

Pathways Linking Air Pollution to Insulin Resistance Through Dysfunctional Bone-Vascular Axis in Healthy Adults

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Research

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Abstract

Background

Epidemiological evidence supports that ambient air pollution exposure is associated with the onset of clinically overt diabetes, but the precise pathways are unknown. This study aimed to investigate whether dysfunctional bone-vascular axis can be a mechanistic linkage between air pollution and insulin resistance.

Methods

Study outcomes were determined by assessing a series of circulating biomarkers indicative of bone-vascular axis, insulin resistance, and immune inflammation among 73 healthy adults who undergo repeated clinical visits in Beijing, China, 2014-2016. Linear mixed-effects models were used to evaluate the impacts of air pollution on outcomes, and the potential interlinked pathways between exposure measures and insulin resistance were examined using mediation analyses.

Results

Participants experienced extremely high levels of ambient particulates and gaseous pollutants exposures throughout the study, with daily concentrations varying from 30.6 to 236.2 $\mu\text{g}/\text{m}^3$ for fine particulate matter (PM_{2.5}) and 54.9 to 235.8 $\mu\text{g}/\text{m}^3$ for oxides of nitrogen. We observed that short-term exposure to air pollutants was associated with changes in biomarkers of the bone-vascular axis and insulin resistance. Specifically, an interquartile range increase in 7-day moving average of PM_{2.5} concentrations was significantly associated with elevations of 30.1% in osteoprotegerin and 20.5% in adiponectin, as well as reductions of 16.6% in bone morphogenetic protein-4 and 8.5% in soluble insulin receptor ectodomain. Further, higher PM_{2.5} exposures were positively related to various indicators of immune inflammation, including elevations of 22.5% in interleukin-10, 19.6% in soluble CD163 and 27.5% in C-C chemokine ligand-2. Mediation analyses revealed that activation of the bone-vascular axis mediated up to 52% of pollutants-associated immune inflammatory responses, both of which could explain 66% of increases in insulin resistance attributed to pollutants exposures.

Conclusions

Short-term air pollution exposure is potentially capable of promoting insulin resistance possibly via generating dysfunctional bone-vascular axis, suggesting a novel mechanism by which air pollution may potentiate the development of diabetes.

Background

Anthropogenic air pollution, consisting of a mixture of particulates and gaseous pollutants from various fossil fuel combustion sources, poses an enormous risk to global public health[1]. Epidemiologic studies

have shown that ambient air pollution exposure over a few days can trigger numerous detrimental cardiometabolic effects (e.g., insulin resistance and diabetes) [2, 3]. Recent evidence suggests that exposure to ambient particulate matter in diameter $<2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) is likely responsible for an estimated 3.2 million incident cases of diabetes worldwide in 2016, and the burden of diabetes is much higher in some heavily polluted regions in Asian countries[4]. Thus far, a constellation of pathophysiologic responses, including systemic inflammation and immune activation, endothelial injury, and defective insulin receptor signaling, has been proposed to be partially responsible for the linkages between air pollution and cardiometabolic disorders[5]. However, the exact mechanisms whereby air pollution exposure contributes to the progression of insulin resistance and diabetes remain unknown.

There is emerging evidence implicating that dysfunctional bone-vascular axis may play a role in air pollution-associated cardiometabolic disorders[6, 7, 8, 9]. Human and experimental studies suggest that air pollution can be a trigger of dysregulated bone-vascular axis[6, 7, 8]. The osteoprotegerin (OPG) /receptor activator of NF- κB (RANK) /RANK ligand (RANKL) system is the core component of the bone-vascular axis, which is closely linked to an array of cardiometabolic disorders, including insulin resistance, diabetes, diabetic vascular complications, and cardiometabolic mortality[10, 11, 12]. As a potent stimulator of NF- κB , binding of RANKL to its receptor (RANK) is capable of activating NF- κB signaling and subsequent inflammatory cascades, as well as amplifying inflammatory responses via interactions with immune cells (e.g., T cells and macrophages) [13]. Studies in animals have shown that blockade of the OPG-RANK-RANKL system significantly improves insulin sensitivity and decreases cytokine production in the liver and skeletal muscle[14, 15]. Another key component of the bone-vascular axis is bone morphogenetic proteins (BMPs), and altered biological activities of BMPs can worsen insulin resistance and inflammatory potential in the vasculature and adipose tissue[16]. Recent mechanistic studies showed that exposure to concentrated ambient $\text{PM}_{2.5}$ resulted in insulin resistance by activation of NF- κB pathways-induced inflammation[17, 18]. Further, upregulated expressions of RANKL and BMPs along with significant elevations of inflammatory mediators are observed in endothelial and immune cells following exposure to particulates pollutants[6, 7]. Nevertheless, it remains unclear how air pollution exposure alters the functioning of the bone-vascular axis in human, and whether these metabolic changes may prompt the genesis of insulin resistance that could occur before developing clinically overt diabetes.

Building on existing evidence, here we hypothesized that recent air pollution exposure during the prior days would worsen metabolic insulin resistance, potentially due to dysregulation of the bone-vascular axis. In this context, we first assessed the impacts of a variety of ambient air pollutants on biomarkers indicative of the core axis of bone-vascular system, and various metrics of insulin resistance and inflammation. We further investigated to what extent the increases in insulin resistance could be explained by air pollution-associated activation of the bone-vascular axis.

Results

Participant Characteristics and Air Pollution Exposure

Descriptive characteristics of study participants, outcome measurements, and environmental factors are summarized in Table 1. As expected, large day-to-day variations in levels of size-fractionated particulates (e.g., PM_{2.5}, PNCs in the diameter size range of 5-100 nm [UFPs]) and traffic-related pollutants (e.g., BC, NO₂ and NO_x) were found across the entire study period, which were reflected by large IQRs and wide ranges for measured pollutants. For instance, the mean 7 MA days of PM_{2.5} and NO_x exposures were 91.8 µg/m³ (range: 30.6 to 236.2 µg/m³) and 124.8 µg/m³ (range: 54.9 to 235.8 µg/m³), respectively.

