulin resistance" OR "insulin sensitivity" OR "insulin" OR "glucose" OR "HOMA-IR"). Studies were selected if they were population-based cohort studies that could potentially assess longitudinal associations, if they assessed long-term air pollution exposure (exposure window ≥1 year), and if they had insulin sensitivity or resistance as the outcome. We identified seven studies, and only one examined longitudinal associations of air pollution with both the level and the rate of change of insulin sensitivity-related biomarkers among Latino children who were overweight or obese. One study analysed repeated measurements of biomarkers and assessed the association of air pollution with only the degree of insulin sensitivity. The remaining five studies did cross-sectional analyses on data collected at a single examination in cohorts, including two studies of German children and adolescents, one on German adults, one on Mexican Americans at high risk for diabetes, and one on African-American and Latino youth in Los Angeles who were overweight or obese. A meta-analysis done in 2018, which included five of the aforementioned studies, reported cross-sectional associations of insulin sensitivity with particulate matter with an aerodynamic diameter of 10 μm and nitrogen dioxide, but not with $PM_{2·5}$. Overall, little is known about the longitudinal association between ambient air pollution and insulin sensitivity, especially in the general adult population.

Added value of this study

To the best of our knowledge, this is the first epidemiological study on the longitudinal association between ambient air pollution and insulin sensitivity in the general adult population. Our study found that long-term exposure to air pollution was positively associated with the level and the rate of change of the homoeostasis model assessment of insulin resistance and fasting insulin, suggesting associations of air pollution with impaired insulin sensitivity and a more pronounced deterioration (or less pronounced improvement) of insulin sensitivity over time. In addition, our study found similar changes for the homoeostasis model assessment of β-cell function, in line with a compensatory increase in insulin secretion. Participants who were older, male, unemployed, had prediabetes or diabetes, or were physically inactive were potentially more susceptible to the adverse air pollution effects on insulin sensitivity.

Implications of all the available evidence

Together with the evidence from previous studies, our study helps understand the mechanisms through which air pollution might be associated with the development of type 2 diabetes. Such findings imply an urgent need for air quality improvement to mitigate the adverse health effects of air pollution. In addition, reducing air pollution exposure could be considered as a prevention strategy for type 2 diabetes at the population level.

overweight or obese showed that long-term exposure to elevated $PM_{2·5}$ and nitrogen dioxide (NO_2) was associated with a faster decrease in insulin sensitivity during a mean follow-up of 3·4 years (SD 3·1). Such associations are yet to be assessed in the general adult population.

In this study, we examined longitudinal associations of air pollution with the repeatedly assessed homoeostasis model assessment of insulin resistance (HOMA-IR), as a surrogate marker of insulin sensitivity, and the homoeostasis model assessment of β-cell function (HOMA-B), as a surrogate marker of fasting insulin secretion, as well as fasting insulin and glucose. We also investigated whether air pollution was associated with a change in those biomarkers over time, and we explored individual characteristics potentially related to the susceptibility to air pollution effects. We hypothesised that air pollution would be positively associated with the level and the rate of change of all investigated biomarkers, especially among more susceptible subgroups, such as older adults.

See Online for appendix

Methods

Study design and participants

In this longitudinal study, we analysed data from the Cooperative Health Research in the Region of Augsburg (KORA) cohort, which was done in the city of Augsburg, Germany, and two adjacent counties.[18] Between 1999 and 2001, 4261 participants aged 25–74 years with German citizenship were recruited in the fourth cross-sectional health survey of the KORA cohort (KORA S4), with examinations between Oct 25, 1999, and April 28, 2001. Two follow-up examinations were carried out: the first follow-up, KORA F4, consisted of 3080 participants with examinations between Oct 9, 2006, and May 31, 2008; and the second follow-up, KORA FF4, consisted of 2279 participants with examinations between June 3, 2013, and Sept 27, 2014. Participants were invited to the KORA study centre, Augsburg, Germany, and completed a computer-assisted personal interview, a self-administered questionnaire, and physical examinations at each visit. Individual characteristics relevant in the current study are defined in the appendix (p 2).

The KORA study was approved by the ethics committee of the Bavarian Chamber of Physicians (Munich, Germany); all participants gave written informed consent.

Procedures and outcomes

Blood samples were drawn between 0700 h and 1100 h after fasting for at least 8 h for the measurements of

fasting insulin and glucose concentrations. Blood samples were kept on ice after withdrawal and transported at 4°C to the laboratories for analysis (to the German Diabetes Center laboratory, Düsseldorf, in KORA S4, and to the central laboratory in Augsburg in KORA F4 and KORA FF4). Detailed information about the standard operating procedure, assays of serum concentrations of fasting insulin and glucose, and the comparability and calibration of different assays is in the appendix (p 3). Fasting insulin and glucose in KORA S4 were only measured in participants older than 54 years (n=1357). HOMA-IR was calculated as fasting insulin (μIU/mL)×fasting glucose (mmol/L)/22·5. HOMA-B was calculated as 20×fasting insulin (μIU/mL)/(fasting glucose [mmol/L]−3·5). A higher HOMA-IR indicates reduced insulin sensitivity, and a lower HOMA-B indicates decreased fasting insulin secretion. For the validity of the assessment, we excluded observations by the timepoint of which glucose-lowering medication (Anatomical Therapeutic Chemical code A10) had been used.

Annual average concentrations of ultrafine particles (particles ≤100 nm in aerodynamic diameter, represented by particle number concentration [PNC]), particulate matter with an aerodynamic diameter of 2·5–10 μm (PM_{coarse}), $PM_{2·5}$, $PM_{2·5}$ absorbance ($PM_{2·5abs}$, a proxy of elemental carbon related to traffic exhaust), NO_2, and ozone (O_3) were estimated using land-use regression (LUR) models. In brief, we carried out three 2-week measurements at 20 locations within the KORA study area between March 6, 2014, and April 7, 2015, covering the warm, cold, and intermediate seasons, and we calculated annual average air pollutant concentrations at those sites. We built LUR models by regressing the measured annual average concentrations in 2014–15 against geographic information system-based spatial predictors, and we applied the fitted models to participants' home addresses to determine residential exposure levels. The adjusted model-explained variance (R^2) ranged from 0·68 (PM_{coarse}) to 0·94 (NO_2), and the adjusted leave-one-out cross-validation R^2s were between 0·55 (PM_{coarse}) and 0·89 (NO_2), indicating good model fit. Further information about this approach is given elsewhere.[19] For participants who moved house during the study period, the updated residential addresses were used for exposure assignment; otherwise, the same exposure levels were assigned across different visits.

To control for potential confounding effects of road traffic noise and greenspace, we assigned annual average day–night sound level and normalised difference vegetation index (NDVI) in a 300 m buffer (as a surrogate for surrounding greenness) to participants' residential addresses. Assessments of noise and NDVI are in the appendix (p 4).

The outcome variables were HOMA-IR, HOMA-B, fasting insulin, and fasting glucose. Participant observations were excluded from analysis if the residential address was unavailable, there was no data on fasting insulin and glucose, they were taking glucose-lowering medication, the blood sample was drawn after 1100 h, or there were missing values in the covariates of the main model.

Statistical analysis

We applied linear mixed-effects models with random intercepts for participants to examine associations of air pollution with repeatedly assessed HOMA-IR, HOMA-B, fasting insulin, and fasting glucose levels. All outcome values were natural log-transformed to increase the conformity to normal distributions of residuals. Covariates in models were selected a priori on the basis of the disjunctive cause criterion,[20] the covariate being the cause of either the exposure or the outcome, or both, but not in the potential causal pathway linking exposure to the outcome. Minimum models were adjusted for age, sex, body-mass index (BMI), visits (KORA S4, KORA F4, or KORA FF4), and the yearly season of blood withdrawal. Main models additionally included educational attainment, occupational status, smoking status and pack-years, alcohol consumption, and physical activity. Extended models were further controlled for waist–hip ratio, high-density lipoprotein, and total cholesterol. To assess the potential confounding effect of residential road traffic noise and greenspace, we built a second extended model by adding noise and NDVI to the main model. Annual average air pollutant concentrations were included separately in each model as a linear term. Effect estimates are presented as percent changes with 95% CIs in the geometric mean of the repeatedly assessed biomarker per IQR increase in air pollutant concentrations. We examined the linearity of the exposure–response relationship using a penalised spline of the air pollutant with degrees of freedom chosen by generalised cross validation.

For participants with biomarkers measured at more than one visit, we calculated the annual rate of change in HOMA-IR, HOMA-B, fasting insulin, and fasting glucose as the slope coefficient of a linear regression of biomarker levels regressed against years since baseline (KORA S4 or KORA F4, whichever the first measurement occurred in). Because the rate values were not normally distributed and the log-transformation was not applicable to negative values, we assessed associations between air pollution and the annual rate of change in biomarkers (original scale) using quantile regression models, which do not make assumptions about the residual distribution and are more robust to outliers in the outcome. To reduce the selection bias introduced by the selection of individuals with more than one measurement, we first estimated the weight for the included participants using the inverse probability weighting approach.[21] Specifically, we modelled the probability of being included in the rate of change analysis among all participants in KORA S4 via logistic regression, using individual characteristics in the

	KORA S4 examination (n=1312)*†	KORA F4 examination (n=2704)†	KORA FF4 examination (n=1992)†
HOMA-IR	3·7 (5·1); median 2·5	2·6 (2·1); median 2·0	2·7 (2·1); median 2·1
HOMA-B	137·8 (202·9); median 99·0	120·0 (66·4); median 104·9	109·6 (62·1); median 95·1
Fasting insulin, µIU/mL	14·1 (19·5); median 10·1	10·6 (7·2); median 8·7	10·6 (6·9); median 8·9
Fasting glucose, mg/dL	102·4 (17·2); median 99·0	95·8 (14·3); median 93·0	98·9 (14·0); median 97·0
Age, years	63·9 (5·5)	55·2 (12·9)	59·6 (12·3)
Sex			
Female	635 (48%)	1407 (52%)	1041 (52%)
Male	677 (52%)	1297 (48%)	951 (48%)
Body-mass index (kg/m²)	28·4 (4·2)	27·4 (4·6)	27·5 (4·9)
Occupation (employed)	313 (24%)	1581 (58%)	1165 (58%)
Education (high)	1049 (80%)	2492 (92%)	1867 (94%)
Smoking pack-years	14·2 (23·1)	11·9 (18·8)	11·4 (18·0)
Smoking status			
Current smoker	183 (14%)	505 (19%)	320 (16%)
Former smoker	493 (38%)	1037 (38%)	803 (40%)
Never	636 (48%)	1162 (43%)	869 (44%)
Alcohol consumption			
No	338 (26%)	790 (29%)	526 (26%)
Moderate	702 (54%)	1430 (53%)	1093 (55%)
High	272 (21%)	484 (18%)	373 (19%)
Physical activity			
Low	534 (41%)	830 (31%)	529 (27%)
Medium	540 (41%)	1191 (44%)	931 (47%)
High	238 (18%)	683 (25%)	532 (27%)
Diabetes status‡			
Normal glucose tolerance	596 (46%)	1690 (64%)	1045 (54%)
Prediabetes	588 (45%)	812 (31%)	751 (39%)
Diabetes	124 (9%)	156 (6%)	137 (7%)
Waist–hip ratio‡	0·90 (0·08)	0·88 (0·09)	0·90 (0·09)
Cholesterol, mg/dL‡	243·3 (41·8)	217·1 (39·4)	217·9 (39·2)
HDL, mg/dL‡	58·3 (16·4)	56·2 (14·4)	66·3 (18·8)

Data are mean (SD) or n (%), unless otherwise indicated. KORA=Cooperative Health Research in the Region of Augsburg. S4=fourth cross-sectional health survey of the KORA cohort. F4=first follow-up examination of KORA S4. FF4=second follow-up examination of KORA S4. HOMA-IR=homeostasis model assessment of insulin resistance. HOMA-B=homeostasis model assessment of β-cell function. HDL=high-density lipoproteins. *Participants in KORA S4 were restricted to individuals older than 54 years. †Medians of some outcomes were reported due to their skewed distributions. ‡Diabetes status was missing for four (<0·5%) participants in in KORA S4, 46 (2%) in KORA F4, and 59 (3%) in KORA FF4; waist–hip ratio was missing for one (<0·5%) participant in KORA FF4; cholesterol was missing for one (<0·5%) participant in KORA S4; and HDL was missing for two (<0·5%) participants in KORA S4.

Table 1: **Descriptive statistics of participant characteristics at each examination**

main mixed-effects model as predictors. The inverse of the predicted probability derived from the regression model was used as the weight in the quantile regression model, aiming to up-weight participants who were under-represented in the rate of change analysis. The quantile regression model was adjusted for baseline covariates including age, sex, BMI, educational attainment, occupational status, smoking status and pack-years, alcohol consumption, physical activity, and baseline levels of the investigated biomarker, as well as annual rates of change in BMI and smoking pack-years, and an indicator for the visits used in the calculation of the rate of change. Results are presented as absolute changes (with 95% CIs) in the annual rate of change at deciles of the distribution of rate values per IQR increase in air pollutant concentrations.

Effect modification was investigated by including an interaction term between the air pollutant and the potential effect modifier, which was assessed at each visit for the mixed-effects models and at the first visit for the quantile regression models. The examined modifiers included age (<60 years *vs* ≥60 years), sex (male *vs* female), educational attainment (high *vs* low), occupational status (employed *vs* not employed), smoking status (current *vs* former smoker or never), physical activity (low *vs* medium or high), obesity (BMI <30 kg/m² *vs* ≥30 kg/m²), and diabetes status (normal glucose tolerance *vs* prediabetes or diabetes).

In sensitivity analyses, we first built two-pollutant models by simultaneously including two pollutants that were not strongly correlated (r <0·7). Second, we excluded observations with fasting insulin higher than the 90th percentile in KORA S4, to generate a similar distribution across the three visits. Third, we excluded participants who moved during the study period to reduce exposure misclassification. Fourth, we excluded observations without a documented time of blood withdrawal. Moreover, we did the following sensitivity analyses for only the repeated measurements (mixed-effects model). We excluded outliers in outcomes defined as natural log-transformed values less than Q1−1·5×IQR or more than Q3+1·5×IQR (appendix p 11). Additionally, we adjusted for fasting insulin or fasting glucose in models of HOMA-IR and HOMA-B, and we used back-extrapolated annual average air pollutant concentrations in the year of each visit instead of the LUR-estimated annual average in 2014–15, which further took into account the temporal variation in exposure. A detailed description of the back-extrapolation approach is in the appendix (pp 4–5). For the annual rate of change analysis, we fitted models without control for the annual rate of change in BMI and smoking pack-years to examine the effect of time-varying adjustment, and models with control for road traffic noise and NDVI.

All statistical analyses were done with R (version 3.6.2), and the significance level was set at two-sided p value of less than 0·05.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, or data interpretation, the writing of the report, or the decision to submit the paper for publication. All authors had full access to all the data in the study, and the corresponding author had final responsibility for the decision to submit for publication.

Results

Among 9620 observations from 4261 participants in the KORA cohort, we included 6008 (62·5%) observations from 3297 (77·4%) participants in our analyses. The exclusion process is detailed in the appendix (p 12). Altogether, 466 (14·1%) of 3297 participants completed all three examinations, 1776 (53·9%) completed two, and the remaining 1055 (32·0%) completed one examination, giving a total of 1312 participants in KORA S4, 2704 in KORA F4, and 1992 in KORA FF4. In general, the distributions of fasting insulin and glucose concentrations were similar across KORA S4, KORA F4, and KORA FF4 among participants of the same age range, except that the 90th percentile of fasting insulin in KORA S4 was higher than that in KORA F4 and KORA FF4. In addition, these concentration distributions did not show substantial diurnal variations between 0700 h and 1100 h (appendix p 10).

Due to the age restriction in KORA S4 (fasting insulin and glucose were only measured in participants aged >54 years), only 1366 (32·1%) of 4261 participants contributed data on HOMA-IR, HOMA-B, fasting insulin, and fasting glucose, and thus, KORA S4 participants were on average older and had higher mean concentrations of these biomarkers than KORA F4 and KORA FF4 participants (table 1). We observed moderate to strong positive correlations between these biomarkers, except for a weak negative correlation between HOMA-B and fasting glucose (appendix p 13). Compared with all KORA participants, participants included in the analysis of repeated measurements had generally similar characteristics, whereas participants in the rate of change analysis had lower BMI and smoke exposure, and higher educational attainment, alcohol consumption, and physical activity at recruitment (appendix p 6).

The annual rate of change in HOMA-IR ranged from –6·20 to 3·96 units per year, with a median of 0·03 (IQR –0·05 to 0·12) units per year (appendix p 7). Participants with increasing HOMA-IR (n=1336) were more likely to have normal glucose tolerance and lower HOMA-IR, HOMA-B, fasting insulin, and fasting glucose at the first visit than were participants with unchanged (n=12) or decreasing (n=894) HOMA-IR over time; this pattern was reversed for the last visit (appendix p 8). Although BMI was similar between the two subgroups with increasing or unchanged and decreasing HOMA-IR at the first visit, it tended to be higher among participants with increasing HOMA-IR at the last visit.

Annual average concentrations of $PM_{2·5}$ and NO_2 at participants' residences were well below the EU air quality standards values of 25 μg/m³ for $PM_{2·5}$ and 40 μg/m³ for NO_2, although the $PM_{2·5}$ level exceeded the WHO guideline value of 10 μg/m³ (table 2). Correlations between air pollutants were moderate to strong, except for weak correlations with O_3 (appendix p 13).

Concerning repeated measurements of biomarkers, elevated PM_{coarse}, $PM_{2·5}$, $PM_{2·5abs}$, NO_2, and, to a lesser extent, O_3, were linearly associated with increases in HOMA-IR, HOMA-B, and fasting insulin (table 3). We did not find associations between air pollution and fasting glucose. The results were robust to the adjustment for additional covariates in the extended models; only $PM_{2·5abs}$ and NO_2 effects on HOMA-IR and fasting insulin slightly increased with further control for noise and NDVI (appendix p 14). Exposure–response relationships did not substantially deviate from linearity except for PNC with HOMA-IR, HOMA-B, and fasting insulin (appendix p 15). When restricting our analyses to the linear section of the relationship (PNC <12·7×10³/cm³), we observed positive associations of PNC with HOMA-IR, HOMA-B, and fasting insulin.

	Mean (SD)	Range	Median (IQR)
PNC, ×10³/cm³	7·3 (1·8)	3·2–15·7	7·3 (6·2–8·2)
PM_{coarse}, μg/m³	5·0 (1·0)	2·5–9·2	5·0 (4·3–5·7)
$PM_{2·5}$, μg/m³	11·8 (1·0)	8·3–14·8	11·9 (11·1–12·5)
$PM_{2·5abs}$, 10^{-5}/m	1·2 (0·2)	0·8–1·9	1·2 (1·1–1·4)
NO_2, μg/m³	14·4 (4·5)	6·9–28·2	14·0 (10·8–17·9)
O_3, μg/m³	39·0 (2·4)	31·3–46·2	39·0 (37·2–40·7)
Road traffic noise, dB	54·8 (6·6)	22·3–75·4	53·9 (50·6–58·6)
NDVI	0·1 (0·1)	0·0–0·3	0·1 (0·0–0·1)

Exposure levels were estimated at participants' residences in KORA S4 in this descriptive analysis. For 19 participants whose residential information was missing in the KORA S4 survey, residential addresses in KORA F4 were used. NDVI=normalised difference vegetation index. NO_2=nitrogen dioxide. O_3=ozone. PM_{coarse}=particulate matter with an aerodynamic diameter of 2·5–10 μm. $PM_{2·5abs}$=$PM_{2·5}$ absorbance. PNC=particle number concentration. KORA=Cooperative Health Research in the Region of Augsburg. S4=fourth cross-sectional health survey of the KORA cohort. F4=first follow-up examination of KORA S4.