Association Between Air pollution and Dysfunctional Bone-Vascular Axis

Based on LME models, the adjusted changes in regulators in the core axis of the bone-vascular system in relation to air pollutants are shown in Figure 1. We found significant increases in OPG of 9.7% (95% CI, 3.2-16.2) to 34.1% (95% CI, 22.9-45.2) associated with IQR increases in PM_{2.5}, PNC₁₀₀₋₅₆₀, PSC₁₀₀₋₅₆₀, CO, NO₂ and NO_x at prior 2 to 7 MA days. The estimated effects were greater when the exposure periods were extended, suggesting that accumulative exposure to air pollution may exert prominent effects on activation of the bone-vascular axis. Inverse associations with OPG were also observed for UFPs exposures. Further, elevations of sRANKL /OPG ratio were positively related to BC, CO, NO₂ and NO_x, with stronger effects observed at prior 3 MA days. With IQR increases in exposure to BC, CO, NO₂ and NO_x at prior 3 to 7 MA days, BMP-2 levels were significantly increased by 8.8% (95% CI, 2.1-15.4) to 14.6% (95% CI, 5.1-24.1), whereas significant reductions of 9.1% (95% CI, -17.2 to -1.0) to 19.9% (95% CI, -28.6 to -11.2) were found for BMP-4 levels.

Association Between Air pollution and Insulin Resistance

For biomarkers indicative of insulin resistance-related metabolic factors, as shown in Figure 2, we found increases in circulating insulin of 8.9% (95% CI, 0.4-17.3) to 49.6% (95% CI, 20.2-79.1) associated with most air pollutants (except for PSC₁₀₀₋₅₆₀). For soluble form of the receptor for insulin, significant reductions of sIRα levels were observed in association with a variety of air pollutants (Figure 2). Among the examined exposure periods, the largest reductions in sIRα levels, ranging from 8.9% (95% CI, -17.7 to -0.1) to 13.8% (95% CI, -21.4 to -6.2), were associated with IQR increases in PM_{2.5}, BC and CO at prior 5 MA days. Additionally, positive associations of adiponectin and leptin with air pollutants were found at prior 1 to 7 MA days, but inverse associations were found for resistin (Figure 2 and Figure 3). For growth factors, significant reductions of CNTF levels were associated with air pollutants, with estimate effects ranging from 4.9% (95% CI, -9.3 to -0.5) to 22.2% (95% CI, -39.2 to -5.1; Figure 3). Concomitantly, elevations of BTC and HGF levels were found for exposure to PM_{2.5}, CO, and NO_x (Figure 3).

Association Between Air Pollution and Immune Inflammation

In line with the hypothesized mechanism that air pollution exposure may activate immune-inflammatory responses, we found significant changes in a suite of biomarkers indicative of systemic immune and inflammation in relation to air pollutants (Figure 4 and 5). As shown in Figure 4, significant increases in T cell-related cytokines (IL-2, 10, 22) of 7.6% (95% CI, 0.8-14.5) to 42.5% (95% CI, 7.2-77.8) and IL-1 family

mediator (sIL1RA) of 13.3% (95% CI, 1.9-24.6) to 32.0% (95% CI, 14.3-49.7), were observed in association with IQR increases in PM_{2.5}, CO, and NO_x at prior 1 to 7 MA days. For chemokines, significant increases in CCL-2 were related to PM_{2.5}, PNC₁₀₀₋₅₆₀, PSC₁₀₀₋₅₆₀, BC, CO, NO₂, and NO_x, with the stronger effects observed at prior 5 to 7 MA days of exposure (Figure 5). Similar association patterns and the magnitude of effect estimates were also observed for CCL-5 (Figure 5). As expected, significant elevations of 7.8% (95% CI, 0.9-14.8) to 20.4% (95% CI, 10.3-30.5) in specific macrophage activation marker sCD163 were observed in association with IQR increases in exposure to air pollutants at prior 1 to 7 days. In addition, greater magnitude of elevated CXCL-8 levels was found ranging from 12.4% (95% CI, 0.3-24.6) to 31.5% (95% CI, 12.1-50.9, Figure 5).

Exploratory Analyses

In mediation analyses, single-mediator models showed that dysregulated bone-vascular axis (e.g., heightened the biological activity of OPG-RANK-RANKL system) could mediate up to 66% of the effects of selected pollutants (e.g., PM_{2.5} and CO) on insulin resistance (Additional Table 1). Further, air pollutants-associated activations of immune inflammation could also be mediated via dysregulation of the bone-vascular axis, and elevated products of immune-inflammatory mediators (e.g., CCL-2) might further increase in circulating levels of regulators in the bone-vascular axis such as OPG (Additional Table 2 and 3). Results obtained from multiple-mediator models were in line with those observed in single-mediator models (Additional Table 4). As shown in Additional Figure 1, the main findings and the study conclusions remained unchanged when sensitivity analyses were performed, including repeated analyses with a subject-normalization approach, excluding individuals with urinary cotinine levels >200 ng/mg Cr, and excluding individuals who lived beyond 1 kilometer from the air monitoring station.

Discussion

As depicted in Figure 6, we have shown here that short-term exposure to various ambient size-fractionated particulates and traffic pollutants (e.g., PM_{2.5}, UFPs, BC, and NO_x) can significantly prompt the genesis of dysfunctional bone-vascular axis and insulin resistance. Higher concentrations of air pollutants were also associated with worsening systemic immune and inflammation responses. As hypothesized, mediation analyses showed that insulin resistance attributable to air pollution exposure might be partially mediated via dysregulated bone-vascular axis as well as immune-inflammatory pathways. Given the growing co-pandemics of air pollution and cardiometabolic disorders across the worldwide, our findings unveil a characteristic signature of metabolic changes predisposing to the onset of diabetes caused by inhaled air pollution. Though the relatively modest changes in metabolic homeostasis could be transient or reversible; however, persistent air pollution exposure, especially for individuals residing in heavily polluted urban environments, may potentially convey the risk burden of metabolic disorders at older ages and heighten future cardiometabolic events.

Limited epidemiological studies assessed the associations of PM originating from biomass burning and fossil fuel combustion with circulating OPG levels, and have yielded mixed results [7, 8]. In this study, we

observed elevations of 10.4% to 34.1 % in OPG levels in relation to a variety of particulates and traffic pollution, which could account for elevated OPG levels of 18.4 to 60.3 pg/mL among exposed individuals. A recent clinical study has demonstrated that each 10 pg/mL increase in serum OPG levels is likely responsible for the increased risk of insulin resistance state by 3 % to 15% in a diabetic population living in China - which has direct relevance for our findings[19]. Mechanistically, it has been reported that OPG, RANKL and BMPs appear to be highly expressed in immune and vascular cells (e.g., T cells and endothelial cells), following cellular activation by inflammatory stimuli[13, 16]. Addition of inflammatory cytokines, such as IL-22 and MCP-1, is capable of promoting RANKL and OPG expressions in vitro and vivo[20, 21, 22]. Higher OPG levels are indicators of reflecting heightened the biological activity of the OPG-RANK-RANKL system[23]. Apart from changes in the circulating levels of OPG and sRANKL, significant alterations in BMPs levels along with higher levels of inflammatory cytokines were also found to be influenced by air pollution exposure, which are largely consistent with prior experimental findings[6, 24]. Collectively, our results extended existing findings to a real-world exposure setting with higher levels of air pollutants, and provided evidence that air pollution may prompt dysfunctional bone-vascular axis, possibly via activation of immune-inflammatory pathways.

To our knowledge, the Beijing AIRCHD study is the first to assess the mediating roles of immune inflammation and bone-vascular axis in air pollution-associated insulin resistance and to gain insight into potential interlinked mechanisms. Immune inflammation driven by activated lymphocytes and monocytes /macrophages is an important contributor involving in the pathogenesis of insulin resistance and diabetes [25]. Experimental studies showed increases in adipose tissue macrophages and impairments in insulin sensitivity following exposure to ambient PM_{2.5}[26]. As a member of the epidermal growth factor family, BTC plays a crucial role in the process of inflammation and tissue repair, which can upregulate cytokines levels by modulating the NF-κB pathway[27]. Interestingly, the impacts of particulates in smaller size fractions on inflammation and different metrics of insulin resistance were not always consistent. For instance, significant increases in insulin and leptin levels associated with UFPs and PSC₅₋₁₀₀ exposures, whereas null or inverse associations were found for immune-inflammatory mediators. These disparate findings suggest that increases in insulin resistance attributable to air pollution exposure might not be exclusively mediated via immune-inflammatory pathways. Indeed, previous studies showed that significant activation of OPG-RANKL system was observed in diabetic patients but not elevated levels of pro-inflammatory cytokines[28]. Genetic disruption of the RANK-RANKL pathway in mouse liver has shown resulting in suppressed phosphorylation of IκB kinases (IKKs) and improving insulin sensitivity[14]. Inhibition of central IKKβ protects against ambient PM_{2.5}-induced insulin resistance and inflammation[29]. A mechanistic study also suggested that insulin sensitivity was reduced in the liver of mice exposed to ambient PM_{2.5} via activation of NF-κB pathways-mediated inflammation[18].

Besides, our study revealed that air pollution-associated changes in components of the insulin signaling pathways might be mediated via alterations in the levels of regulators in the bone-vascular axis (e.g., OPG and BMPs) and metabolism-related factors (e.g., HGF). A critical step in the cascade of insulin action is

that insulin binding to α -subunits of insulin receptor (IR- α) activates the tyrosine kinase of β subunits (IR- β) and recruits IR substrates (e.g., IRS1) for tyrosine phosphorylation [30]. Down-regulation of the density of cell surface IR can lead to hyperinsulinemia and peripheral insulin resistance[30, 31]. Circulating sIR α , which contains one of the IR- α and a part of the IR- β , is primarily shed from the cell surface IR in hepatocytes, and altered sIR α levels may reflect the expression of cellular membrane-bound IR[32, 33]. Prior mechanistic studies showed that significant reductions of IR expressions were caused by concentrated ambient PM exposures, and the phosphorylation levels of IRS1 were also inhibited by air pollution-induced inflammation [34, 35]. In addition, sIR α can bind a larger proportion of insulin in the circulation system and thereby buffer the amount of insulin available to exert biological effects [33]. Further, CNTF can improve insulin sensitivity via regulation of the metabolic clearance rate of insulin from circulation [36]. BMP-4 signaling can regulate the differentiation and proliferation of pancreatic cells[37]. More recently, modulation of IR by HGF has been found to alter IR response to insulin, and higher serum levels of HGF have been linked to air pollution exposure [38, 39]. Considering all these evidence, our results indicated that dysregulated bone-vascular axis as well as increased metabolic inflammatory status could be partially responsible for ambient air pollution-associated insulin resistance.

Our study has several strengths. The Beijing AIRCHD study was conducted in a real-world scenario with a wide spectrum of air pollutant concentrations and over an extended follow-up period, making our findings of critical implications for reducing the global burden of cardiometabolic diseases attributable to air pollution. Further, there was minimal potential for confounding by environmental tobacco smoke exposure or the stress response of human body because urinary cotinine and cortisol levels were both assessed in this healthy population and controlled in the analyses. Moreover, mediation analysis was conducted to examine potential mediators of interest on the associations between air pollutants and metabolic responses, which allowed to explore the pathophysiologic mechanisms linking air pollution exposure to insulin resistance. Concomitantly, several limitations should also be merited in discussion. First, the fixed-location monitoring data as a surrogate for individual exposure may introduce non-differential exposure error that may bias effect estimates towards the null (e.g., underestimates of effect). Third, enrollment of a homogenous group of healthy and young adults with similar demographic characteristics and the relative uniformity of lifestyle patterns might have limited the generalizability of our findings to a broader clinical setting. Despite these, however, the study design also allowed to reduce the potential confounding from medication use, age-related susceptibility, indoor sources of air pollution, and lifestyle related factors.

Conclusions

We have shown that short-term exposure to ambient air pollution is associated with significant increases in insulin resistance, which might be partially mediated via dysfunctional bone-vascular axis. These findings provide novel insights into pathophysiologic interlinks between exposure to air pollution and occurrence of diabetes, and highlight the importance to mitigate the health risk from reducing air pollution exposure during early life stage.

Methods

Study Participants

The Beijing AIRCHD study (Air Pollution and Cardiovascular Dysfunctions in Healthy Adults Living in Beijing) was a prospective follow-up of 73 non-smoking healthy adults with repeated measurements (the baseline examination and subsequent 4 clinical visits) in 2014-2016, yielding a total of 327 person-visits in the final analytic dataset. Detailed design and selection criteria of this project have been described previously[40]. Briefly, blood and urine samples, participants' information on demographic characteristics, smoking status, medical history, and residential address were obtained at each examination following standardized protocols. All individuals gave written informed consent at enrollment, and the Institutional Review Board of PUHSC approved the study.