Table 2: **Distribution of annual average air pollutant concentrations, road traffic noise, and NDVI at residences (n=3297)**

	HOMA-IR	HOMA-B	Fasting insulin	Fasting glucose
PNC	0·7 (–1·0 to 2·4)	0·8 (–0·8 to 2·4)	0·7 (–0·8 to 2·3)	–0·1 (–0·4 to 0·3)
PNC (linear)*	2·1 (0·2 to 4·0)	2·3 (0·5 to 4·1)	2·1 (0·4 to 3·9)	0·0 (–0·5 to 0·4)
PM_{coarse}	2·5 (0·3 to 4·7)	2·0 (–0·1 to 4·0)	2·4 (0·4 to 4·4)	0·1 (–0·4 to 0·6)
$PM_{2·5}$	3·1 (0·9 to 5·3)	2·7 (0·6 to 4·7)	3·0 (1·0 to 5·0)	0·1 (–0·4 to 0·6)
$PM_{2·5abs}$	3·6 (1·0 to 6·3)	2·7 (0·2 to 5·2)	3·4 (1·0 to 5·9)	0·2 (–0·4 to 0·8)
NO_2	3·2 (0·6 to 5·8)	2·5 (0·1 to 4·9)	3·1 (0·7 to 5·4)	0·2 (–0·4 to 0·7)
O_3	2·2 (–0·1 to 4·5)	1·0 (–1·1 to 3·2)	1·9 (–0·2 to 4·0)	0·3 (–0·3 to 0·8)

Mixed-effects models for repeated measurements of biomarkers were adjusted for age, sex, BMI, visits, season, educational attainment, occupational status, smoking status and pack-years, alcohol consumption, and physical activity. Outcome variables were natural log-transformed in analyses, and the effect estimates are presented as the percentage changes in the geometric mean of repeatedly assessed biomarkers. The geometric mean was 2·2 for HOMA-IR, 102·3 for HOMA-B, 9·4 μIU/mL for fasting insulin, and 97·3 mg/dL for fasting glucose. An IQR increase was 2·0×10³/cm³ for PNC, 1·4 μg/m³ for PM_{coarse}, 1·4 μg/m³ for $PM_{2·5}$, 0·3×10^{-5}/m for $PM_{2·5abs}$, 7·1 μg/m³ for NO_2, and 3·5 μg/m³ for O_3. HOMA-IR=homeostasis model assessment of insulin resistance. HOMA-B=homeostasis model assessment of β-cell function. PNC=particle number concentration. PM_{coarse}=particulate matter with an aerodynamic diameter of 2·5–10 μm. $PM_{2·5abs}$=$PM_{2·5}$ absorbance. NO_2=nitrogen dioxide. O_3=ozone. *Exposure–response relationships between the whole range of PNC and repeated measurements of HOMA-IR, HOMA-B, and fasting insulin were not linear. We restricted the analyses to PNC <12·7×10³/cm³ (cutoff values suggested by the exposure–response curve; n=5927) to assess the linear relationship. The association between PNC and fasting glucose was also investigated in the reduced PNC range.

Table 3: **Percentage changes (95% CIs) in the repeated measurements (n=6008) of biomarkers per IQR increase in air pollutant concentrations**

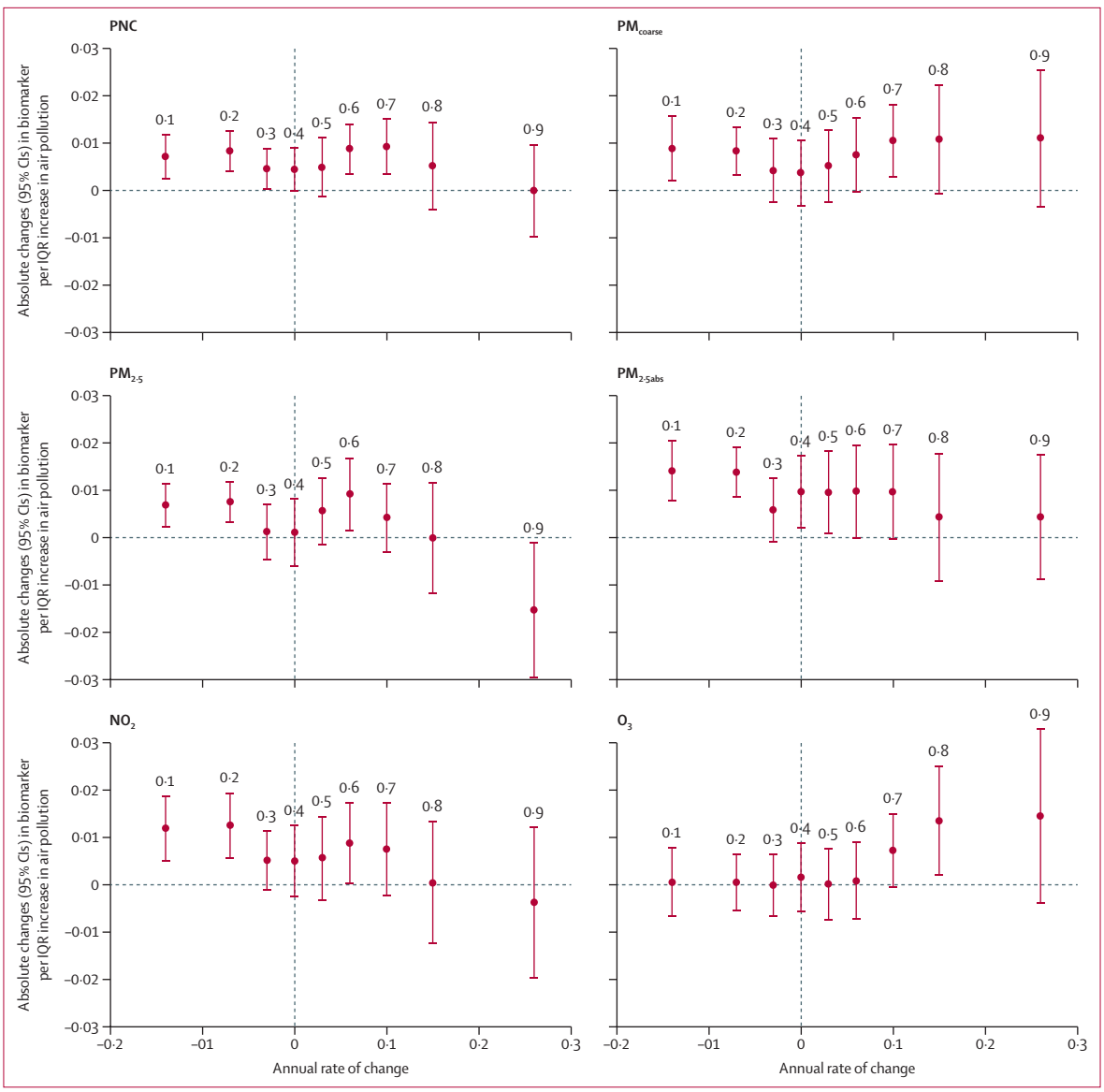

Figure 1: **Absolute changes (95% CIs) in the annual rate of change in HOMA-IR at deciles of the distribution per IQR increase in air pollutant concentrations**
Quantile regression models for the annual rate of change were adjusted for baseline levels of the investigated biomarker, age (baseline), sex, BMI (baseline), annual rate of change in BMI, educational attainment (baseline), occupational status (baseline), smoking status and pack-years (baseline), annual rate of change in smoking pack-years, physical activity (baseline), and an indicator for the visits used in the calculation of the rate of change. Area on the left side of the dashed line indicates increasing insulin sensitivity over years (annual rate of change below zero); area on the right side of the dashed line indicates decreasing insulin sensitivity over years (annual rate of change above zero). Values (ie, 0·1–0·9) above the error bars indicate deciles of the distribution of the annual rate of change. An IQR increase was $2·0 \times 10^3$/cm^3 for PNC, 1·4 μg/m^3 for PM$_{coarse}$, 1·4 μg/m^3 for PM$_{2·5}$, $0·3 \times 10^{-5}$/m for PM$_{2·5abs}$, 7·1 μg/m^3 for NO$_2$, and 3·5 μg/m^3 for O$_3$. HOMA-IR=homeostasis model assessment of insulin resistance. PNC=particle number concentration. PM$_{coarse}$=particulate matter with an aerodynamic diameter of 2·5–10 μm. PM$_{2·5abs}$=PM$_{2·5}$ absorbance. NO$_2$=nitrogen dioxide. O$_3$=ozone.

The annual rate of change in HOMA-IR was positively associated with PNC, PM$_{2·5abs}$, and NO$_2$ at the 10th to 70th percentiles of the rate value distribution (ie, rate of change ≤0·10 units per year), with PM$_{coarse}$ at all deciles, with PM$_{2·5}$ at lower percentiles, and with O$_3$ at higher percentiles (figure 1; appendix p 9). Positive associations at rate values above zero (ie, the 40th to 90th percentiles) indicate air pollution-related greater decline in insulin sensitivity over time, whereas positive associations at rate values below zero indicate that air pollution attenuated improvement in insulin sensitivity. Particles and NO$_2$ were positively associated with the annual rate of change in HOMA-B and fasting insulin, with exceptions at the lowest or highest end, or both, for PM$_{2·5}$, PM$_{2·5abs}$, and NO$_2$ (figure 2; appendix pp 9, 16). Associations of O$_3$ with HOMA-B and fasting insulin were similar to those with HOMA-IR. No consistent associations were observed for the annual rate of change in fasting glucose (appendix pp 9, 17).

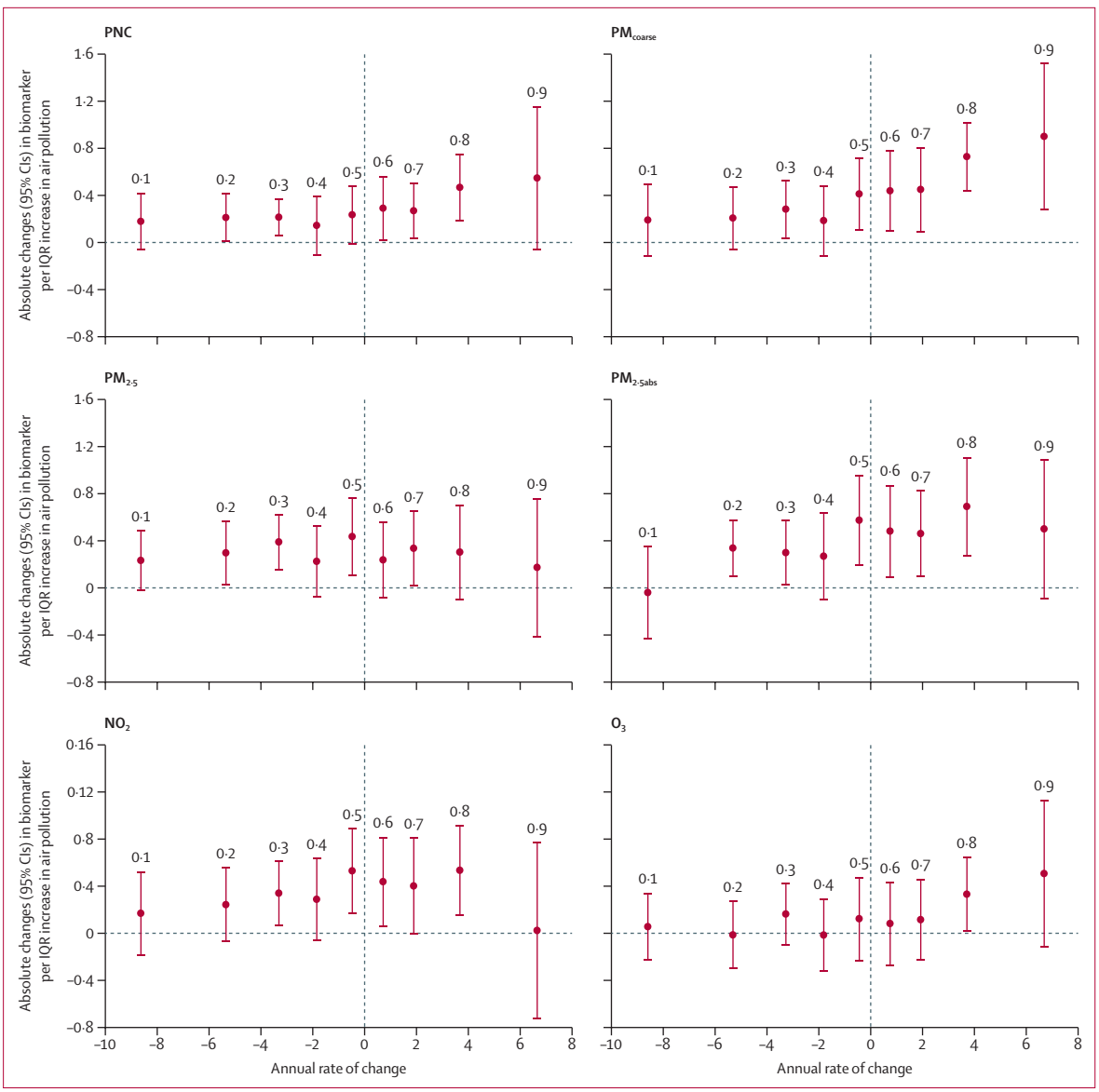

Figure 2: **Absolute changes (95% CIs) in the annual rate of change in HOMA-B at deciles of the distribution per IQR increase in air pollutant concentrations**
Quantile regression models for the annual rate of change were adjusted for baseline levels of the investigated biomarker, age (baseline), sex, BMI (baseline), annual rate of change in BMI, educational attainment (baseline), occupational status (baseline), smoking status and pack-years (baseline), annual rate of change in smoking pack-years, physical activity (baseline), and an indicator for the visits used in the calculation of the rate of change. Area on the left side of the dashed line indicates decreasing insulin secretion over years (annual rate of change below zero); area on the right side of the dashed line indicates increasing insulin secretion over years (annual rate of change above zero). Values (ie, 0·1–0·9) above the error bars indicate deciles of the distribution of the annual rate of change. An IQR increase was $2·0 \times 10^3$/cm^3 for PNC, 1·4 μg/m^3 for PM$_{coarse}$, 1·4 μg/m^3 for PM$_{2·5}$, $0·3 \times 10^{-5}$/m for PM$_{2·5abs}$, 7·1 μg/m^3 for NO$_2$, and 3·5 μg/m^3 for O$_3$. HOMA-B=homeostasis model assessment of β-cell function. PNC=particle number concentration. PM$_{coarse}$=particulate matter with an aerodynamic diameter of 2·5–10 μm. PM$_{2·5abs}$=PM$_{2·5}$ absorbance. NO$_2$=nitrogen dioxide. O$_3$=ozone.

The associations of particles and NO$_2$ with repeated measurements of HOMA-IR, HOMA-B, and fasting insulin were significantly stronger among participants who were older than 60 years, male, or not employed, and suggestively stronger among physically inactive individuals (appendix pp 18–19). Males also showed higher susceptibility to air pollution effects on fasting glucose. For the annual rate of change, we observed stronger associations of particles and NO$_2$ with HOMA-IR, fasting insulin, and fasting glucose (rate values above zero) among older adults, and with HOMA-IR, HOMA-B, and fasting insulin among males and participants with prediabetes or diabetes (examples in the appendix pp 20–21). No effect modification was found for other potential modifiers (data not shown).

In terms of sensitivity analysis, the associations of particles and NO$_2$ with repeated measurements of biomarkers were robust to additional adjustment for O$_3$,

and vice versa (appendix p 22). Adjustment for $PM_{2·5}$ attenuated the effect estimates of other particles, and the effect estimates of $PM_{2·5}$ slightly decreased after including PM_{coarse} and $PM_{2·5abs}$. For the annual rate of change, the effect estimates of $PM_{2·5}$ were attenuated by adjustment for PNC, PM_{coarse}, and $PM_{2·5abs}$. The other associations remained stable in two-pollutant models (appendix pp 23–26).

Associations between air pollution and biomarkers were generally robust in sensitivity analyses (appendix pp 27, 29–32). However, air pollution effects on repeated measurements of HOMA-IR and HOMA-B substantially decreased when controlled for fasting insulin (appendix p 28). Additionally, further adjustment for road traffic noise and NDVI increased the effects of air pollution on the annual rate of change in HOMA-IR, fasting insulin, and fasting glucose. Excluding observations with fasting insulin in KORA S4 that were higher than the 90th percentile attenuated effects on the annual rate of change in fasting insulin at specific percentiles.

Discussion

In this longitudinal study with biomarkers measured up to three times, participants exposed to elevated particulate matter, NO_2, and O_3 had higher levels of HOMA-IR, HOMA-B, and fasting insulin. Moreover, we observed positive associations between air pollution and the annual rate of change in HOMA-IR, HOMA-B, and fasting insulin over time. No significant associations were found between air pollution and fasting glucose in the whole study population. Participants who were older than 60 years, male, not employed, physically inactive, or who had prediabetes or diabetes were potentially more susceptible to the effects of air pollution on the investigated biomarkers.

Insulin resistance is characterised by a lower response of tissues to insulin stimulation and is usually measured as impaired insulin-stimulated skeletal muscle glucose uptake and glycogen synthase activity.[22] It has an important role in the development of type 2 diabetes and is also associated with higher incident cardiovascular disease.[23] Our finding of positive associations between air pollution and HOMA-IR and fasting insulin levels suggest an air pollution-related decrease in insulin sensitivity. Consistent findings were reported in our previous cross-sectional analyses[10] on data from KORA F4, as well as among children and adolescents in two German birth cohorts[8,9] and a US childhood obesity study,[12] and among Mexican-American adults with higher risks of type 2 diabetes.[13] However, other long-term exposure studies did not find significant associations between air pollution and insulin sensitivity, including the Framingham Heart Study[15] and the Meta-AIR study.[24] The mixed results could be partly due to different population susceptibility. Studies have shown that children are more susceptible to adverse health effects of air pollution because of their higher minute ventilation, higher levels of physical activity, and dynamic developmental physiology.[25] Individuals with higher genetic risk of type 2 diabetes were also shown to be more susceptible to the effects of particulate matter on diabetes.[26] Moreover, effects on insulin sensitivity could vary across different air pollutants; for example, stronger effects have been observed for traffic-related exposure metrics than for $PM_{2·5}$.[15]

In addition, we observed positive associations of air pollution with the annual rate of change in HOMA-IR and fasting insulin, suggesting a faster deterioration of insulin sensitivity related to air pollution exposure. In the subgroup with increasing insulin sensitivity over time (annual rate of change below zero), which might be attributable to beneficial lifestyle changes, weight loss, or both, such positive associations indicate that elevated air pollution exposure could slow down the process of improvement. So far, air pollution effects on the change in insulin sensitivity were assessed only among 314 Latino children (8–15 years) in Los Angeles who were overweight or obese, showing that long-term exposure to $PM_{2·5}$ and NO_2 was associated with an increased decline in insulin sensitivity.[14] Our study replicated these findings among the general adult population, and further provided evidence for the heterogeneity of air pollutant effects across different degrees of change in insulin sensitivity.

Our study did not find associations between air pollution and fasting glucose, and the air pollution effects on HOMA-IR were attenuated only by further adjustment for fasting insulin. These findings indicate that the positive associations between air pollution and HOMA-IR are mainly driven by the air pollution-related increase in fasting insulin rather than fasting glucose. This conclusion is supported by the theory that impaired insulin sensitivity might first lead to an increase in insulin secretion to compensate for reduced insulin signalling and maintain normal glucose tolerance.[27] Therefore, our positive associations between air pollution and HOMA-B indicate increased fasting β-cell insulin secretion in response to impaired insulin sensitivity, rather than improved β-cell function.

Several mechanisms have been proposed whereby air pollution could potentially affect insulin sensitivity. For example, air pollution exposure has been shown to increase systemic levels of pro-inflammatory cytokines, such as tumour necrosis factor-α and interleukin-1. These cytokines could contribute to the development of insulin resistance by activating c-Jun N-terminal kinase, which inhibits insulin signalling through serine phosphorylation of insulin receptor substrate proteins.[28] In addition, $PM_{2·5}$ exposure was shown to induce pulmonary oxidative stress, thereby decreasing AKT and endothelial nitric oxide synthase phosphorylation, and impairing insulin signalling via the PI3-kinase–AKT pathway.[29,30] Moreover, air pollution-mediated over-activity of the sympathetic nervous system could further exacerbate insulin resistance.[31]

Our study identified subgroups with stronger responses to the effect of air pollution on insulin sensitivity. Such predisposition is determined by both intrinsic and external factors. The greater susceptibility of older adults has been frequently reported, which could be explained by declines in physiological processes (eg, reduced clearance of particulate matter) and a higher prevalence of pre-existing diseases that might confer increased risks for adverse health effects.[32] The sex-related difference in the effects of air pollution is potentially associated with sex-specific biological, social, or behavioural traits that could affect the deposition rate of pollutants and exposure patterns. Given the currently mixed evidence regarding effect modification by sex,[33] further investigation is needed to elucidate possible mechanisms. Stronger associations in participants with prediabetes or diabetes could be related to their chronic inflammatory state,[34] which might enhance the inflammatory response to air pollution.[26,35] Additionally, these participants might have a higher genetic risk of type 2 diabetes and thus be more susceptible to air pollution effects than individuals without diabetes.[26] The interpretation of effect modification should also consider the potential non-differential exposure misclassification, which has been proved to underestimate the effects of air pollution assessed at a residence. In our study, employed participants who commuted to their workplaces had a greater risk of exposure misclassification than participants who were not employed and were likely to spend more time around their residences, and thus smaller effect estimates were expected in employed individuals.