Biomarker Assessment

Serum samples were taken between 8 AM and 10 AM after an overnight fasting and stored at -80°C upon collection. Multiplex bead-based flow cytometry assays were used to simultaneously detect biomarkers indicative of the bone-vascular axis (OPG, soluble RANKL [sRANKL], BMP-2 and 4), metabolic insulin resistance (insulin, adiponectin, leptin, resistin, betacellulin [BTC], ciliary neurotrophic factor [CNTF], and hepatocyte growth factor [HGF]), and immune inflammation including T-cell /interleukin-1 (IL-1) family-related mediators (IL-2, 10, 22, and soluble IL-1 receptor antagonist [sIL1RA]), and monocyte /macrophage activation indicators (C-C chemokine ligand-2 [CCL-2], CCL-5, C-X-C chemokine motif ligand-8 [CXCL-8], soluble CD163 [sCD163]). Serum concentrations of soluble insulin receptor ectodomain (sIRα) were determined by an enzyme-linked immunosorbent assay (ELISA) approach (BioVendor, Brno, Czech Republic). The ratio between sRANKL and OPG (sRANKL /OPG) was calculated reflecting the overall biological activity of OPG-RANK-RANKL system[23]. Because potential environmental tobacco smoking exposure and stress response of human body might affect the ability to capture cardiometabolic effects attributable to air pollution exposure[41, 42], thus spot urine samples were analyzed for urinary concentrations of cotinine (IMMUNAL YSIS, Pomona, CA) and cortisol (LIUHEBIO, Wuhan, China) using ELISA approaches for further analyses.

Ambient Air Pollution and Meteorology Assessment

Hourly measures of PM_{2.5}, temperature and relative humidity, minute-to-minute measures of black carbon (BC), carbon monoxide (CO), nitrogen dioxide (NO₂) and oxides of nitrogen (NO_x), and 5-minute number and surface area concentrations of particulates with sizes of 5.6-560 nm were monitored continuously throughout the study. To assess the health impacts of size-fractionated particulates, particulate number concentrations (PNCs) and particulate surface area concentrations (PSCs) were grouped into 5.6-100 nm and 100-560 nm for further analysis, respectively[43]. All environmental factors were measured at a fixed monitoring station, which was located within 1 kilometer in distance to the vast majority (93%) of study participants. Previous studies have shown that air pollution-associated insulin resistance could likely occur within several days of exposure[2]. Thus, 1- to 7-day moving average (MA) concentrations of air

pollutants before each participant's clinic visit were calculated as exposure metrics, including pollutant-specific averaged levels over the last 24 hours (MA day 1 [1 MA day]), 1 to 2 days (2 MA days), and so on up to 7 MA days. Daily averages of all environmental factors were computed from 9 AM to 9 AM the next day because blood samples were obtained from participants at approximately 9 AM during the study period.

Statistical Analysis

Summary statistics were computed for all measurements of study outcomes and environmental exposures, including means (standard deviation), medians (range), and interquartile range (IQR). Linear mixed-effects (LME) models were conducted to characterize associations between ambient air pollutants and outcome measures. Outcome variables with skewed distributions were log-transformed to approximate a normal distribution. To control potential impacts of meteorological parameters, 24-hour averages (1 MA day) of temperature and relative humidity were included in all LME models using natural splines with degrees of freedom (≤ 3) based on the minimizing Akaike's Information Criterion. Seasonality was explained by adding sine and cosine terms of calendar dates of blood withdrawal. A backward stepwise model selection procedure was operated to discern the key covariates for each outcome measure, including age, sex, body mass index, waist-to-hip ratio, day of week of clinical visit, month of blood withdrawal, creatinine-corrected urinary cotinine and cortisol. The identified covariates in final LME models are presented in Additional Table 5.

In exploratory analyses, mediation analyses with a single mediator were firstly performed to examine the potential mechanisms responsible for insurance resistance attributable to air pollution, focusing on dysregulated bone-vascular axis[40]. We also assessed the mediating roles of the bone-vascular axis in pollutants-associated activation of immune inflammation, and whether heightened immune-inflammatory responses could in turn worsen the functioning of the bone-vascular axis. Further, considering significant correlations observed among measured study biomarkers (Additional Table 6), multiple-mediator models were further developed by including all potential mediators with statistical significance (p -value < 0.05) in the single-mediator model[40, 44]. Lastly, several sensitivity analyses were conducted to examine whether the associations differed from the findings obtained in main models if we: (1) repeated regression analyses with subject-normalized measurements of laboratory data of measured biomarkers instead of the raw data of the measurements[45]; (2) excluded individuals that had urinary levels of cotinine > 200 ng/mg Cr to reduce the potential impacts of environmental tobacco smoking exposure; (3) restricted individuals to those who lived within 1 kilometer from the ambient air monitoring station.

All estimates are presented as percent changes with 95% confidence intervals (CIs) associated with IQR increases in air pollutant concentrations. Statistical significance was determined at p -value < 0.05 . To control for potential type I error rate, we considered a p -value < 0.0025 ($0.05/20$) as significant after a Bonferroni correction for multiple comparisons among outcome variables. All analyses were conducted using R, version 3.4.3 (R Project for Statistical Computing).

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Peking University Health Science Center.

Consent for publication

Not applicable.

Availability of data and material

The datasets used for current analysis are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Hongbing Xu, Shengcong Liu, and Yang Wang analyzed the data and prepared the manuscript. Qian Zhao, Jie Chen, Yang Wang, Yutong Zhu, Hongbing Xu, Shengcong Liu, Tiece Yi, Tong Wang, Jiakun Fang, Yunfei Xie, Rongshan Wu, Baihuan Feng, and Xiaoming Song performed the clinical outcomes and air pollutant measurements. Sanjay Rajagopalan, Robert D. Brook, Jianping Li, Lemin Zheng, and Wei Huang designed the study and interpreted clinical relevance of the study findings. All authors read and approved the final manuscript.

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Tables

Table 1. Descriptive statistics of study participants (N=73, 48 females), outcome measurements, and environmental factors during the study period.