Our study used HOMA-IR rather than direct measures of insulin sensitivity, such as the glucose clamp technique and the minimal model assessment, in consideration of convenience and cost savings. Of note, HOMA-IR reflects fasting-state insulin sensitivity, whereas the dynamic tests reflect post-prandial insulin stimulated conditions. The limitations of HOMA-IR have been previously documented. For instance, Bergman and colleagues[36] reported that HOMA-IR did not measure the same genetic contribution to insulin resistance as is reflected in minimal model-based insulin sensitivity, but that it captured more in terms of environmental factors. Furthermore, differences related to ethnicity and sex have been found in the ability of HOMA-IR to predict insulin sensitivity.[37] However, several studies have shown a strong correlation between HOMA-IR and insulin sensitivity as determined by the glucose clamp in various populations ($r=-0{\cdot}82$; $p<0{\cdot}0001$ in one study;[38] $r=-0{\cdot}71$; $p<0{\cdot}01$ in another;[39] and $R_s=0{\cdot}88$; $p<0{\cdot}0001$ in a third study[40]). HOMA-IR has also been recently validated against the hyperinsulinaemic clamp in a German cohort and both tests identified groups of diabetes clusters.[41] In sum, HOMA-IR has developed as a reliable and practical measure of insulin sensitivity in comparable cohorts. In addition, it should be noted that HOMA-B only assesses fasting insulin secretion, and data for dynamic measures of insulin secretion, such as the insulinogenic index, were not available in our study.

The KORA cohort is a well characterised study with a standardised and comprehensive collection of individual information, which enhanced the reliability of our results. The longitudinal study design with repeated measurements of biomarkers strengthened statistical power and reduced potential residual confounding from unmeasured factors. In addition, the design enabled examination of the change of insulin sensitivity over time, which provided a better understanding of the longitudinal air pollution effects on the development of type 2 diabetes. Furthermore, the residential air pollutant concentrations, which were estimated using well defined LUR models, captured the spatial variation in exposure and enabled us to draw conclusions from consistent patterns across various air pollutants, reducing the risk of chance findings. The finding of associations between PNC and insulin sensitivity provide evidence for the adverse long-term health effect of ultrafine particles, which has been understudied so far.

One limitation of our study is that the air pollutant concentrations were estimated using spatial models for 2014–15. Although we believe that these exposure estimates are valid for the historical spatial contrasts, because previous studies have shown that the spatial variation in exposure remained stable over time,[42] we did not take into account the temporal variation in exposure. By applying back-extrapolated air pollution concentrations, we assessed the potential effect of temporal variation, and the robust results validated our exposure assessment approach. Second, we assigned air pollution concentrations to residential addresses and did not allow for the mobility of participants. This non-differential exposure misclassification might have biased the effect estimates towards the null. Third, we did not adjust for time-varying covariates other than BMI and smoking pack-years in the rate of change analysis, which might have resulted in residual confounding. Additionally, there could also be residual confounding by unmeasured factors.

In conclusion, our study suggests that long-term exposure to elevated air pollution was associated with decreased insulin sensitivity and a more pronounced deterioration (or less pronounced improvement) of insulin sensitivity over time, with compensatory increased insulin secretion. These findings support one underlying mechanism of the effects of air pollution on the development of type 2 diabetes in the general adult population. From a public health perspective, our study indicates that it would be beneficial to reduce air pollution exposure, in addition to making lifestyle interventions, to mitigate the health burden of type 2 diabetes.

Contributors

SZ and SM did the data analyses and wrote the manuscript. SZ, CHu, WR, CHe, AP, KW, and AS, have verified the underlying data. RP, SB, AP, KW, and AS were involved in the study design, results

interpretation, and review of the manuscript. AP provided oversight on the KORA study design. CHu, WK, WR, CHe, and MR were involved in the acquisition of the KORA data and reviewed the manuscript. JC and KW were involved in the measurement and modelling of the air pollution data. All authors read and approved the final manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Data collected for the study, including de-identified individual participant data and a data dictionary defining each field in the set, are available on reasonable request. Study protocol and statistical analysis plan will be available on reasonable request. These data will be available with publication from the corresponding author (siqi.zhang@helmholtz-muenchen.de). Data will be shared by the following access criteria: with investigator support; after approval of a scientific research proposal; and with a signed data access agreement.

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Appendix

Longitudinal associations between ambient air pollution and insulin sensitivity: results from the KORA cohort study

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Text S1. Definitions of individual characteristics

Body mass index (BMI) was calculated as weight divided by height squared. Occupational status was defined as employed if participants were employed, self-employed, or in training, and as unemployed/retired if participants were unemployed, homemakers, or retired. Educational attainment higher than secondary school was categorized as high, otherwise as low. Cumulative exposure to tobacco smoke (i.e. smoking pack-years) was assessed as the number of packs of cigarettes (20 cigarettes per pack) smoked per day multiplied by the years of smoking. Alcohol consumption was categorized into no (0 g/day), moderate (men 0·1–39·9 g/day and women 0·1–19·9 g/day), and high (men ≥ 40 g/day and women ≥ 20 g/day) consumption.[1] Physical activity was categorized based on the time spent on physical exercise into low (no or almost no physical exercise), medium (about one hour per week), and high (more than two hours per week) levels. Diabetes was defined as self-reported diabetes with confirmation by the respective physician or medical records and/or use of glucose-lowering medication (Anatomical Therapeutic Chemical code = A10), fasting glucose ≥ 126 mg/dl, or 2-h post glucose load glucose ≥ 200 mg/dl. Prediabetes included impaired glucose tolerance (defined as fasting glucose < 126 mg/dl and 2-h post glucose load 140 mg/dl ≤ glucose < 200 mg/dl) and impaired fasting glucose (defined as 110 mg/dl ≤ fasting glucose < 126 mg/dl). Normal glucose tolerance was defined as fasting glucose < 110 mg/dl and 2-h post glucose load glucose < 140 mg/dl.[2] Seasons of blood withdrawal were defined as spring: March-May; summer: June–August; autumn: September–November; winter: December–February.

Text S2. Measurement of biomarkers

Participants were asked to fast for at least 12 hours before the visits to the KORA study center, during which time no food or liquid were allowed for intake except for mineral water. Physical exertion and smoking were also to be avoided on the day before and the morning before blood sampling. After a rest for 5–10 min, blood samples were collected in a sitting position.

Serum fasting insulin concentrations were measured by a microparticle enzyme immunoassay (Abbott, Wiesbaden, Germany) in S4, by an electrochemiluminescence immunoassay on a Cobas e602 instrument (Roche Diagnostics GmbH, Mannheim, Germany) in F4, and by a solid-phase enzyme-labeled chemiluminescent immunometric assay on an Immulite 2000 systems analyzer (Siemens) or by an electrochemiluminescence immunoassay on a Cobas e602 instrument (Roche) in FF4.[3,4] Serum fasting glucose concentrations were assessed by a hexokinase method (Gluco-quant, Roche) in S4, by a hexokinase method on a Dimension RxL (GLU Flex, Dade Behring, Deerfield, IL, USA) in F4, and by an enzymatic, colorimetric method using the GLU assay on a Dimension Vista 1500 instrument (Siemens) or using the GLUC3 assay on a Cobas c702 instrument (Roche) in FF4.[3,4]

The measurement instruments and assays of fasting insulin and glucose changed in KORA FF4 from Siemens to Roche halfway during the study. Calibration formulas were developed using 194 (122 for fasting glucose) FF4-samples measured with both methods during the time of the change. The Siemens fasting insulin results were calibrated to the Roche measurements using the following formula: Insulin_Roche = 1·307 µIU/mL + Insulin_Siemens × 1·016. No calibration was needed for fasting glucose because the double measurements were very similar, so that the intercept and the slope of the Passing-Bablok regression used for calibration were estimated to be zero and one, respectively. The distribution of fasting insulin concentrations among participants of the same age range was consistent across KORA S4, F4, and FF4, except that the 90th percentile of fasting insulin in S4 was higher than that in F4 and FF4, which might be due to a higher proportion of undiagnosed or untreated diabetic participants in S4. Thus, to make the fasting insulin and corresponding HOMA-IR and HOMA-B levels more comparable across three examinations, we excluded the observations with fasting insulin levels higher than the 90th percentile in S4 in a sensitivity analysis.

Text S3. Exposure assessment

Noise
We calculated the annual average day-night sound level four meters above the ground using a model developed by ACCON GmbH (Greifenberg, Germany).[5] This three-dimensional ground model considered all breaking edges, bridge constructions, and noise abatement walls at public roads, and took into account ground plan, occupancy, height, and reflection characters of around 87,000 buildings. Roads width, type, surface, traffic volume were used to describe roads in a network containing an overall length of 750 km in 2009.

NDVI (Normalized Difference Vegetation Index)
NDVI was derived from Landsat 5 Thematic Mapper satellite images captured in June 2000 (S4), 2007 (F4), and 2013 (FF4) at a spatial resolution of 30 m.[6] NDVI is calculated based on the difference of surface reflectance in visible (0·4–0·7 µm) and near-infrared (0·7–1·1 µm) wavelengths. NDVI values range from -1 to 1. Values close to one indicate a high density of green vegetation and values close to zero indicate barren areas of rock or sand. Negative values refer to blue spaces (water). In our study, we set all negative values to zero.

Back-extrapolated air pollutant concentrations
In the city of Augsburg, daily monitoring of background air pollution by routine continuous monitoring sites has been in operation since 1984. A map of the monitoring sites is shown in the figure below.

We generated the time series of daily pollutant concentrations covering the study period of KORA S4–FF4. Using data from routine monitoring sites, we calculated the absolute differences in annual average concentrations between the period of each visit (01.01.2000–31.12.2000 for S4, 01.01.2007–31.12.2007 for F4, and 01.07.2013–30.06.2014 for FF4) and the period of ULTRA III measurements (01.03.2014–15.04.2015). To account for the difference in monitoring devices used at routine monitoring sites and in ULTRA III measurements, we calculated the ratio of average concentrations at the monitoring sites for the ULTRA III measurement period to average concentrations at the 20 measurement sites in ULTRA III, and calibrated the absolute difference by multiplying the absolute difference by the ratio. We then calculated for each study participant the back-extrapolated concentration at each visit by adding the calibrated absolute difference to the LUR-model-estimated ULTRA III annual average concentrations. The back-extrapolated air pollution concentrations reflected not only the spatial variation but also the temporal variation in exposure.

The mixed-effects models using back-extrapolated exposure were additionally adjusted for the year of visit to control for the potential temporal trend in both exposure and outcomes.

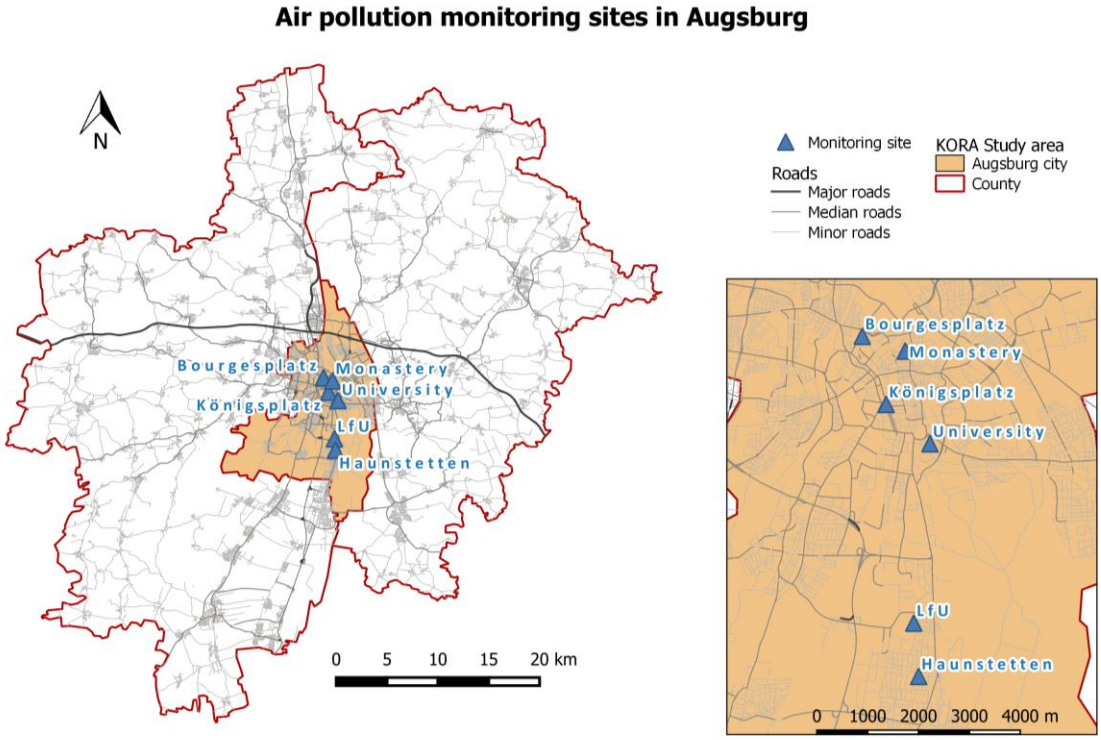
Air pollution monitoring sites in Augsburg
N
Bourgesplatz
Monastery
University
Königsplatz
LfU
Haunstetten
0 5 10 15 20 km
Monitoring site
KORA Study area
Augsburg city
County
Roads
Major roads
Median roads
Minor roads
Bourgesplatz
Monastery
Königsplatz
University
LfU
Haunstetten
0 1000 2000 3000 4000 m

Table S1. Comparison of individual characteristics in KORA S4 among all KORA participants and participants included in the repeated measurements and annual rate of change analyses.

	KORA (*N* = 4,261)	**Repeated measurements (*N* = 3,297)**		**Rate of change (*N* = 2,242)**	
	Mean ± SD / *n* (%)	**Mean ± SD / *n* (%)**	***p*-value***	**Mean ± SD / *n* (%)**	***p*-value***
Age (years)	49·2 ± 13·9	50·3 ± 13·7	<0·001	49·5 ± 13·2	0·34
Sex (male)	2,090 (49)	1,590 (48)	0·49	1,086 (48)	0·66
Body mass index (kg/m^2)	27·2 ± 4·7	27·2 ± 4·5	0·94	26·8 ± 4·3	0·0091
Occupation (employed)	2,584 (61)	1,943 (59)	0·12	1,403 (63)	0·15
Education (high)	3,802 (89)	2,957 (90)	0·71	2,051 (91)	0·0085
Smoking pack-years	12·2 ± 19·6	11·6 ± 18·7	0·17	10·2 ± 16·9	<0·001
Smoking status			0·022		<0·001
Current smoker	1,118 (26)	776 (24)		467 (21)	
Former smoker	1,393 (33)	1,103 (33)		762 (34)	
Never smoker	1,745 (41)	1,418 (43)		1,013 (45)	
Alcohol consumption			0·22		<0·001
No	1,183 (28)	861 (26)		527 (24)	
Moderate	2,200 (52)	1,762 (54)		1,264 (56)	
High	860 (20)	669 (20)		448 (20)	
Physical activity			0·48		0·0013
Low	1,448 (34)	1,080 (33)		665 (30)	
Medium	1,933 (46)	1,532 (47)		1,081 (48)	
High	861 (20)	679 (20)		493 (22)	

**p*-values indicate the significance of differences in characteristics among participants included in the repeated measurements and annual rate of change analyses compared with that among all KORA participants. *p*-values were derived from Kruskal-Wallis rank sum test for continuous variables and Chi-square test of independence for categorical variables.

Table S2. Distribution of the annual rate of change in biomarkers.

	Minimum	Q10	Q20	Q30	Q40	Median	Q60	Q70	Q80	Q90	Maximum
HOMA-IR (unit/year)	-6·20	-0·14	-0·07	-0·03	0·00	0·03	0·06	0·10	0·15	0·26	3·96
HOMA-B (unit/year)	-245·64	-8·62	-5·34	-3·30	-1·84	-0·47	0·72	1·90	3·68	6·66	94·52
Fasting insulin (µIU/ml/year)	-24·34	-0·59	-0·32	-0·16	-0·03	0·08	0·19	0·31	0·50	0·86	8·14
Fasting glucose (mg/dl/year)	-6·13	-0·78	-0·31	0·00	0·23	0·47	0·77	1·07	1·43	2·06	26·37

Table S3. Descriptive statistics of participant characteristics at the first and last visits stratified by the direction of annual rate of change in HOMA-IR.

	First visit			Last visit		
	Increasing HOMA-IR (*N* = 1,336)	**Unchanged / Decreasing HOMA-IR (*N* = 906)**	***p*-value***	**Increasing HOMA-IR (*N* = 1,336)**	**Unchanged / Decreasing HOMA-IR (*N* = 906)**	***p*-value***
HOMA-IR	2·2 ± 1·4	3·2 ± 4·1	<0·001	3·4 ± 2·5	2·0 ± 1·3	<0·001
HOMA-B	109·2 ± 56·5	148·8 ± 207·8	<0·001	125·5 ± 68·9	93·5 ± 54·2	<0·001
Fasting insulin (µIU/ml)	9·2 ± 5·2	13·2 ± 17·2	<0·001	13·1 ± 8·1	8·3 ± 4·8	<0·001
Fasting glucose (mg/dl)	94·3 ± 9·9	96·2 ± 10·4	<0·001	102·3 ± 18·0	96·2 ± 11·4	<0·001
Age (years)	54·2 ± 10·4	53·3 ± 10·8	0·052	62·5 ± 12·1	61·2 ± 12·2	0·015
Sex (male)	660 (49)	426 (47)	0·29	665 (49)	426 (47)	0·29
Body mass index (kg/m^2)	27·1 ± 4·2	27·3 ± 4·6	0·45	28·1 ± 4·8	27·3 ± 4·7	<0·001
Occupation (employed)	803 (60)	579 (64)	0·076	617 (46)	472 (52)	0·0068
Education (high)	1,223 (92)	828 (91)	0·96	1,223 (92)	828 (91)	0·96
Smoking pack-years	11·9 ± 19·2	10·0 ± 15·8	0·047	12·5 ± 20·0	10·5 ± 17·1	0·038
Smoking status			0·17			0·20
Current smoker	239 (18)	149 (16)		190 (14)	120 (13)	
Former smoker	516 (39)	327 (36)		566 (42)	358 (40)	
Never smoker	581 (43)	430 (48)		580 (44)	428 (47)	
Alcohol consumption			0·46			0·49
No	352 (27)	231 (25)		370 (28)	231 (25)	
Moderate	726 (54)	515 (57)		716 (54)	505 (56)	
High	258 (19)	160 (18)		250 (19)	170 (19)	
Physical activity			0·55			0·065
Low	386 (29)	280 (31)		418 (31)	242 (27)	
Medium	627 (47)	407 (45)		587 (44)	422 (46)	
High	323 (24)	219 (24)		331 (25)	242 (27)	
Diabetes status			0·0028			<0·001
Normal glucose tolerance	905 (69)	560 (62)		577 (44)	514 (59)	
Prediabetes	383 (29)	320 (36)		577 (44)	316 (36)	
Diabetes	33 (2)	16 (2)		149 (12)	45 (5)	

**p*-values were derived from Kruskal-Wallis rank sum test for continuous variables and Chi-square test of independence for categorical variables.

Table S4. Absolute changes (95% CIs) in the annual rate of change of biomarkers at deciles of the distribution per IQR increase in air pollutant concentrations.

	Percentile	PNC	PM_{coarse}	$PM_{2·5}$	$PM_{2·5abs}$	NO_2	O_3
HOMA-IR	10th	0·007 (0·002, 0·012)	0·009 (0·002, 0·016)	0·007 (0·002, 0·012)	0·014 (0·008, 0·021)	0·012 (0·005, 0·019)	0·001 (-0·007, 0·008)
	20th	0·008 (0·004, 0·013)	0·008 (0·003, 0·014)	0·008 (0·003, 0·012)	0·014 (0·009, 0·020)	0·013 (0·006, 0·020)	0·001 (-0·005, 0·007)
	30th	0·005 (0·000, 0·009)	0·004 (-0·003, 0·011)	0·001 (-0·005, 0·007)	0·006 (-0·001, 0·013)	0·005 (-0·001, 0·012)	0·000 (-0·007, 0·007)
	40th	0·004 (0·000, 0·009)	0·004 (-0·003, 0·011)	0·001 (-0·006, 0·008)	0·010 (0·002, 0·018)	0·005 (-0·003, 0·013)	0·002 (-0·006, 0·009)
	50th	0·005 (-0·002, 0·011)	0·005 (-0·003, 0·013)	0·006 (-0·002, 0·013)	0·010 (0·001, 0·019)	0·006 (-0·003, 0·015)	0·000 (-0·008, 0·008)
	60th	0·009 (0·003, 0·014)	0·008 (0·000, 0·016)	0·009 (0·001, 0·017)	0·010 (0·000, 0·020)	0·009 (0·000, 0·018)	0·001 (-0·007, 0·009)
	70th	0·009 (0·003, 0·015)	0·011 (0·003, 0·018)	0·004 (-0·003, 0·012)	0·010 (0·000, 0·020)	0·008 (-0·002, 0·018)	0·007 (-0·001, 0·015)
	80th	0·005 (-0·004, 0·014)	0·011 (-0·001, 0·023)	0·000 (-0·012, 0·012)	0·005 (-0·009, 0·018)	0·000 (-0·013, 0·013)	0·014 (0·002, 0·025)
	90th	0·000 (-0·010, 0·010)	0·011 (-0·003, 0·026)	-0·015 (-0·030, -0·001)	0·005 (-0·009, 0·018)	-0·004 (-0·020, 0·012)	0·015 (-0·004, 0·033)
HOMA-B	10th	0·175 (-0·072, 0·421)	0·199 (-0·116, 0·514)	0·232 (-0·025, 0·488)	-0·041 (-0·438, 0·356)	0·166 (-0·193, 0·525)	0·053 (-0·236, 0·343)
	20th	0·207 (0·000, 0·414)	0·216 (-0·059, 0·491)	0·296 (0·020, 0·572)	0·337 (0·093, 0·582)	0·240 (-0·078, 0·558)	-0·017 (-0·308, 0·274)
	30th	0·210 (0·048, 0·372)	0·292 (0·041, 0·544)	0·389 (0·152, 0·626)	0·298 (0·020, 0·576)	0·337 (0·057, 0·618)	0·161 (-0·106, 0·428)
	40th	0·140 (-0·113, 0·393)	0·194 (-0·113, 0·501)	0·224 (-0·083, 0·531)	0·268 (-0·107, 0·643)	0·285 (-0·070, 0·640)	-0·018 (-0·332, 0·295)
	50th	0·231 (-0·020, 0·482)	0·424 (0·107, 0·740)	0·435 (0·100, 0·770)	0·574 (0·185, 0·963)	0·528 (0·161, 0·894)	0·121 (-0·237, 0·479)
	60th	0·287 (0·009, 0·564)	0·451 (0·100, 0·801)	0·237 (-0·088, 0·561)	0·481 (0·087, 0·875)	0·435 (0·053, 0·817)	0·080 (-0·278, 0·437)
	70th	0·265 (0·023, 0·507)	0·462 (0·095, 0·830)	0·335 (0·015, 0·656)	0·460 (0·091, 0·829)	0·399 (-0·015, 0·813)	0·113 (-0·237, 0·462)
	80th	0·464 (0·176, 0·751)	0·745 (0·450, 1·041)	0·303 (-0·102, 0·707)	0·691 (0·269, 1·112)	0·532 (0·148, 0·917)	0·330 (0·010, 0·650)
	90th	0·544 (-0·070, 1·157)	0·919 (0·286, 1·552)	0·173 (-0·418, 0·764)	0·500 (-0·095, 1·095)	0·020 (-0·732, 0·773)	0·506 (-0·126, 1·137)
Fasting insulin	10th	0·035 (0·012, 0·057)	0·032 (0·006, 0·059)	0·013 (-0·014, 0·039)	0·042 (0·009, 0·074)	0·041 (0·008, 0·073)	-0·004 (-0·031, 0·023)
	20th	0·037 (0·019, 0·055)	0·036 (0·011, 0·060)	0·036 (0·012, 0·060)	0·057 (0·027, 0·088)	0·049 (0·020, 0·077)	0·000 (-0·024, 0·023)
	30th	0·030 (0·014, 0·046)	0·031 (0·014, 0·048)	0·023 (0·002, 0·043)	0·038 (0·012, 0·063)	0·042 (0·016, 0·067)	-0·003 (-0·027, 0·021)
	40th	0·030 (0·008, 0·053)	0·030 (0·000, 0·060)	0·031 (0·001, 0·060)	0·048 (0·012, 0·084)	0·041 (0·007, 0·075)	0·017 (-0·013, 0·046)
	50th	0·025 (0·004, 0·045)	0·034 (0·002, 0·065)	0·031 (0·001, 0·061)	0·055 (0·019, 0·091)	0·040 (0·003, 0·077)	0·004 (-0·027, 0·035)
	60th	0·042 (0·018, 0·065)	0·051 (0·019, 0·083)	0·032 (0·004, 0·060)	0·055 (0·019, 0·090)	0·055 (0·023, 0·087)	0·018 (-0·012, 0·049)
	70th	0·021 (-0·004, 0·046)	0·038 (0·007, 0·069)	0·013 (-0·019, 0·045)	0·030 (-0·006, 0·066)	0·029 (-0·007, 0·064)	0·028 (0·000, 0·056)
	80th	0·025 (-0·008, 0·058)	0·038 (0·001, 0·075)	0·011 (-0·034, 0·056)	0·040 (-0·012, 0·091)	0·021 (-0·028, 0·071)	0·029 (-0·012, 0·071)
	90th	0·021 (-0·011, 0·052)	0·067 (0·009, 0·125)	-0·038 (-0·083, 0·006)	0·014 (-0·045, 0·074)	0·014 (-0·038, 0·065)	0·056 (0·003, 0·110)
Fasting glucose	10th	-0·028 (-0·092, 0·035)	-0·020 (-0·088, 0·047)	-0·047 (-0·116, 0·023)	-0·001 (-0·096, 0·095)	-0·001 (-0·090, 0·087)	0·045 (-0·033, 0·124)
	20th	0·022 (-0·033, 0·078)	0·004 (-0·071, 0·079)	0·005 (-0·068, 0·078)	0·083 (-0·008, 0·174)	0·049 (-0·042, 0·141)	-0·009 (-0·084, 0·067)
	30th	0·011 (-0·040, 0·063)	0·001 (-0·063, 0·066)	0·004 (-0·060, 0·067)	0·051 (-0·026, 0·129)	0·031 (-0·041, 0·103)	-0·042 (-0·107, 0·022)
	40th	-0·001 (-0·055, 0·052)	-0·039 (-0·095, 0·017)	-0·001 (-0·064, 0·061)	0·010 (-0·069, 0·088)	-0·021 (-0·094, 0·052)	-0·011 (-0·066, 0·045)
	50th	0·029 (-0·025, 0·082)	-0·020 (-0·085, 0·045)	-0·011 (-0·078, 0·057)	0·042 (-0·037, 0·120)	0·016 (-0·056, 0·089)	0·002 (-0·062, 0·066)
	60th	0·025 (-0·022, 0·072)	0·034 (-0·020, 0·087)	-0·053 (-0·114, 0·009)	0·060 (-0·007, 0·127)	0·049 (-0·016, 0·114)	-0·003 (-0·064, 0·059)
	70th	0·019 (-0·038, 0·076)	0·012 (-0·056, 0·081)	-0·058 (-0·130, 0·015)	0·033 (-0·052, 0·117)	0·012 (-0·068, 0·092)	0·007 (-0·059, 0·072)
	80th	0·020 (-0·053, 0·093)	-0·006 (-0·095, 0·083)	-0·029 (-0·120, 0·062)	-0·003 (-0·121, 0·115)	-0·019 (-0·135, 0·097)	-0·007 (-0·112, 0·098)
	90th	-0·056 (-0·136, 0·024)	-0·048 (-0·137, 0·041)	-0·055 (-0·168, 0·058)	-0·056 (-0·168, 0·056)	-0·083 (-0·185, 0·019)	-0·093 (-0·190, 0·004)

Quantile regression models for the annual rate of change were adjusted for baseline levels of the investigated biomarker, age (baseline), sex, BMI (baseline), annual rate of change in BMI, educational attainment (baseline), occupational status (baseline), smoking status (baseline), smoking pack-years (baseline), annual rate of change in smoking pack-years, physical activity (baseline), and an indicator for the visits used in the calculation of the rate of change. An IQR increase was $2·0\times10^3/cm^3$ for PNC, 1·4 $\mu g/m^3$ for PM_{coarse}, 1·4 $\mu g/m^3$ for $PM_{2·5}$, $0·3\times10^{-5}/m$ for $PM_{2·5abs}$, 7·1 $\mu g/m^3$ for NO_2, and 3·5 $\mu g/m^3$ for O_3.

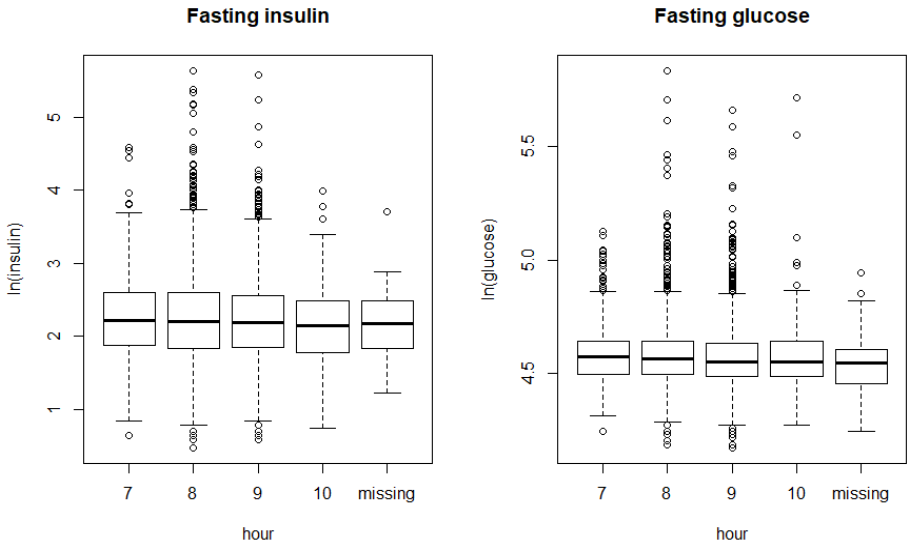

Figure S1. Distribution of ln-transformed fasting insulin and fasting glucose stratified by the hour of blood withdrawal.
Blood samples drawn during 7:00 AM–8:00 AM (hour=7): *N*=645; 8:00 AM–9:00 AM (hour=8): *N*=2,969; 9:00 AM–10:00 AM (hour=9): *N*=2,122; 10:00 AM–11:00 AM (hour=10): *N*=238; without documented time (missing): *N*=34.

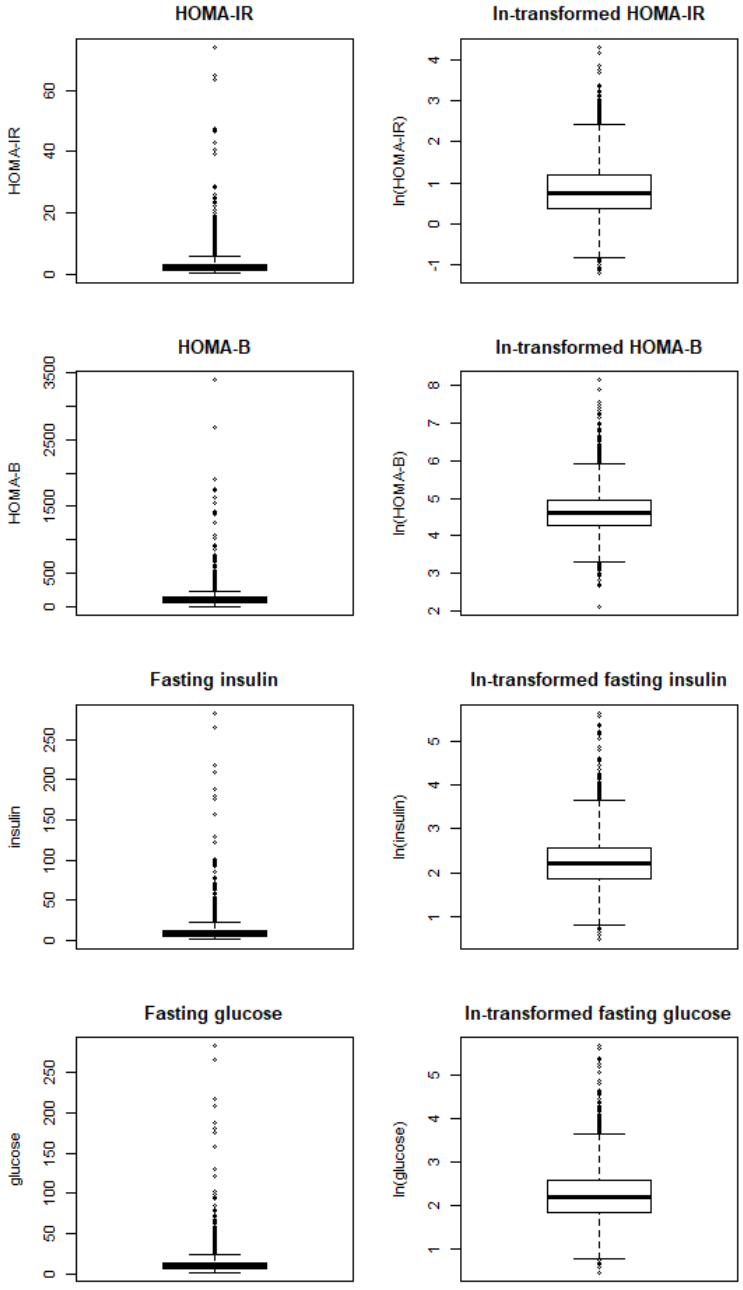

Figure S2. Boxplots of biomarker concentrations on the original and natural log (ln)-transformed scales.

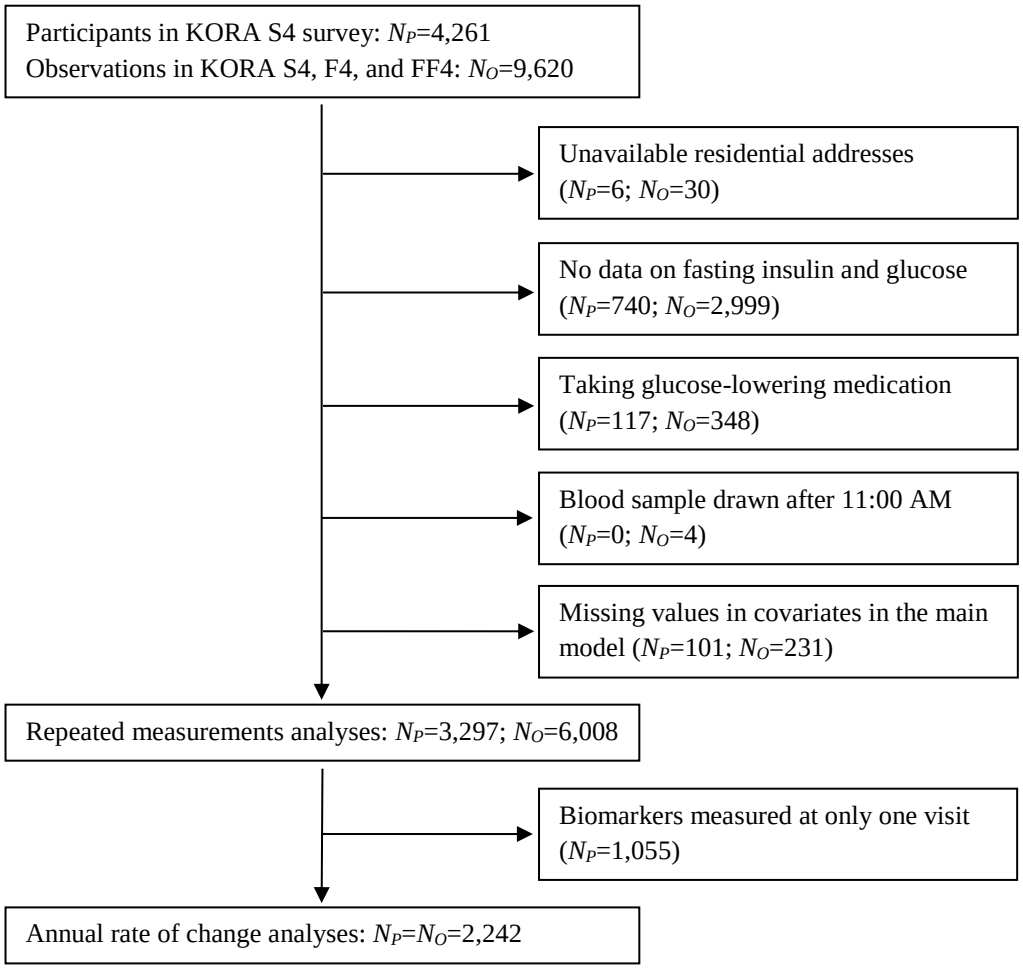

Figure S3. Flow chart of the exclusion process.

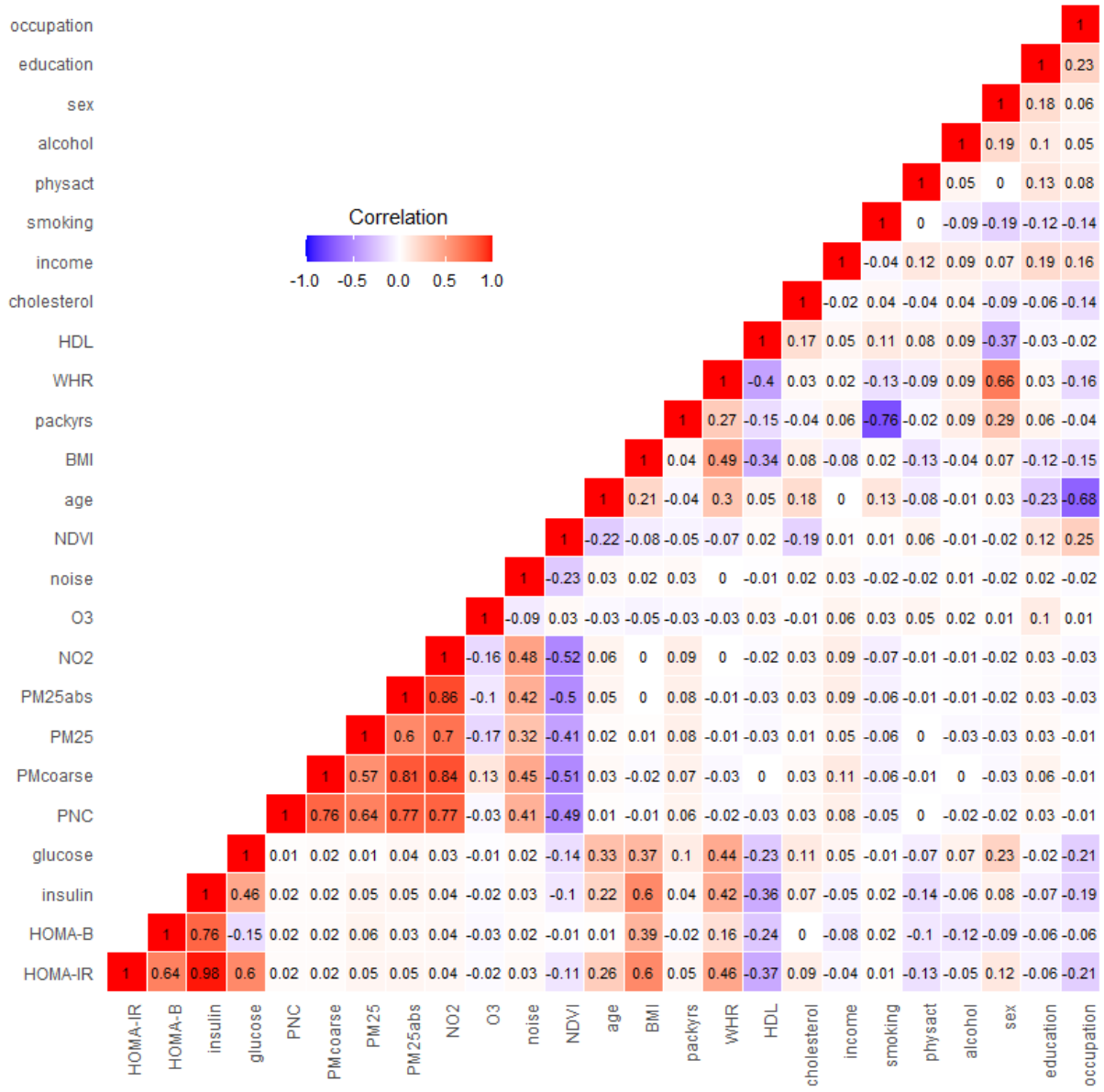

Figure S4. Correlation between outcome, exposure, and covariate variables.
Correlation coefficients in the figure were (1) Spearman's rank correlation coefficients for two continuous variables; (2) Kendall rank correlation coefficients for one continuous and one ordinal variable, or two ordinal variables; (3) Point-Biserial correlation coefficients for one dichotomous and one continuous/ordinal variable; (4) Cramer's V correlation coefficients for two categorical variables. BMI=body mass index; HDL= high-density lipoproteins; income=per capita income; HOMA-IR=homeostasis model assessment of insulin resistance; HOMA-B=homeostasis model assessment of β-cell function; NDVI=normalized difference vegetation index; NO_2=nitrogen dioxide, O_3=ozone; PM_{coarse}= particulate matter with an aerodynamic diameter between 2·5 μm and 10 μm; $PM_{2·5}$=particulate matter with an aerodynamic diameter ≤ 2·5 μm; $PM_{2·5abs}$=$PM_{2·5}$ absorbance; PNC=particle number concentration; WHR=waist-hip-ratio