	Mean ± SD	Median	Range	IQR
Age, years	23.3 ± 5.4	23.0	18.0-50.0	4.0
Body mass index, kg/m ²	22.5 ± 3.5	21.5	16.9-32.9	5.8
Waist-to-hip ratio	0.80 ± 0.07	0.80	0.63-0.94	0.10
Urinary cotinine, ng/mg Cr	20.8 ± 102.7	2.6	0.1-954.8	3.4
Urinary cortisol, ng/mg Cr	1.9 ± 3.2	1.2	0.1-43.1	1.6
Health outcomes				
Bone-vascular axis				
OPG, pg/mL	176.9 ± 150.7	145.8	15.1-1640.3	106.7
sRANKL, pg/mL	132.6 ± 460.8	41.1	11.4-5098.1	42.6
sRANKL/OPG ratio	1.07 ± 2.90	0.30	0.05-30.36	0.50
BMP-2, pg/mL	47.5 ± 61.2	30.5	5.0-467.9	51.9
BMP-4, pg/mL	216.2 ± 79.7	214.8	10.6-567.8	105.7
Insulin resistance				
Insulin, µU/mL	2.35 ± 2.84	1.39	0.23-32.79	2.52
sIRα, ng/mL	32.5 ± 21.6	26.6	13.3-237.6	14.0
Adiponectin, ng/mL	635.1 ± 519.8	475.2	7.3-3631.8	477.7
Leptin, ng/mL	2.05 ± 1.69	1.66	0.01-10.51	2.07
Resistin, ng/mL	21.1 ± 9.1	19.4	5.7-59.3	11.5
BTC, pg/mL	64.4 ± 148.8	28.5	5.0-1384.0	31.7
CNTF, pg/mL	314.6 ± 601.7	134.5	14.5-4987.1	151.6
HGF, pg/mL	347.8 ± 148.0	341.6	50.5-891.8	202.5
Immune-inflammatory mediators				
IL-1 family /T cell-related cytokines				
IL-2, pg/mL	21.0 ± 59.5	4.2	1.5-591.4	11.7
IL-10, pg/mL	7.9 ± 29.3	3.2	1.1-307.2	2.0
IL-22, pg/mL	8.2 ± 29.8	2.6	1.5-265.5	2.3
sIL1RA, pg/mL	82.2 ± 98.2	51.9	5.7-912.2	50.2
Monocyte /macrophage activation				

CCL-2, pg/mL	159.1 ± 87.4	136.6	25.9-918.7	79.1
CCL-5, ng/mL	5.9 ± 2.7	5.2	1.9-23.2	2.5
CXCL-8, pg/mL	9.8 ± 9.7	7.8	0.5-114.8	5.2
sCD163, ng/mL	23.5 ± 11.8	20.7	15.1-81.7	7.9
Environmental measurements				
Air pollutants				
PM _{2.5} , µg/m ³	91.8 ± 48.3	85.6	30.6-236.2	63.8
PNC ₅₋₁₀₀ , 10 ³ particles/cm ³	18.8 ± 4.1	19.2	9.2-31.1	3.2
PNC ₁₀₀₋₅₆₀ , 10 ³ particles/cm ³	4.5 ± 1.9	4.6	1.2-8.6	3.0
PSC ₅₋₁₀₀ , cm ² /m ³	1.8 ± 0.8	1.5	0.6-2.9	1.6
PSC ₁₀₀₋₅₆₀ , cm ² /m ³	5.3 ± 4.3	4.9	0.5-29.9	3.8
BC, µg/m ³	5.9 ± 2.9	5.6	1.7-13.6	4.0
CO, ppm	1.16 ± 0.60	1.04	0.40-3.42	0.68
NO ₂ , µg/m ³	70.3 ± 15.4	68.8	45.3-110.4	16.9
NO _x , µg/m ³	124.8 ± 43.3	123.0	54.9-235.8	48.7
Meteorological parameters				
Temperature, °C	14.6 ± 12.0	17.4	-1.8-31.3	22.3
Relative humidity, %	33.1 ± 10.6	29.3	18.3-59.8	15.4

Results of health outcomes represent the values averaged from each clinical visit for all study participants. Environmental measurements levels were averaged separate 7-day moving average periods before clinical visits. Abbreviations: OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor-κB ligand; BMP, bone morphogenetic protein; sIRα, soluble insulin receptor ectodomain; BTC, betacellulin; CNTF, ciliary neurotrophic factor; HGF, hepatocyte growth factor; IL, interleukin; sIL1RA, soluble IL-1 receptor antagonist; CXCL-8, C-X-C chemokine motif ligand-8; CCL, C-C chemokine ligand; sCD163, soluble CD163; PM_{2.5}, particulate matter in diameter <2.5 µm; PNC_x, number concentration of particulate in given size ranges (nm); PSC_x, surface area concentration of particulate in given size ranges (nm); BC, black carbon; CO, carbon monoxide; NO₂, nitrogen dioxide; NO_x, oxides of nitrogen.

Figures

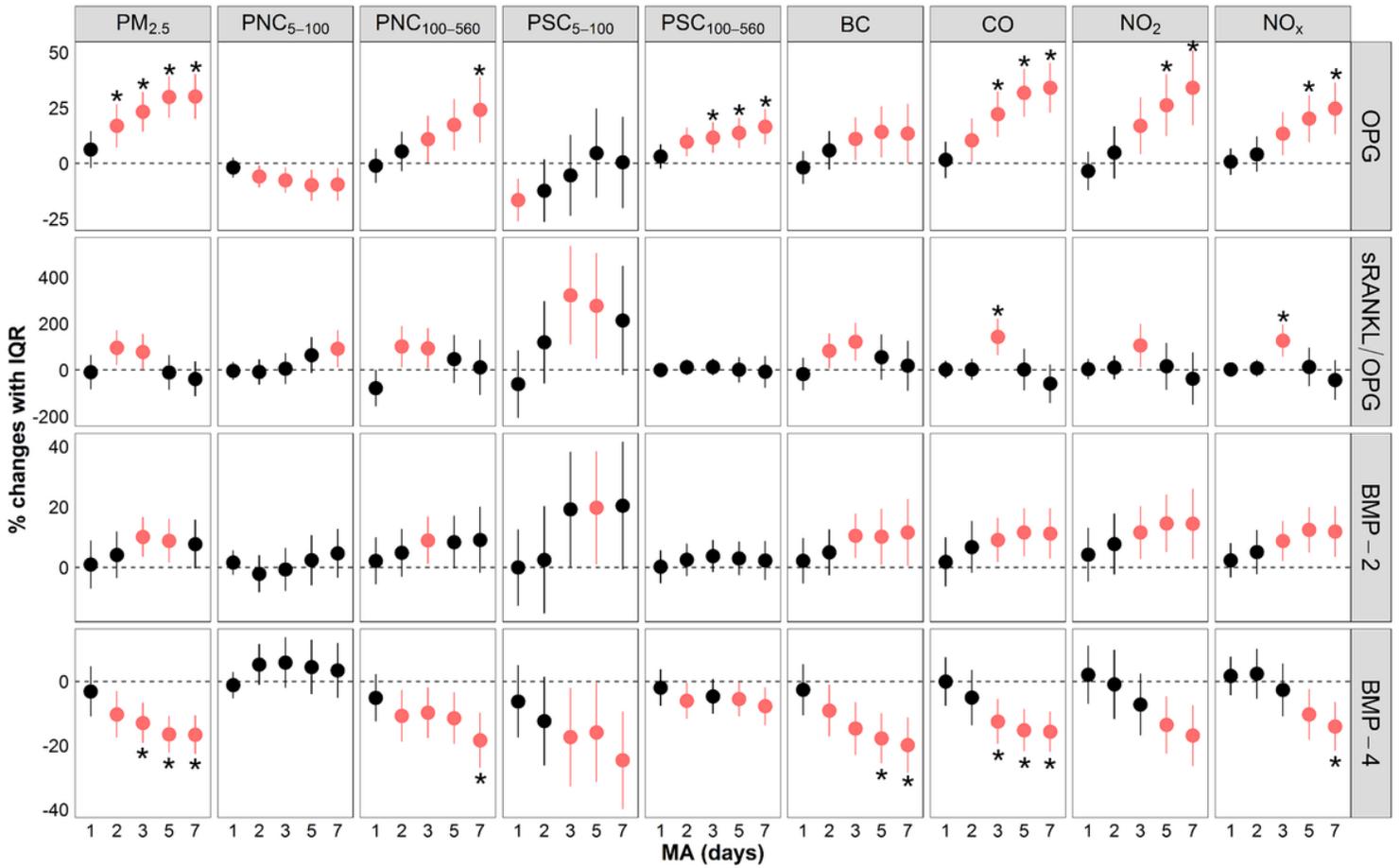


Figure 1

Percent changes in biomarkers of the bone-vascular axis associated with IQR increases in pollutant concentrations. Error bars indicate 95% confidence intervals. Significant associations (p-value < 0.05) are shown in red; Bonferroni corrections with significance (p-value < 0.0025) are indicated by asterisks. Moving average concentrations of air pollutants over the last 24 hours prior to each participant's clinic visit are presented as 1 MA day, 1 to 2 days as 2 MA days, 1 to 3 days as 3 MA days and up to 7 MA days. Abbreviations: IQR, interquartile range; MA, moving average; PM_{2.5}, particulate matter in diameter < 2.5 μm; PNC_X, number concentration of particulate in given size ranges (nm); PSC_X, surface area concentration of particulate in given size ranges (nm); BC, black carbon; CO, carbon monoxide; NO₂, nitrogen dioxide; NO_x, oxides of nitrogen; sRANKL, soluble receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; BMP, bone morphogenetic protein.