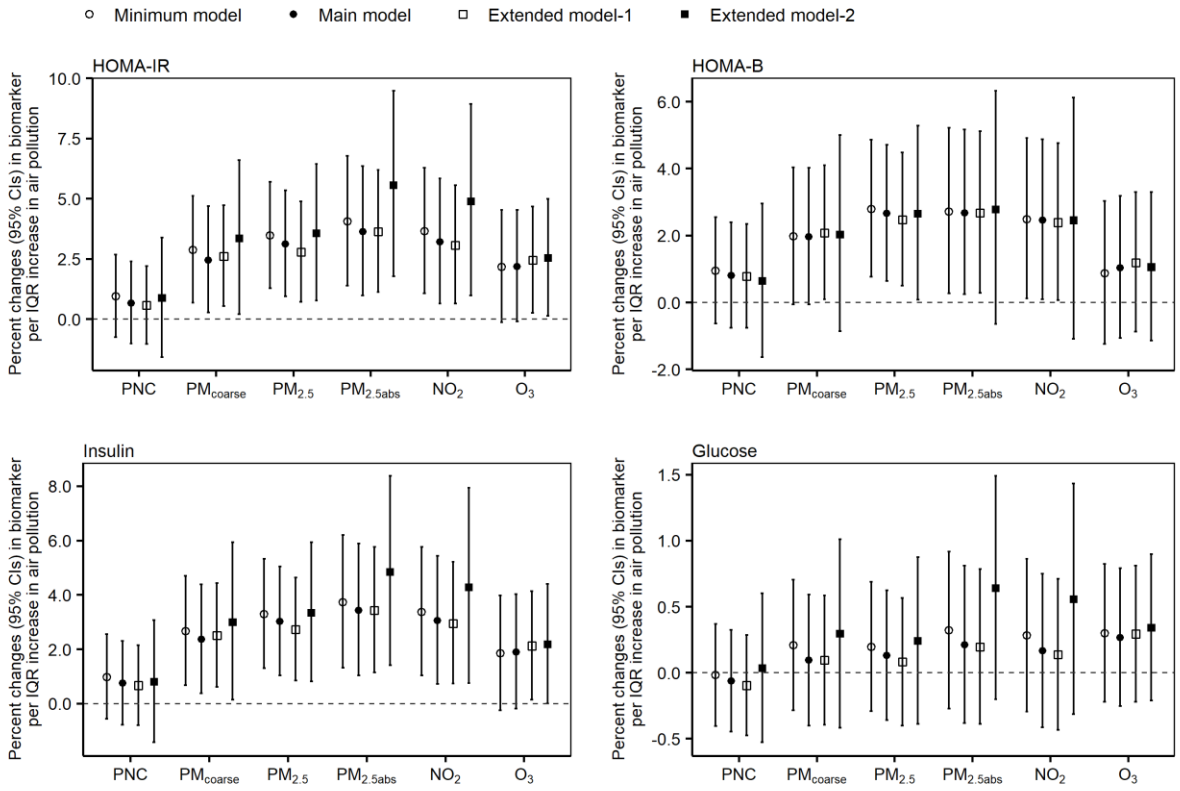

Figure S5. Percent changes (95% CIs) in the repeated measurements of biomarkers per IQR increase in air pollutant concentrations in models with different adjustments of covariates.
Minimum models were adjusted for age, sex, BMI, season, visits; main models were further adjusted for educational attainment, occupational status, smoking status, smoking pack-years, alcohol consumption, and physical activity; extended model-1 was further adjusted for waist-hip-ratio, high-density lipoprotein, and total cholesterol in addition to covariates in main models; extended model-2 were further adjusted for NDVI and noise in addition to covariates in main models. An IQR increase was $2{\cdot}0\times10^{3}/cm^{3}$ for PNC, 1·4 $\mu g/m^{3}$ for PM_{coarse}, 1·4 $\mu g/m^{3}$ for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}/m$ for $PM_{2{\cdot}5abs}$, 7·1 $\mu g/m^{3}$ for NO_2, and 3·5 $\mu g/m^{3}$ for O_3.

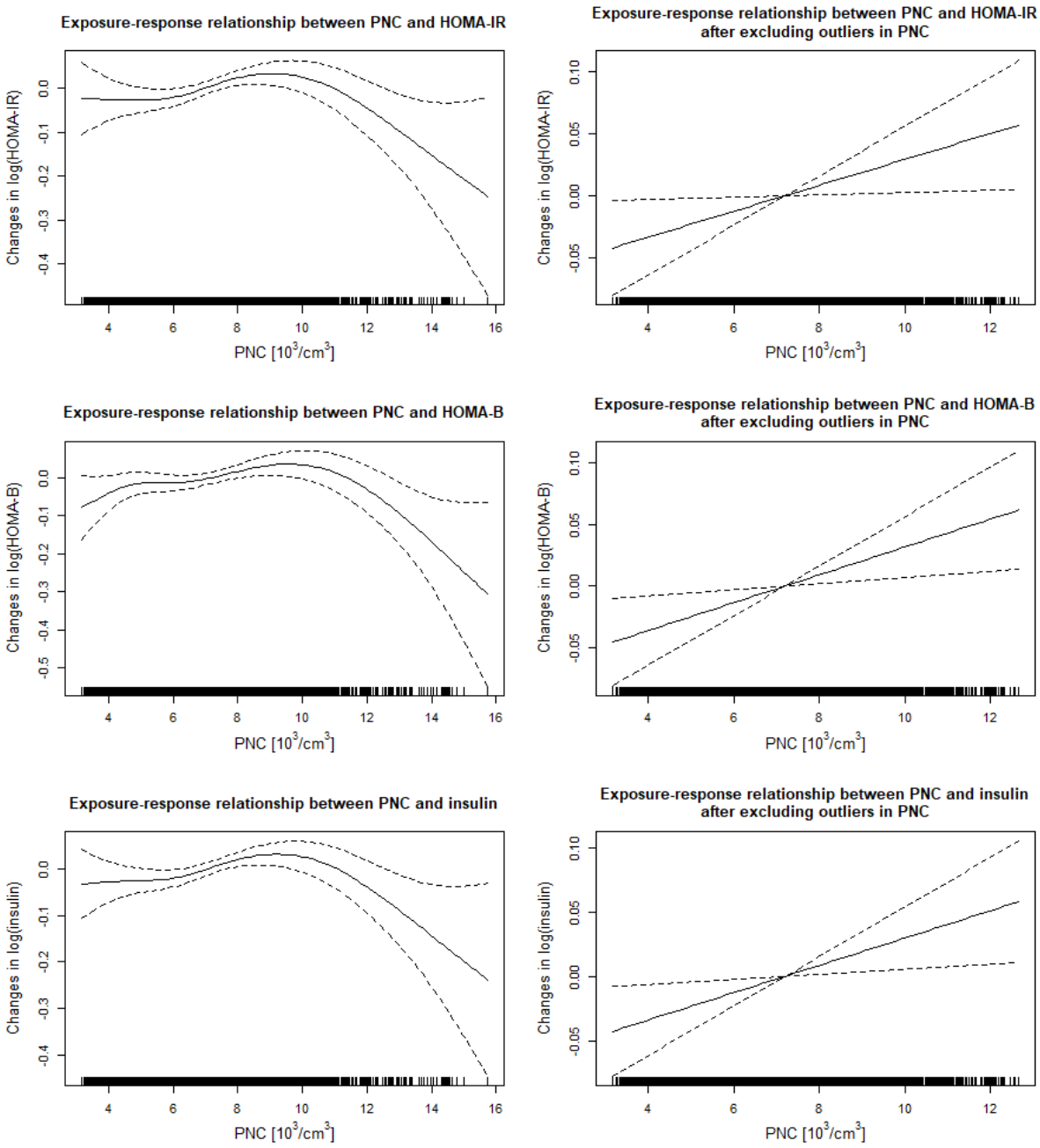

Figure S6. Exposure-response relationships between PNC and HOMA-IR, HOMA-B, and fasting insulin.
Panels on the left side show the exposure-response relationship for the whole range of PNC; panels on the right side show the exposure-response relationship for PNC < $12{\cdot}7\times10^3/cm^3$.

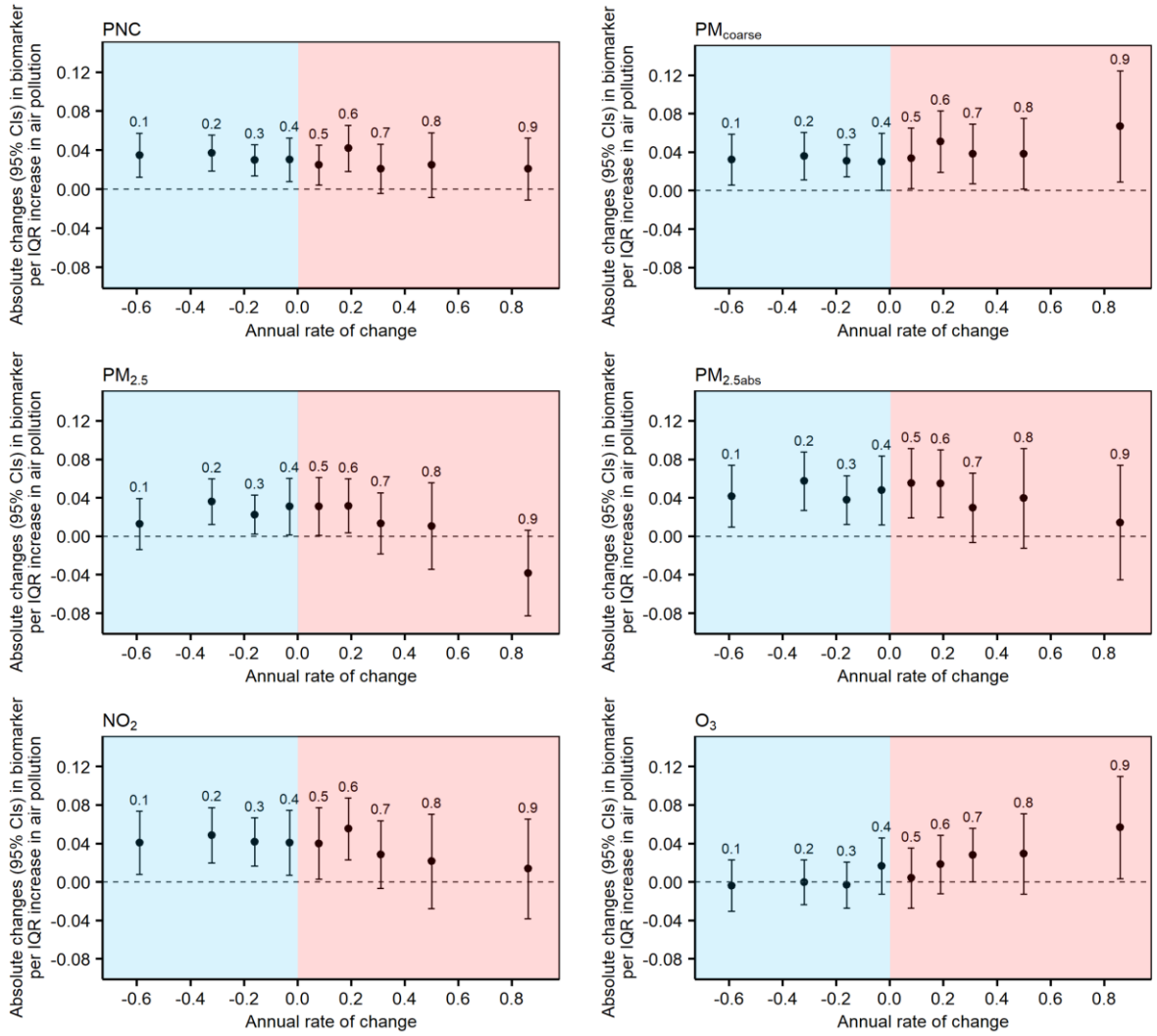

Figure S7. Absolute changes (95% CIs) in the annual rate of change in fasting insulin at deciles of the distribution per IQR increase in air pollutant concentrations.

Quantile regression models for the annual rate of change were adjusted for baseline levels of the investigated biomarker, age (baseline), sex, BMI (baseline), annual rate of change in BMI, educational attainment (baseline), occupational status (baseline), smoking status (baseline), smoking pack-years (baseline), annual rate of change in smoking pack-years, physical activity (baseline), and an indicator for the visits used in the calculation of the rate of change. Blue shaded area on the left side indicates decreasing insulin secretion over years (annual rate of change below zero); red shaded area on the right side indicates increasing insulin secretion over years (annual rate of change above zero). Values (i.e. 0·1–0·9) above the error bars indicate the deciles of the distribution of annual rate of change. An IQR increase was $2{\cdot}0\times10^3$/cm^3 for PNC, 1·4 μg/m^3 for PM$_{coarse}$, 1·4 μg/m^3 for PM$_{2·5}$, $0{\cdot}3\times10^{-5}$/m for PM$_{2·5abs}$, 7·1 μg/m^3 for NO$_2$, and 3·5 μg/m^3 for O$_3$.

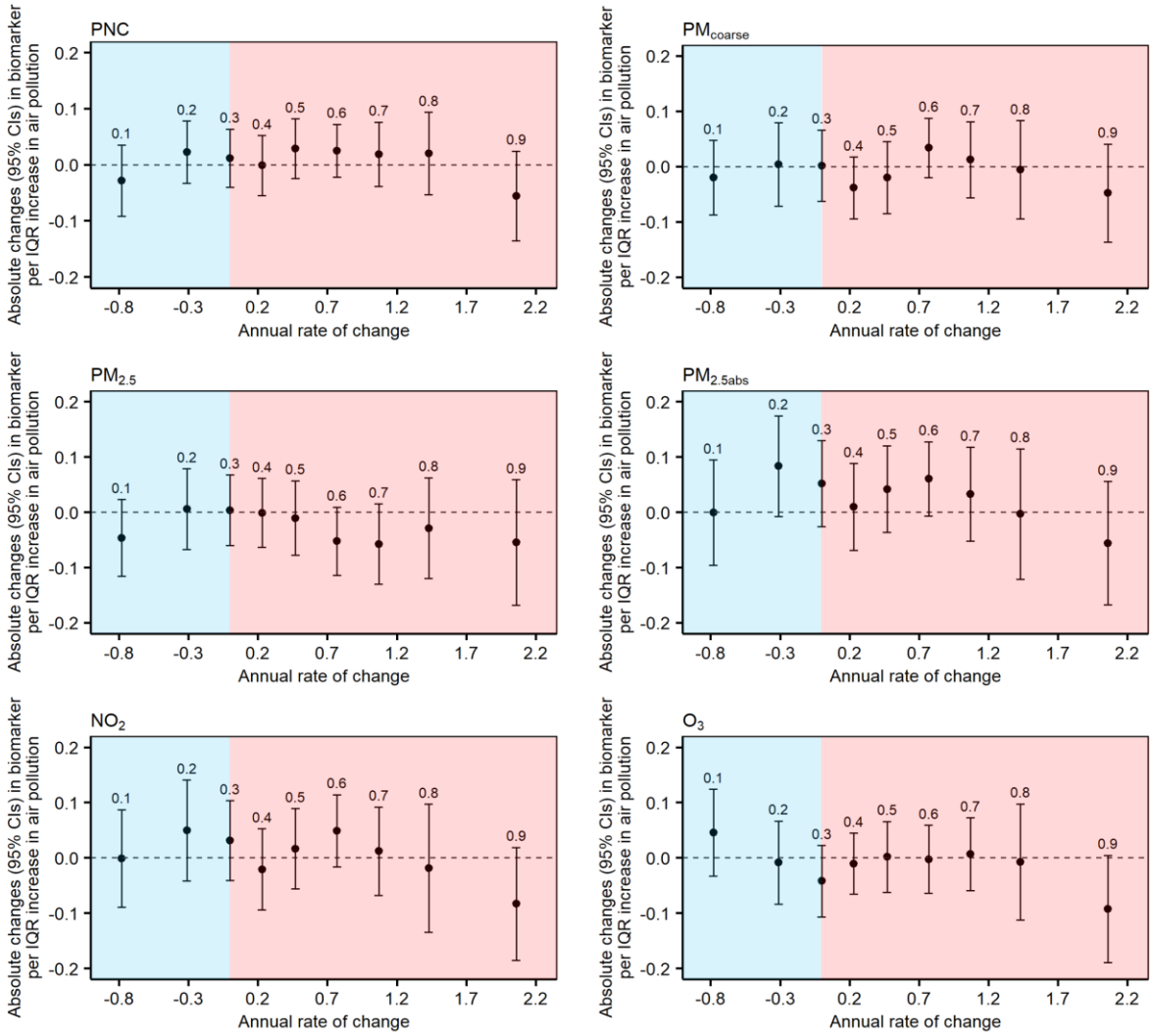

Figure S8. Absolute changes (95% CIs) in the annual rate of change in fasting glucose at deciles of the distribution per IQR increase in air pollutant concentrations.

Quantile regression models for the annual rate of change were adjusted for baseline levels of the investigated biomarker, age (baseline), sex, BMI (baseline), annual rate of change in BMI, educational attainment (baseline), occupational status (baseline), smoking status (baseline), smoking pack-years (baseline), annual rate of change in smoking pack-years, physical activity (baseline), and an indicator for the visits used in the calculation of the rate of change. Blue shaded area on the left side indicates decreasing fasting glucose concentrations over years (annual rate of change below zero); red shaded area on the right side indicates increasing fasting glucose concentrations over years (annual rate of change above zero). Values (i.e. 0·1–0·9) above the error bars indicate the deciles of the distribution of annual rate of change. An IQR increase was $2{\cdot}0\times10^3$/cm^3 for PNC, 1·4 μg/m^3 for PM$_{coarse}$, 1·4 μg/m^3 for PM$_{2\cdot5}$, $0{\cdot}3\times10^{-5}$/m for PM$_{2\cdot5abs}$, 7·1 μg/m^3 for NO$_2$, and 3·5 μg/m^3 for O$_3$.

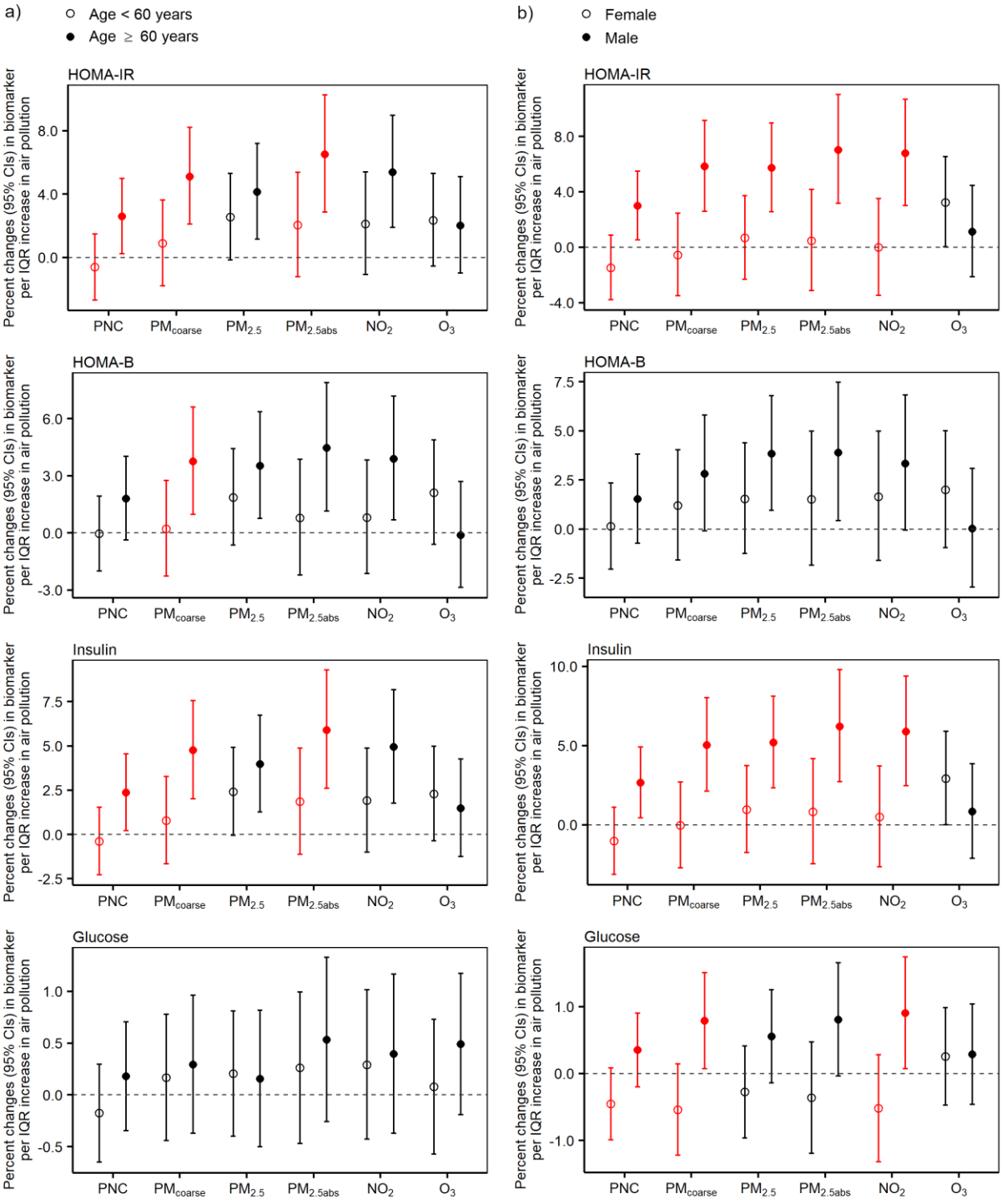

Figure S9. Percent changes (95% CIs) in repeated measurements of biomarkers per IQR increase in air pollutant concentrations by a) age and b) sex.
Panels on the left side show the effect modification by age (age < 60 years: number of observations (*N*) = 3,019, age ≥ 60 years: *N*=2,989); panels on the right side show the effect modification by sex (female: *N*=3,083, male: *N*=2,925). Error bars in red indicate significant differences in effect estimates between subgroups (*p*-value for the interaction term < 0·05). An IQR increase was $2{\cdot}0\times10^3$/cm^3 for PNC, 1·4 μg/m^3 for PM_{coarse}, 1·4 μg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 μg/m^3 for NO_2, and 3·5 μg/m^3 for O_3.

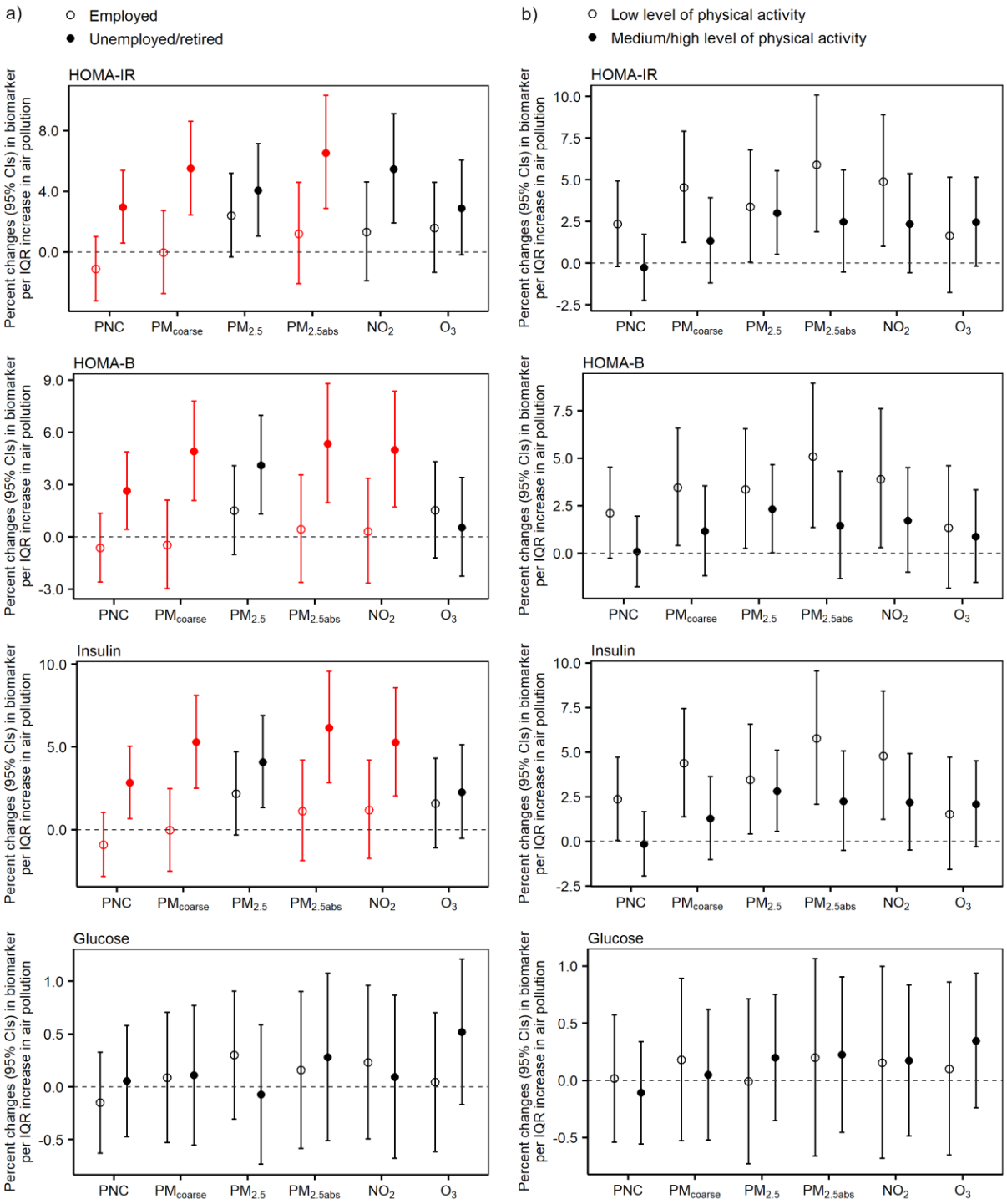

Figure S10. Percent changes (95% CIs) in repeated measurements of biomarkers per IQR increase in air pollutant concentrations by a) occupational status and b) physical activity.
Panels on the left side show effect modification by occupational status (employed: *N*=3,053, unemployed/retired: *N*=2,955); panels on the right side show the effect modification by physical activity (low level: *N*=1,893, medium/high level: *N*=4,115). Error bars in red indicate significant differences in effect estimates between subgroups (*p*-value for the interaction term < 0·05). An IQR increase was $2{\cdot}0\times10^3$/cm^3 for PNC, 1·4 μg/m^3 for PM$_{coarse}$, 1·4 μg/m^3 for PM$_{2·5}$, $0{\cdot}3\times10^{-5}$/m for PM$_{2·5abs}$, 7·1 μg/m^3 for NO$_2$, and 3·5 μg/m^3 for O$_3$.

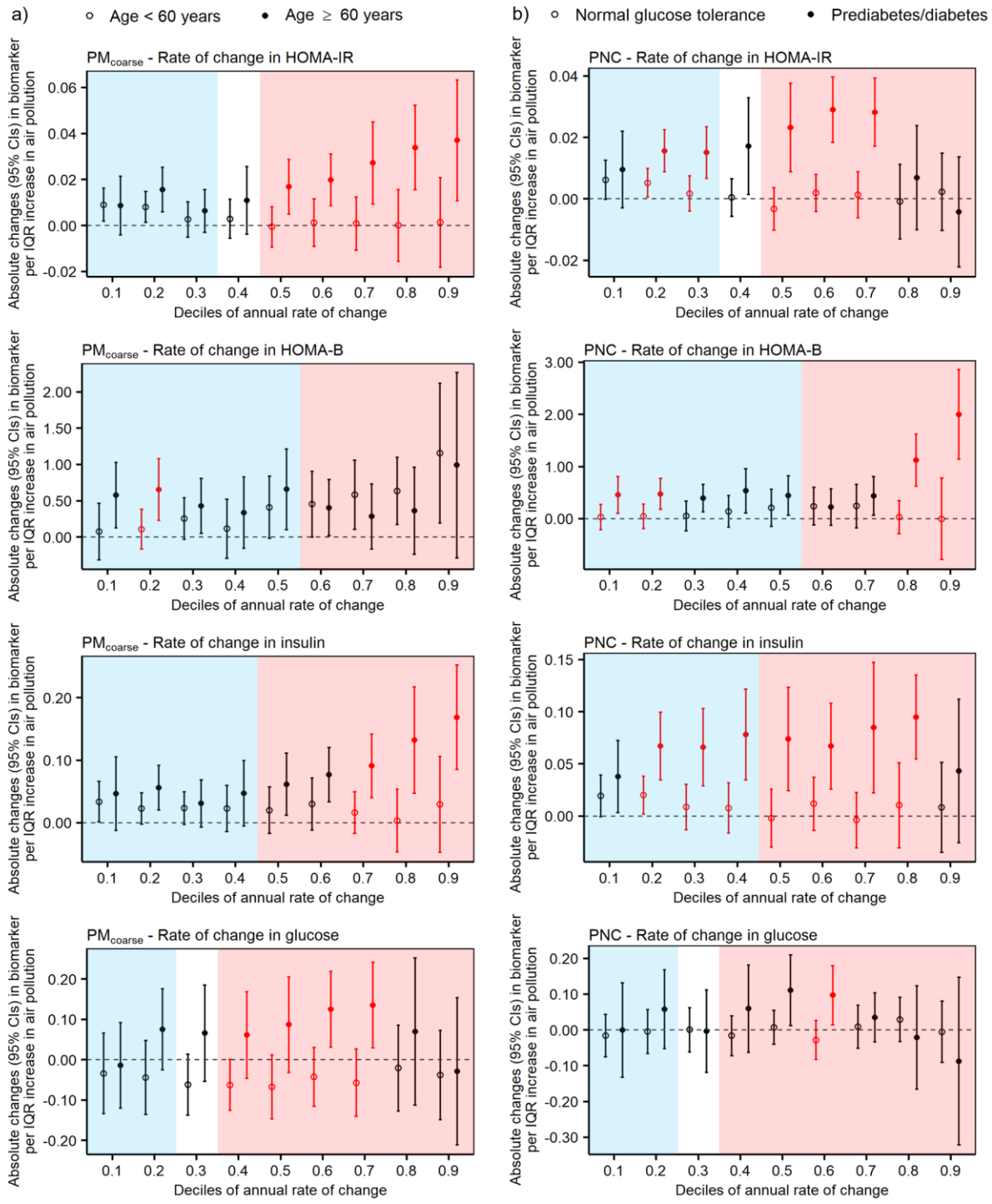

Figure S11. Absolute changes (95% CIs) in the annual rate of change in biomarkers per IQR increase in air pollutant concentrations by a) age and b) diabetes status.
Panels on the left side show the effect modification by age at baseline (age < 60 years: *N*=1,519, age ≥ 60 years: *N*=723); panels on the right side show the effect modification by diabetes status at baseline (normal glucose tolerance: *N*=1,465, prediabetes/diabetes: *N*=752). Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero; unshaded area in the middle indicates stable biomarker levels over years. Error bars in red indicate significant differences in effect estimates between subgroups (*p*-value for the interaction term < 0·05). An IQR increase was $2{\cdot}0\times10^3$/cm^3 for PNC, 1·4 μg/m^3 for PM$_{coarse}$, 1·4 μg/m^3 for PM$_{2·5}$, $0{\cdot}3\times10^{-5}$/m for PM$_{2·5abs}$, 7·1 μg/m^3 for NO_2, and 3·5 μg/m^3 for O_3.

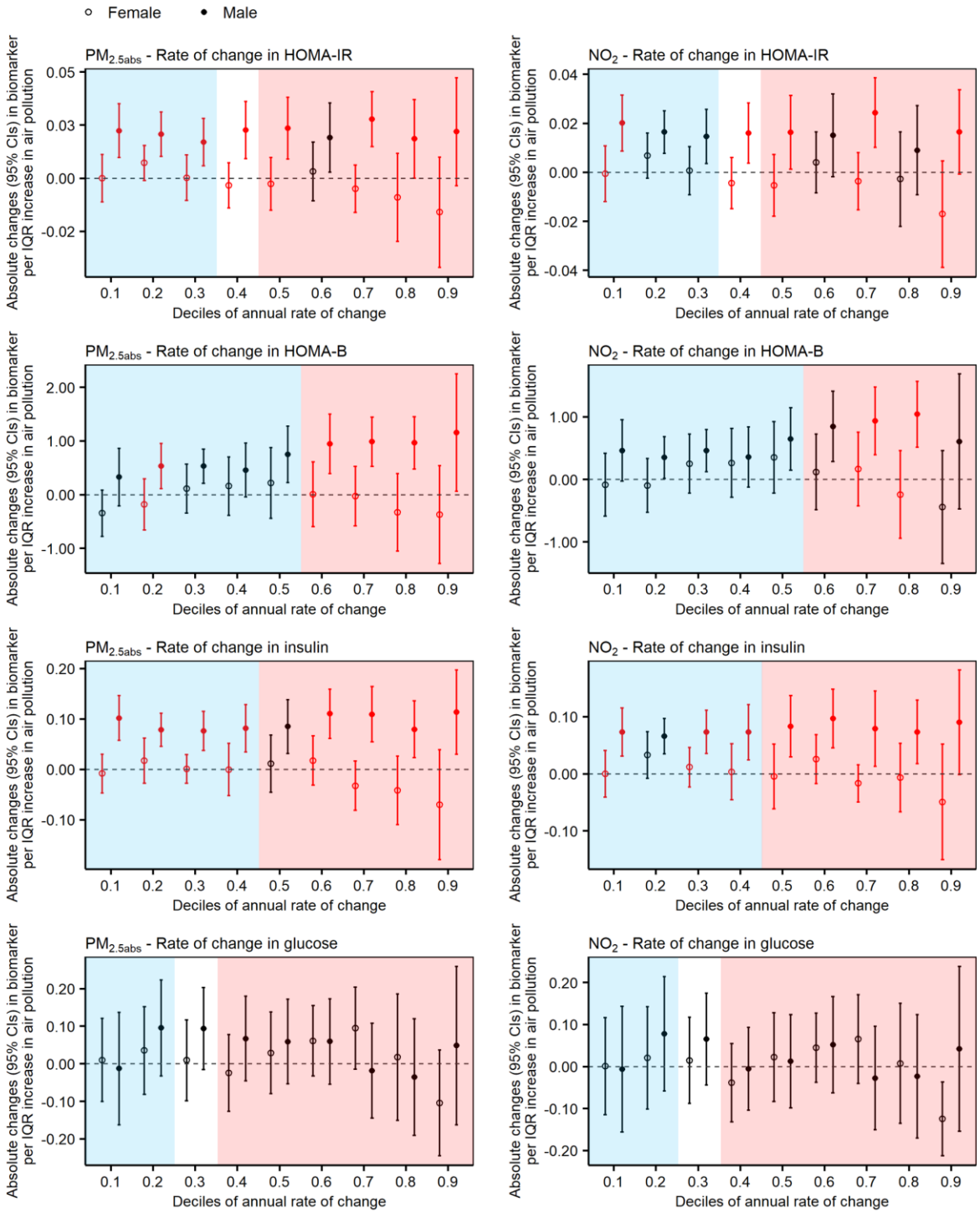

Figure S12. Absolute changes (95% CIs) in the annual rate of change in HOMA-IR and fasting insulin per IQR increase in air pollutant concentrations by sex.
Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero; unshaded area in the middle indicates stable biomarker levels over years. Error bars in red indicate significant differences in effect estimates between subgroups (*p*-value for the interaction term < 0·05). Numbers of females and males were 1,156 and 1,086, respectively. An IQR increase was $2{\cdot}0\times10^{3}$/cm^3 for PNC, 1·4 μg/m^3 for PM_{coarse}, 1·4 μg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 μg/m^3 for NO_2, and 3·5 μg/m^3 for O_3.

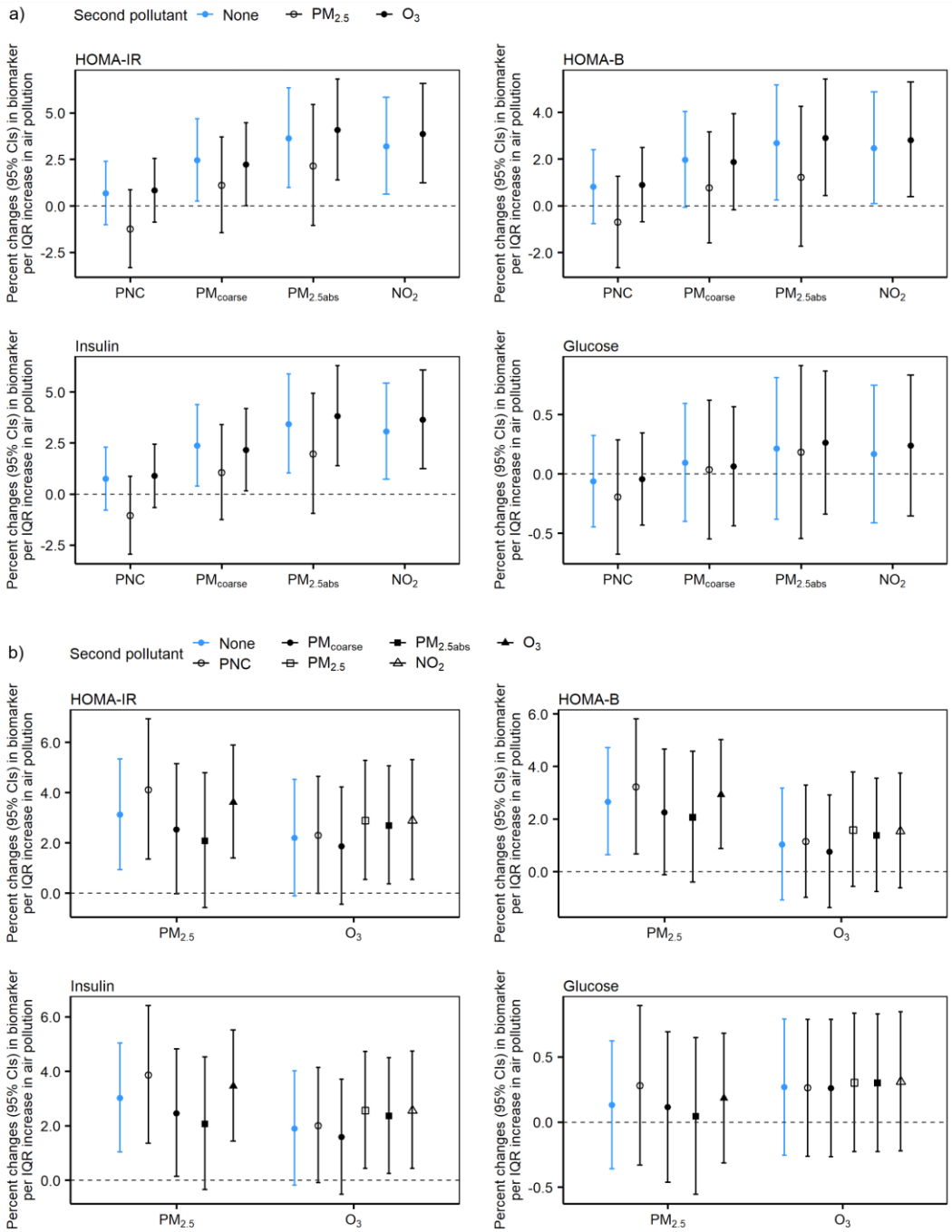

Figure S13. Percent changes (95% CIs) in repeated measurements of biomarkers per IQR increase in air pollutant concentrations in two-pollutant models.
Error bars in blue represent the effect estimates in single-pollutant models. An IQR increase was $2{\cdot}0\times10^{3}$/cm^3 for PNC, 1·4 μg/m^3 for PM_{coarse}, 1·4 μg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 μg/m^3 for NO_2, and 3·5 μg/m^3 for O_3.