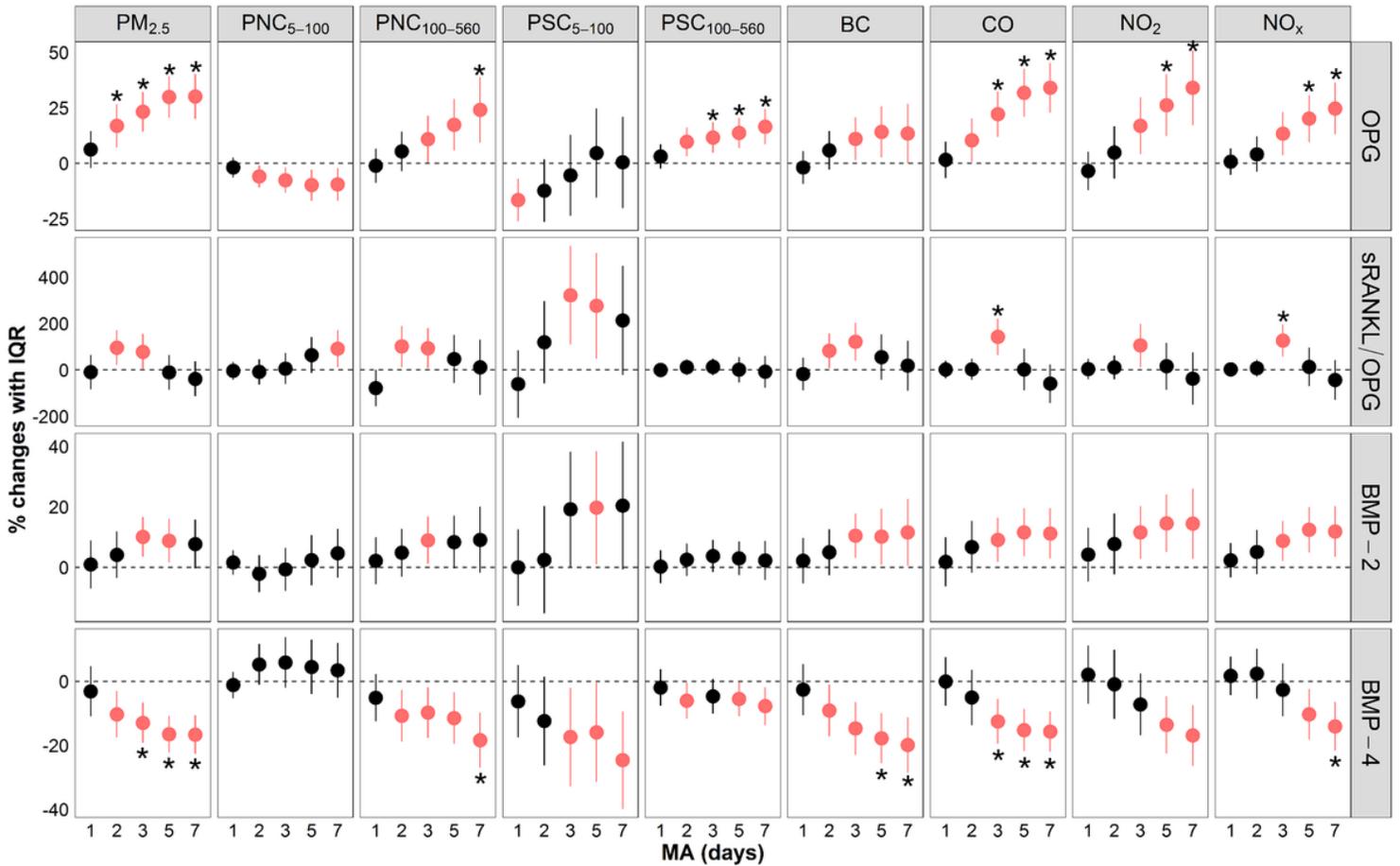


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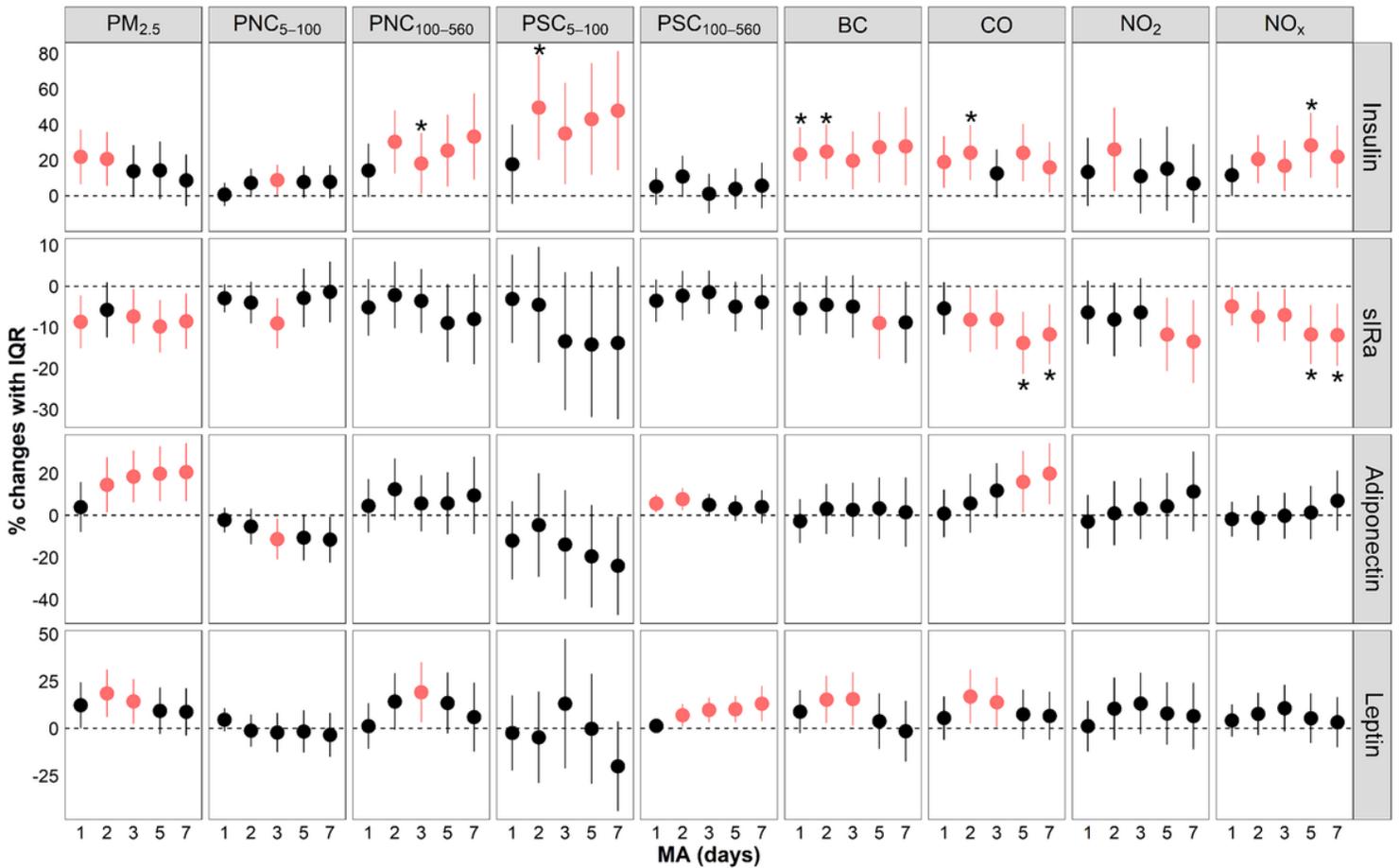


Figure 2

Percent changes in biomarkers of insulin resistance associated with IQR increases in pollutant concentrations. Error bars indicate 95% confidence intervals. Significant associations (p-value < 0.05) are shown in red; Bonferroni corrections with significance (p-value < 0.0025) are indicated by asterisks. Moving average concentrations of air pollutants over the last 24 hours prior to each participant's clinic visit are presented as 1 MA day, 1 to 2 days as 2 MA days, 1 to 3 days as 3 MA days and up to 7 MA days. Abbreviations: IQR, interquartile range; MA, moving average; PM_{2.5}, particulate matter in diameter < 2.5 μm; PNCX, number concentration of particulate in given size ranges (nm); PSCX, surface area concentration of particulate in given size ranges (nm); BC, black carbon; CO, carbon monoxide; NO₂, nitrogen dioxide; NO_x, oxides of nitrogen; sIRα, soluble insulin receptor ectodomain.