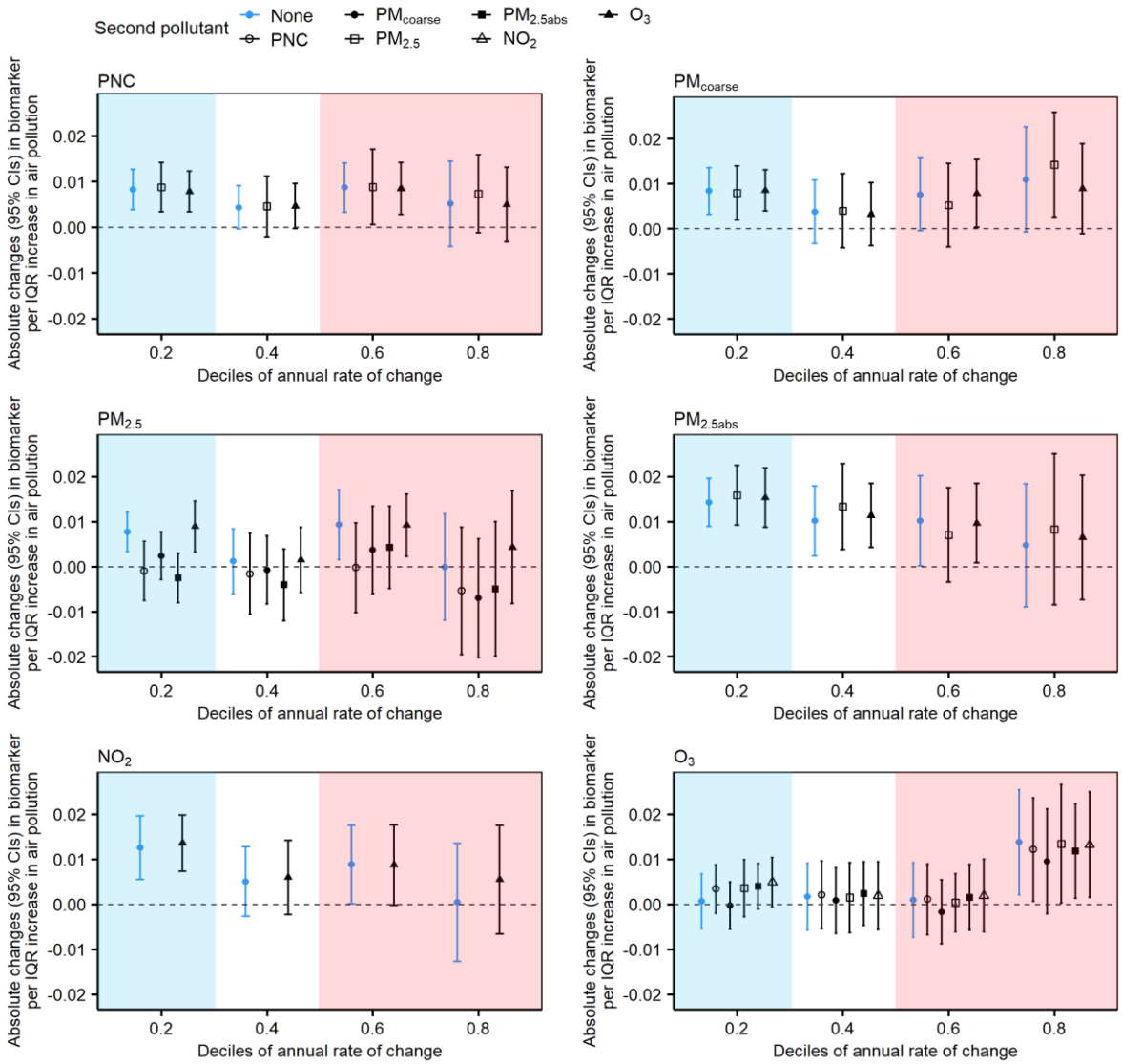

Figure S14. Absolute changes (95% CIs) in the annual rate of change in HOMA-IR at different percentiles of the distribution per IQR increase in air pollutant concentrations in two-pollutant models.

Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero; unshaded area in the middle indicates stable biomarker levels over years. Error bars in blue represent the effect estimates in single-pollutant models. An IQR increase was $2{\cdot}0\times10^{3}$/cm^3 for PNC, 1·4 μg/m^3 for PM_{coarse}, 1·4 μg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 μg/m^3 for NO_2, and 3·5 μg/m^3 for O_3.

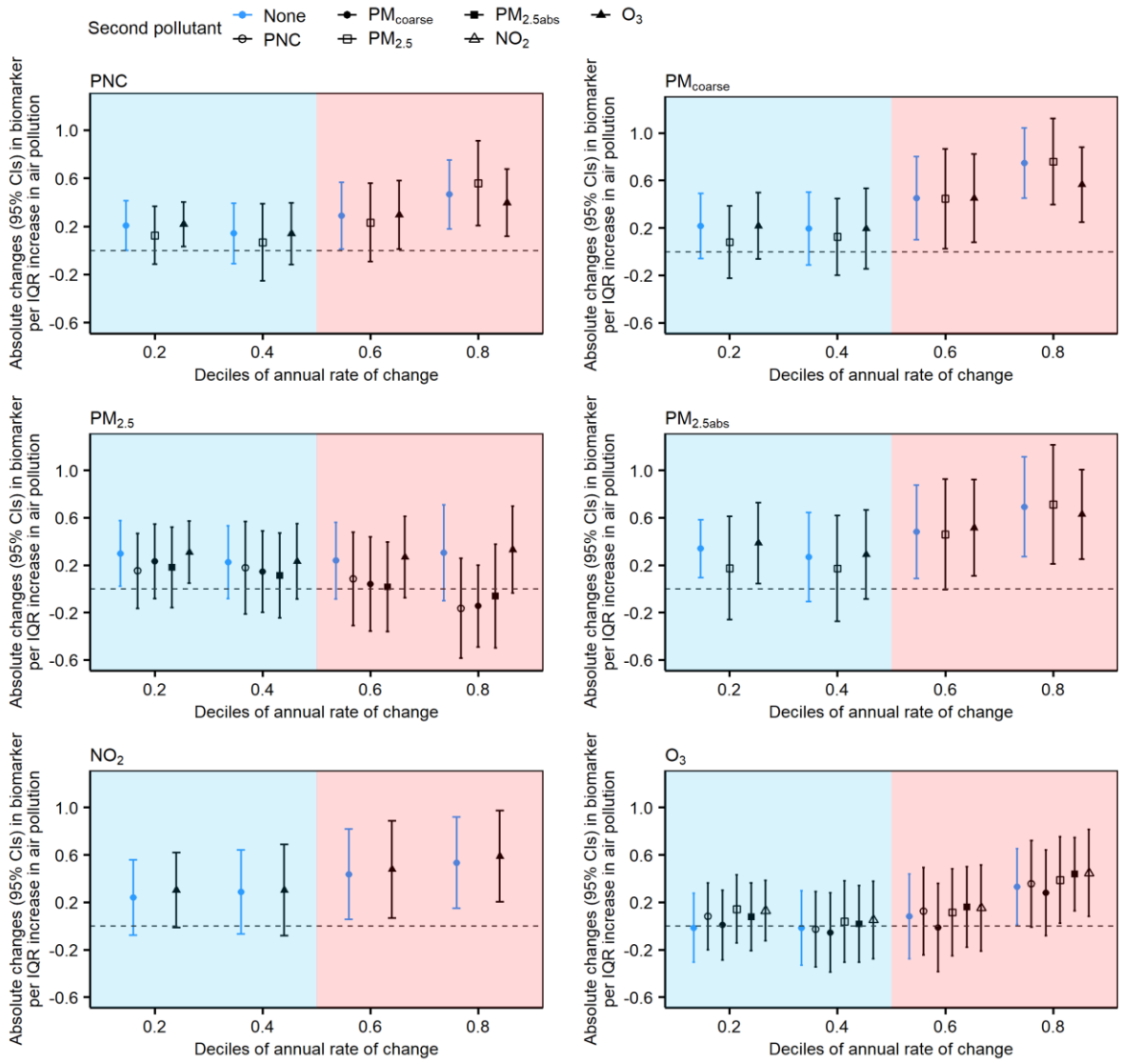

Figure S15. Absolute changes (95% CIs) in the annual rate of change in HOMA-B at different percentiles of the distribution per IQR increase in air pollutant concentrations in two-pollutant models.

Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero. Error bars in blue represent the effect estimates in single-pollutant models. An IQR increase was $2{\cdot}0\times10^{3}$/cm^3 for PNC, 1·4 μg/m^3 for PM$_{coarse}$, 1·4 μg/m^3 for PM$_{2·5}$, $0{\cdot}3\times10^{-5}$/m for PM$_{2·5abs}$, 7·1 μg/m^3 for NO$_2$, and 3·5 μg/m^3 for O$_3$.

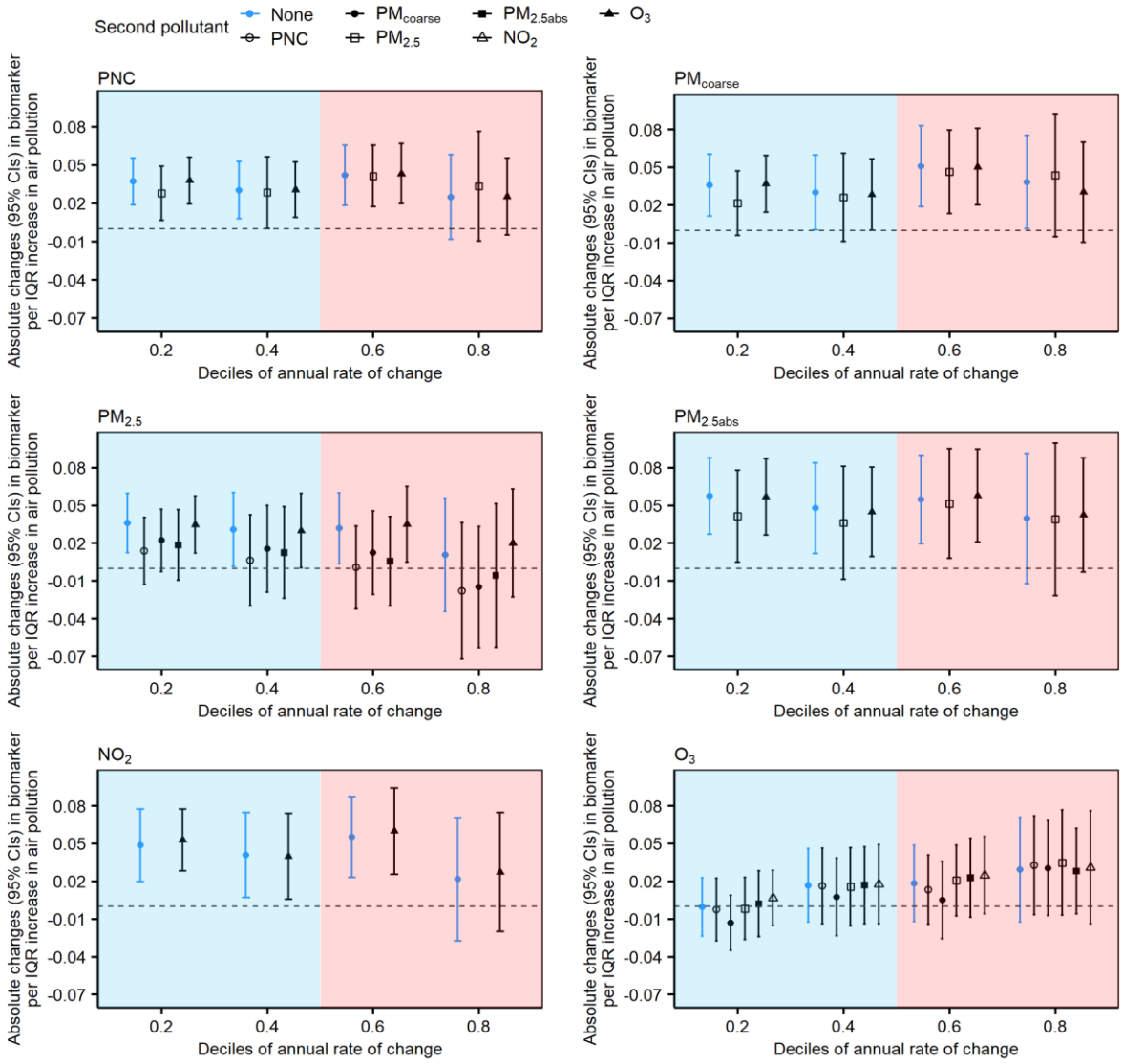

Figure S16. Absolute changes (95% CIs) in the annual rate of change in fasting insulin at different percentiles of the distribution per IQR increase in air pollutant concentrations in two-pollutant models.

Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero. Error bars in blue represent the effect estimates in single-pollutant models. An IQR increase was $2{\cdot}0\times10^{3}$/cm^3 for PNC, 1·4 μg/m^3 for PM_{coarse}, 1·4 μg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 μg/m^3 for NO_2, and 3·5 μg/m^3 for O_3.

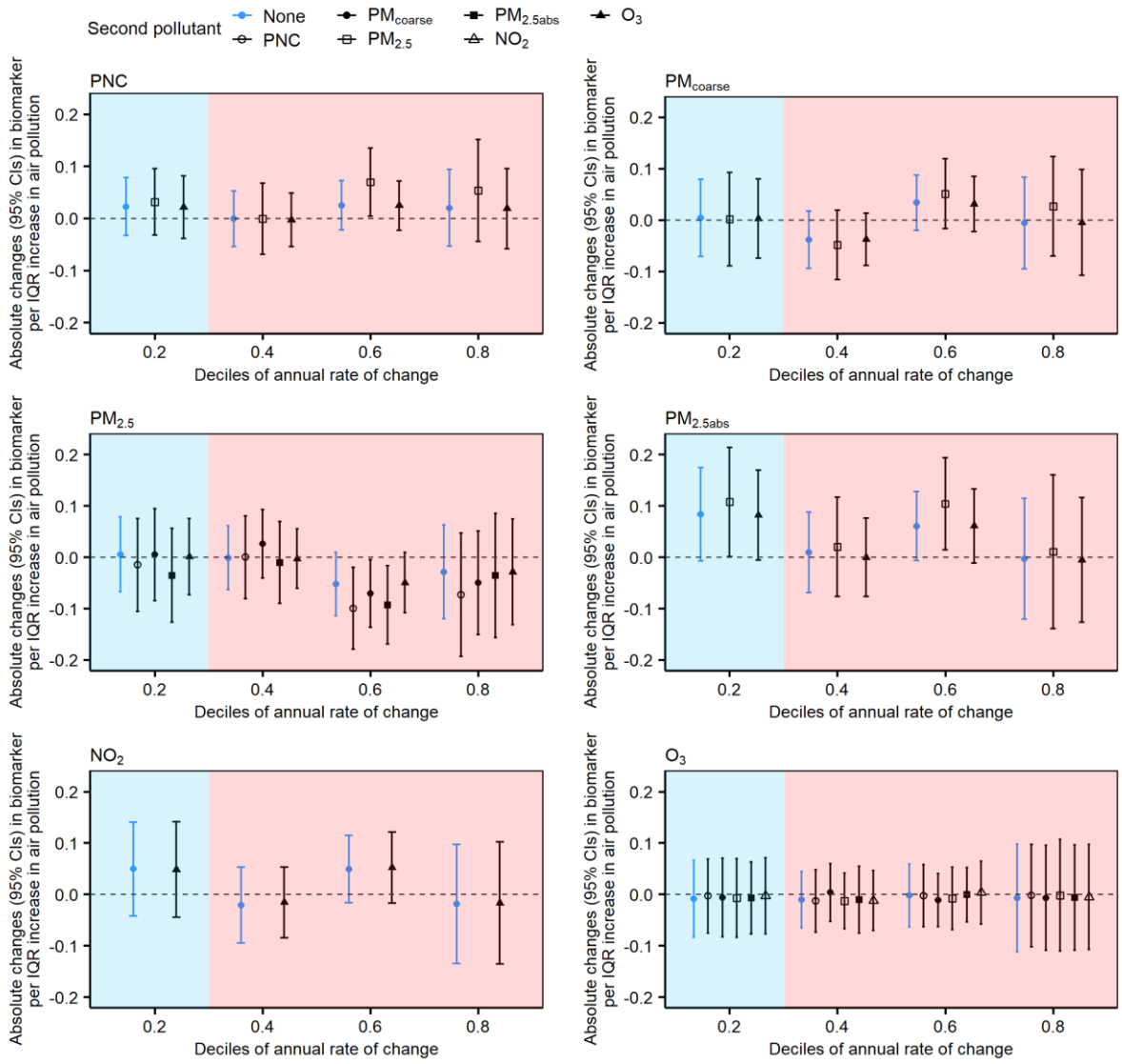

Figure S17. Absolute changes (95% CIs) in the annual rate of change in fasting glucose at different percentiles of the distribution per IQR increase in air pollutant concentrations in two-pollutant models.

Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero. Error bars in blue represent the effect estimates in single-pollutant models. An IQR increase was $2{\cdot}0\times10^{3}$/cm^3 for PNC, 1·4 μg/m^3 for PM$_{coarse}$, 1·4 μg/m^3 for PM$_{2·5}$, $0{\cdot}3\times10^{-5}$/m for PM$_{2·5abs}$, 7·1 μg/m^3 for NO$_2$, and 3·5 μg/m^3 for O$_3$.

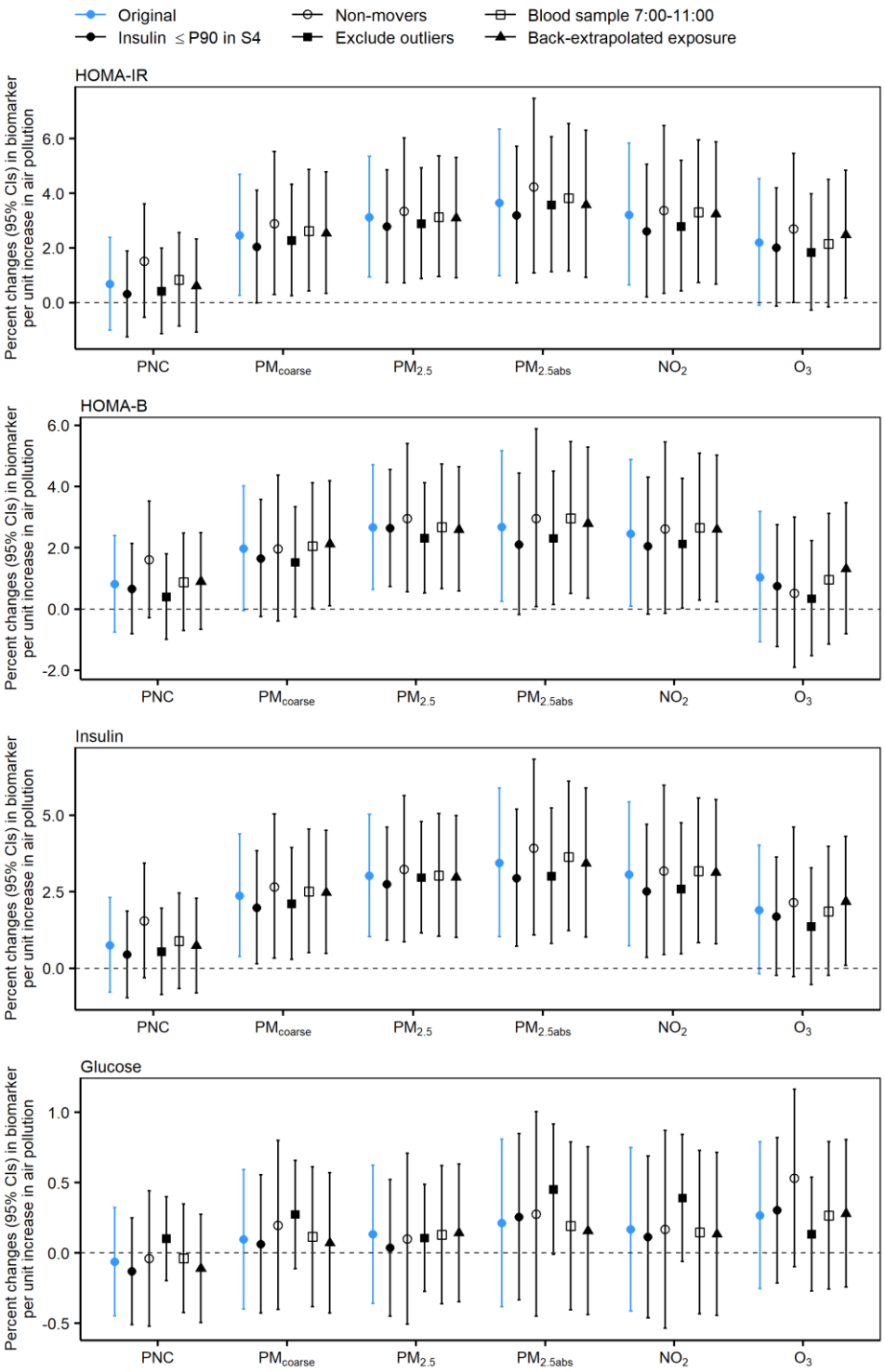

Figure S18. Percent changes (95% CIs) in repeated measurements of biomarkers per IQR increase in air pollutant concentrations in sensitivity analyses.
Original: main ME model (number of observations remained in the analysis (*N*) = 6,008). Insulin ≤ P90 in S4: observations with fasting insulin concentrations ≤ the 90th percentile (21·9 µIU/mL) in KORA S4 (*N*=5,783). Non-movers: participants who did not move during S4 to FF4 (*N*=4,748). Exclude outliers: exclude outliers in outcome variables (*N*=5,911 for HOMA-IR, *N*=5,886 for HOMA-B, *N*=5,907 for insulin, *N*=5,829 for glucose). Blood sample 7:00-11:00: blood samples drawn between 7:00 AM and 11:00 AM (*N*=5,974). Back-extrapolated exposure: use back-extrapolated exposure data with further adjustment for the year of examinations (*N*=6,008).

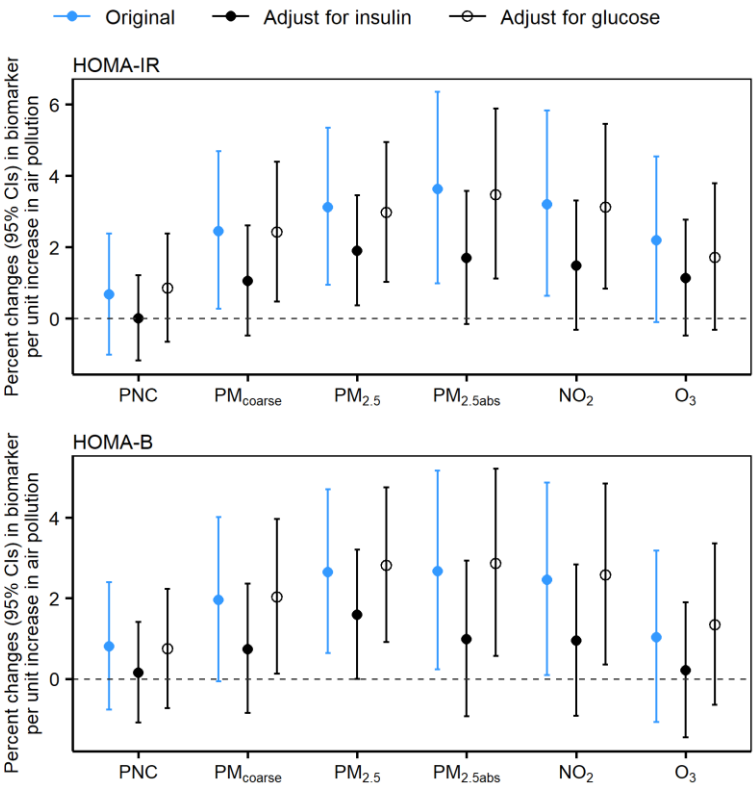

Figure S19. Percent changes (95% CIs) in repeated measurements of HOMA-IR and HOMA-B per IQR increase in air pollutant concentrations in models with further adjustment for fasting insulin or fasting glucose.

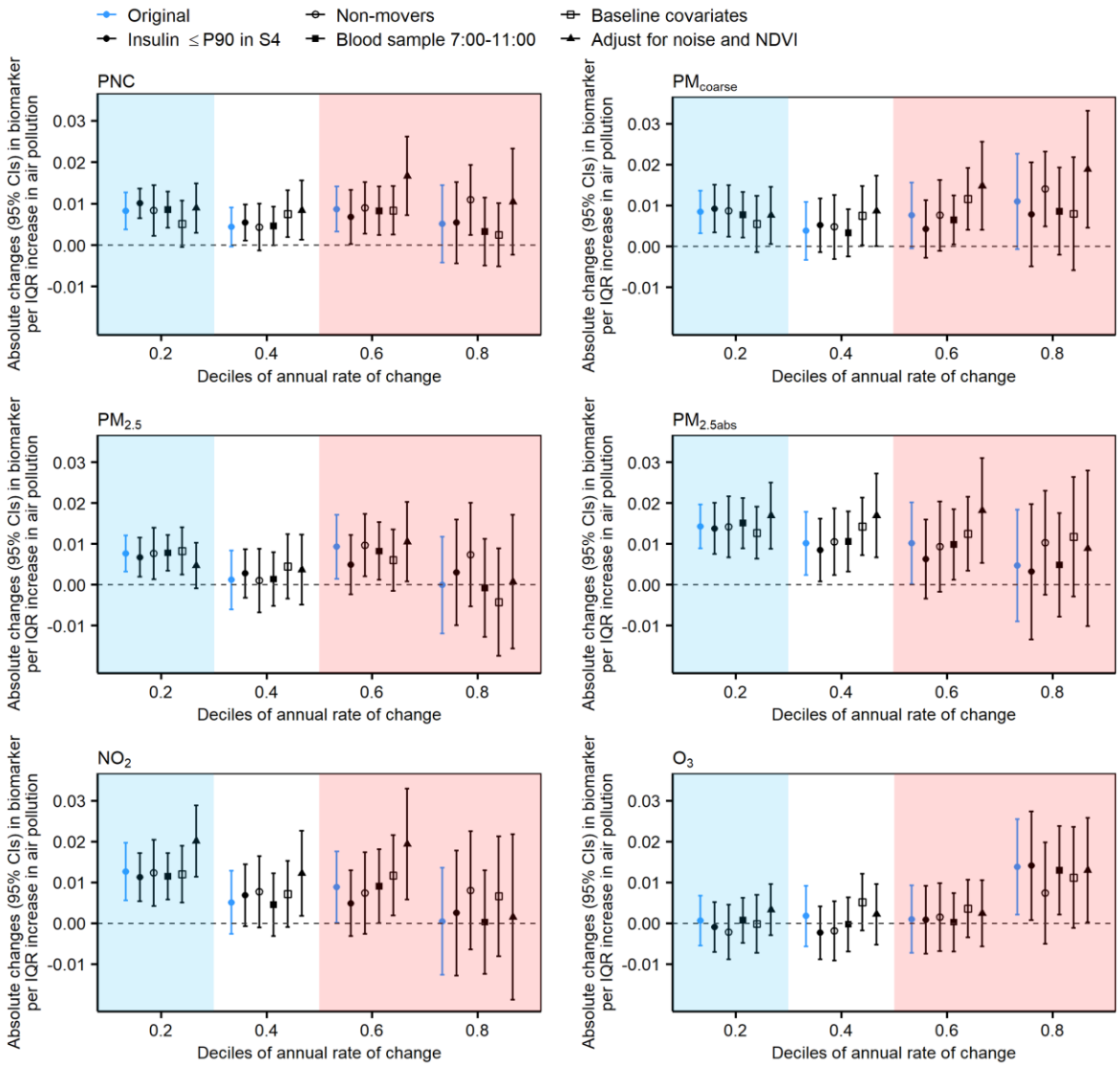

Figure S20. Absolute changes (95% CIs) in the annual rate of change in HOMA-IR per IQR increase in air pollutant concentrations in sensitivity analyses.
Original: main quantile regression model (number of observations remained in the analysis (*N*) = 2,242). Insulin ≤ P90 in S4: participants with fasting insulin concentrations ≤ the 90th percentile (21·9 µIU/ml) in KORA S4 (*N*=2,173). Non-movers: participants who did not move during S4 to FF4 (*N*=1,840). Blood sample 7:00-11:00: blood samples drawn between 7:00 AM and 11:00 AM (*N*=2,224). Baseline covariates: without adjustment for the annual rate of change in BMI and smoking pack-years (*N*=2,242). Adjust for noise and NDVI: with further adjustment for road traffic noise and NDVI in the main models (*N*=2,242). Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero; unshaded area in the middle indicates stable biomarker levels over years. An IQR increase was $2{\cdot}0\times10^{3}$/cm^3 for PNC, 1·4 µg/m^3 for PM_{coarse}, 1·4 µg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 µg/m^3 for NO_2, and 3·5 µg/m^3 for O_3.

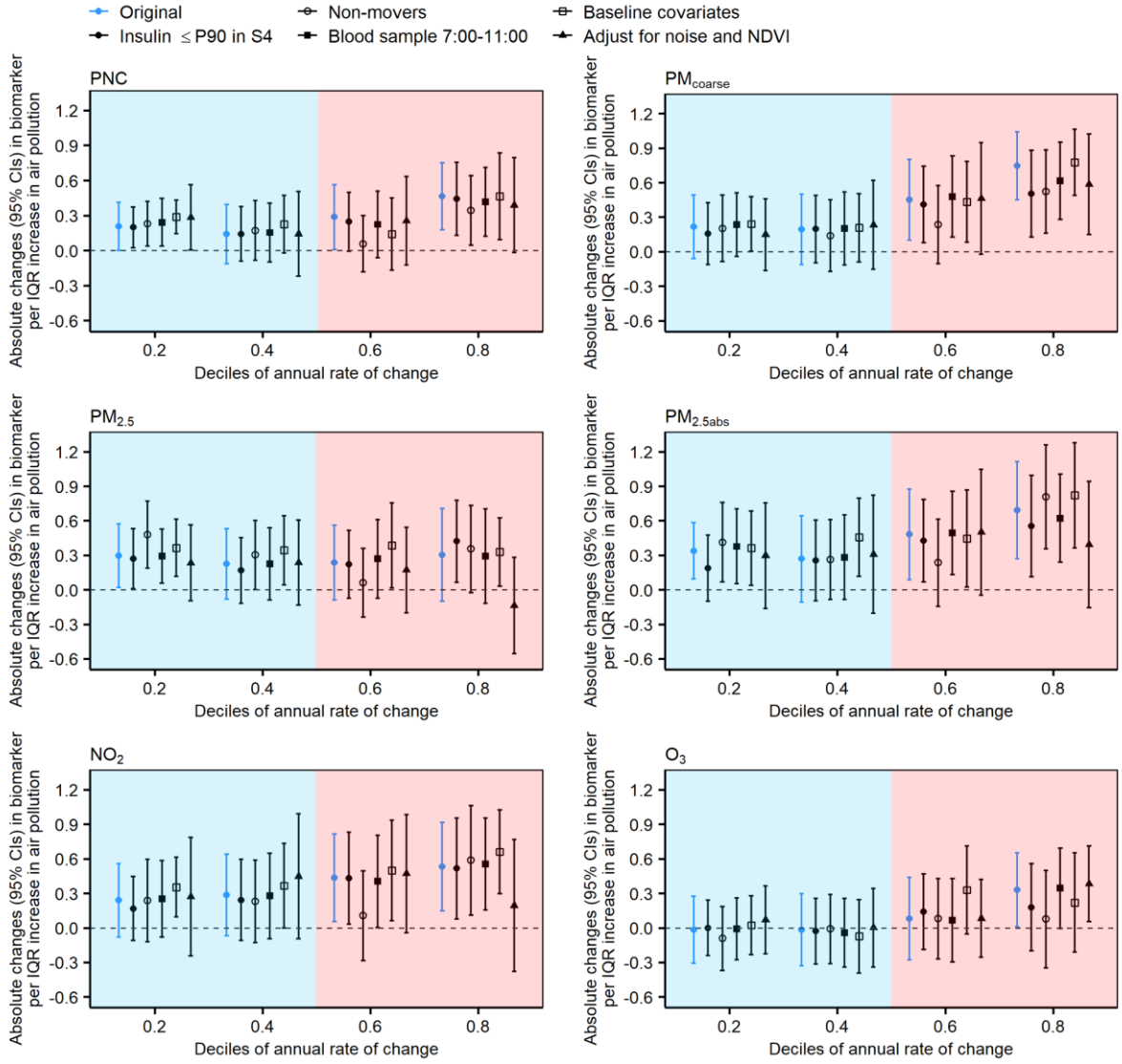

Figure S21. Absolute changes (95% CIs) in the annual rate of change in HOMA-B per IQR increase in air pollutant concentrations in sensitivity analyses.
Original: main quantile regression model (number of observations remained in the analysis (*N*) = 2,242). Insulin ≤ P90 in S4: participants with fasting insulin concentrations ≤ the 90th percentile (21·9 µIU/ml) in KORA S4 (*N*=2,173). Non-movers: participants who did not move during S4 to FF4 (*N*=1,840). Blood sample 7:00-11:00: blood samples drawn between 7:00 AM and 11:00 AM (*N*=2,224). Baseline covariates: without adjustment for the annual rate of change in BMI and smoking pack-years (*N*=2,242). Adjust for noise and NDVI: with further adjustment for road traffic noise and NDVI in the main models (*N*=2,242). Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero. An IQR increase was $2{\cdot}0\times10^{3}/cm^{3}$ for PNC, 1·4 µg/m^3 for PM_{coarse}, 1·4 µg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 µg/m^3 for NO_2, and 3·5 µg/m^3 for O_3.

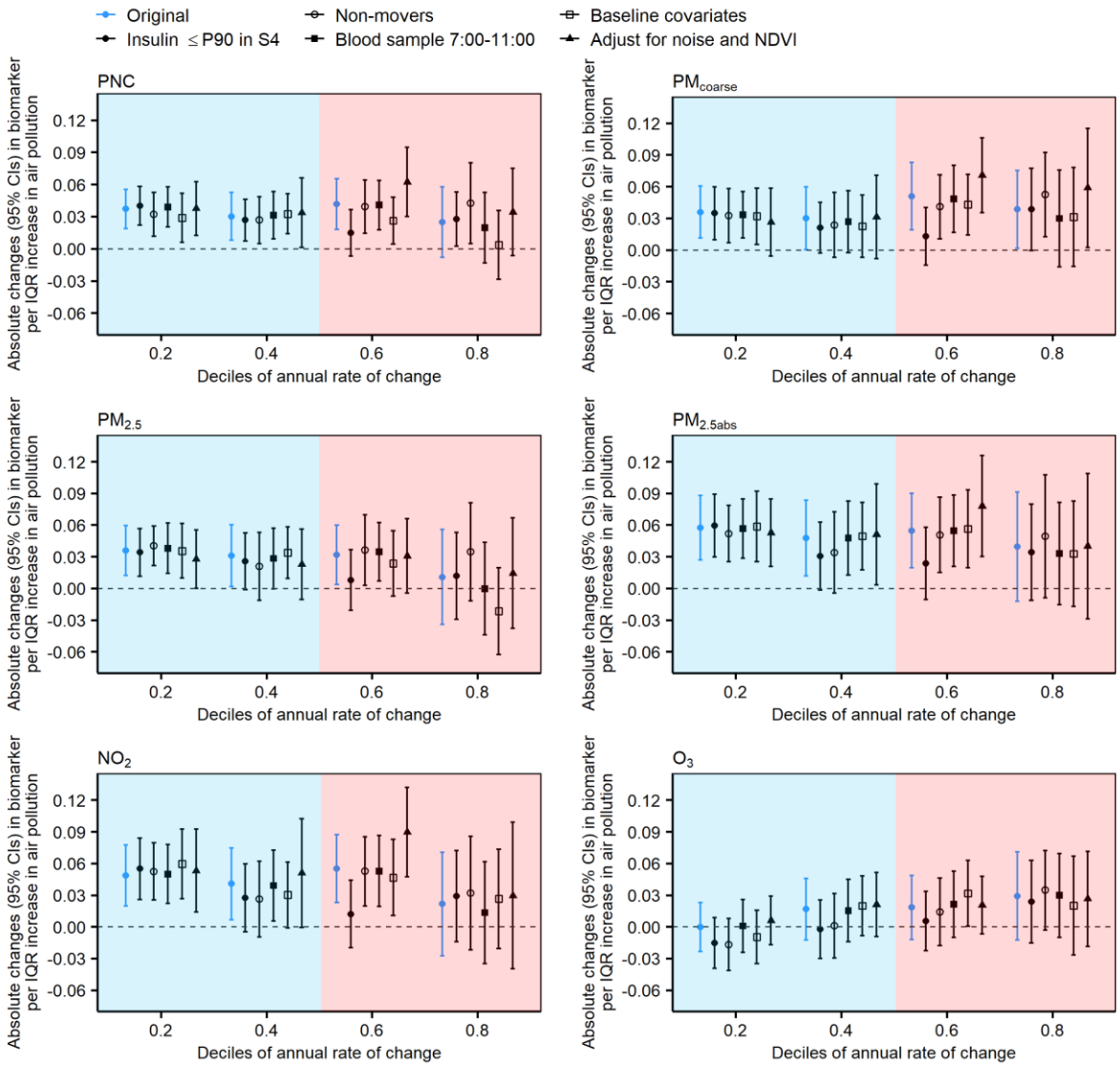

Figure S22. Absolute changes (95% CIs) in the annual rate of change in fasting insulin per IQR increase in air pollutant concentrations in sensitivity analyses.
Original: main quantile regression model (number of observations remained in the analysis (*N*) = 2,242). Insulin ≤ P90 in S4: participants with fasting insulin concentrations ≤ the 90th percentile (21·9 µIU/ml) in KORA S4 (*N*=2,173). Non-movers: participants who did not move during S4 to FF4 (*N*=1,840). Blood sample 7:00-11:00: blood samples drawn between 7:00 AM and 11:00 AM (*N*=2,224). Baseline covariates: without adjustment for the annual rate of change in BMI and smoking pack-years (*N*=2,242). Adjust for noise and NDVI: with further adjustment for road traffic noise and NDVI in the main models (*N*=2,242). Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero. An IQR increase was $2{\cdot}0\times10^{3}$/cm^3 for PNC, 1·4 µg/m^3 for PM_{coarse}, 1·4 µg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 µg/m^3 for NO_2, and 3·5 µg/m^3 for O_3.

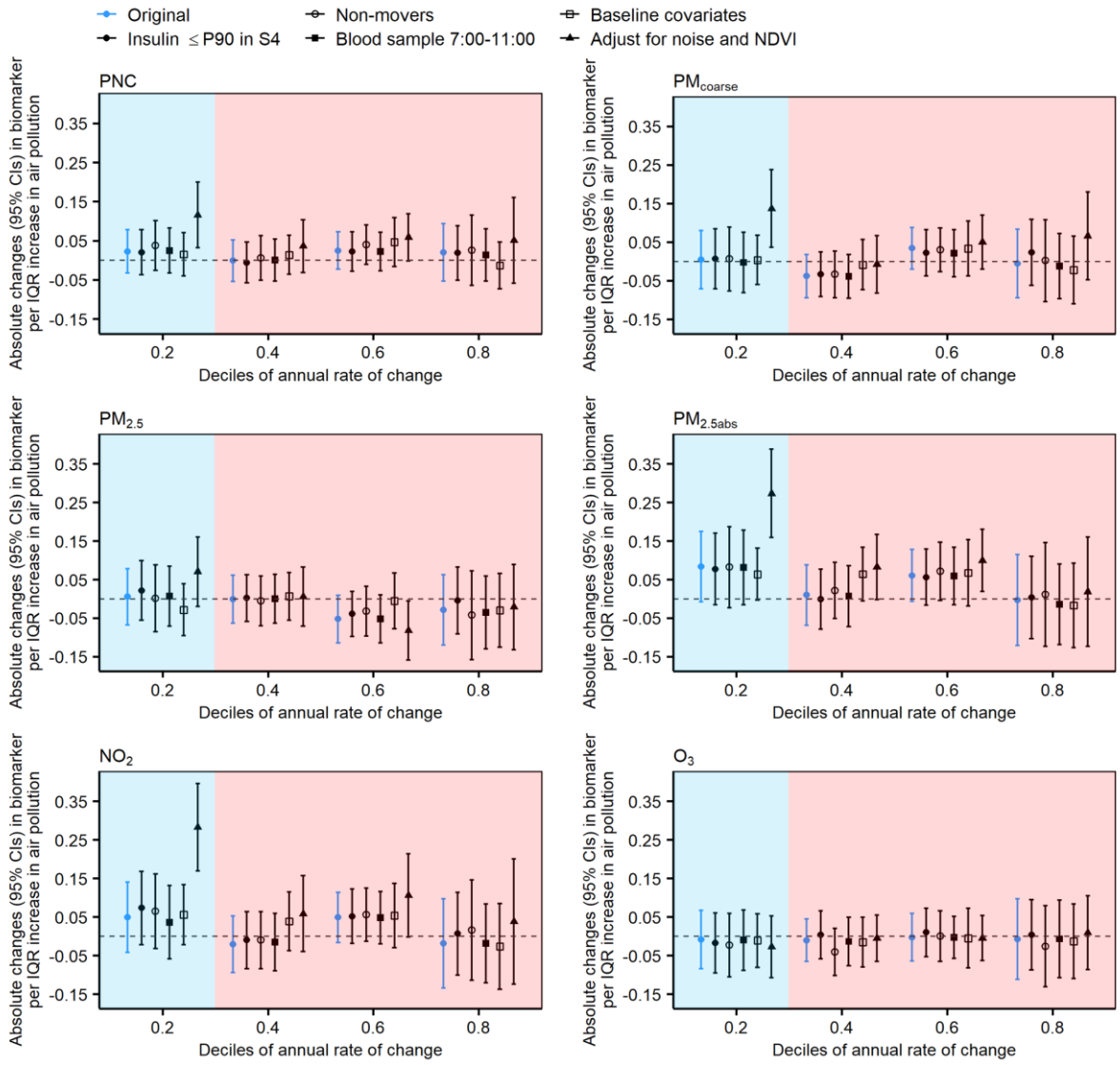

Figure S23. Absolute changes (95% CIs) in the annual rate of change in fasting glucose per IQR increase in air pollutant concentrations in sensitivity analyses.
Original: main quantile regression model (number of observations remained in the analysis (*N*) = 2,242). Insulin ≤ P90 in S4: participants with fasting insulin concentrations ≤ the 90th percentile (21·9 µIU/ml) in KORA S4 (*N*=2,173). Non-movers: participants who did not move during S4 to FF4 (*N*=1,840). Blood sample 7:00-11:00: blood samples drawn between 7:00 AM and 11:00 AM (*N*=2,224). Baseline covariates: without adjustment for the annual rate of change in BMI and smoking pack-years (*N*=2,242). Adjust for noise and NDVI: with further adjustment for road traffic noise and NDVI in the main models (*N*=2,242). Blue shaded area on the left side indicates annual rate of change below zero; red shaded area on the right side indicates annual rate of change above zero. An IQR increase was $2{\cdot}0\times10^3$/cm^3 for PNC, 1·4 µg/m^3 for PM_{coarse}, 1·4 µg/m^3 for $PM_{2{\cdot}5}$, $0{\cdot}3\times10^{-5}$/m for $PM_{2{\cdot}5abs}$, 7·1 µg/m^3 for NO_2, and 3·5 µg/m^3 for O_3.

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