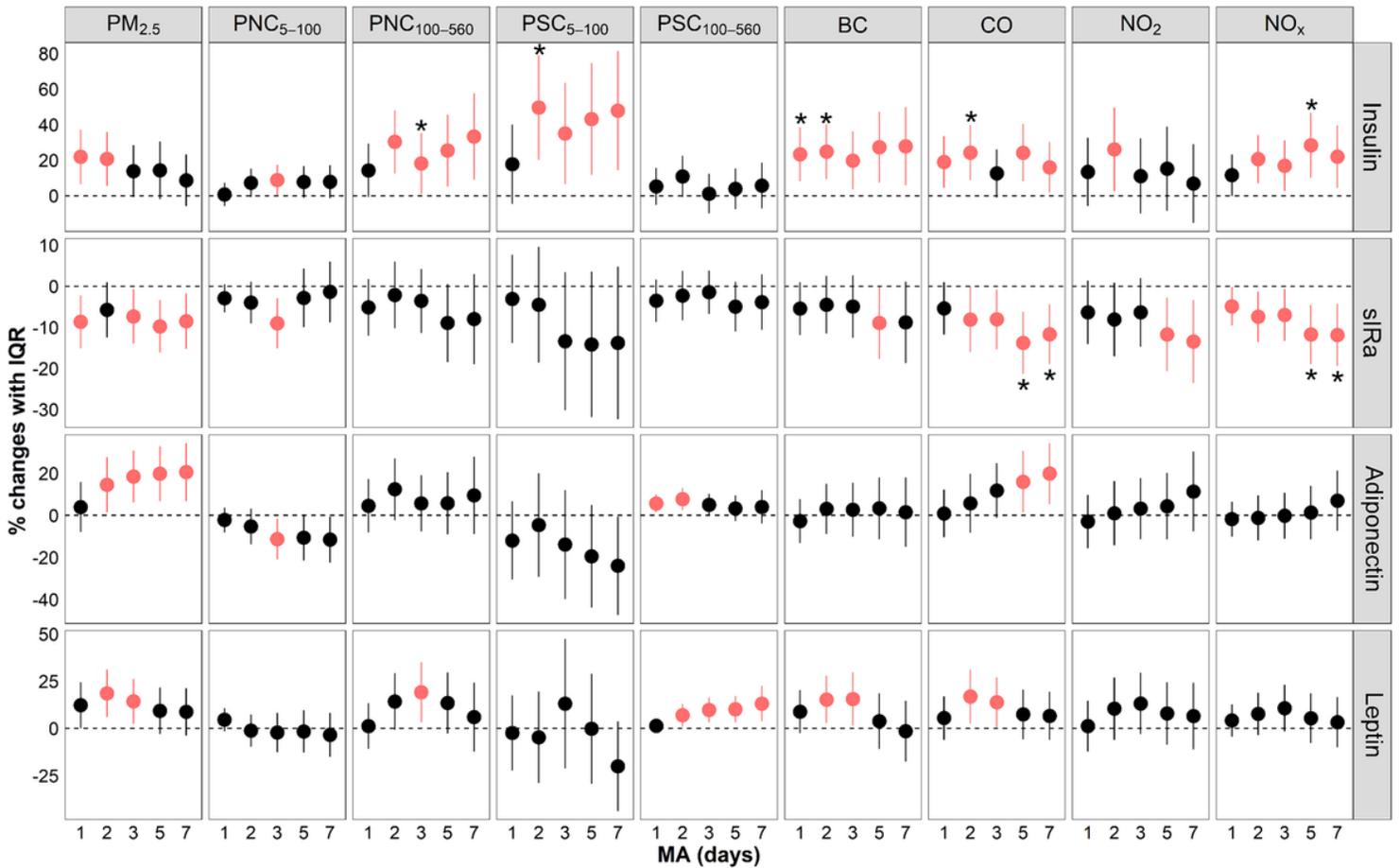


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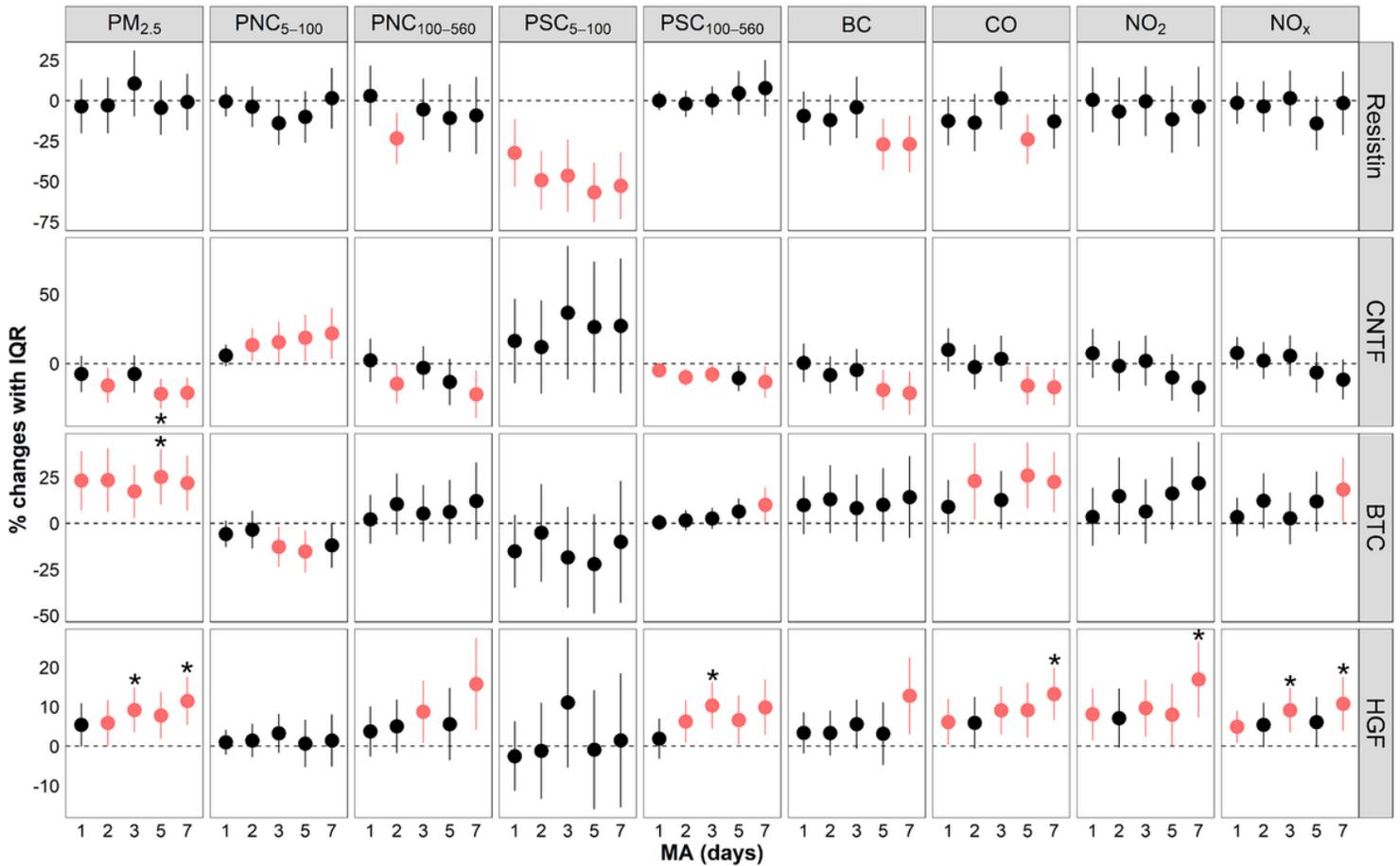


Figure 3

Percent changes in biomarkers of insulin resistance associated with IQR increases in pollutant concentrations. Error bars indicate 95% confidence intervals. Significant associations (p -value < 0.05) are shown in red; Bonferroni corrections with significance (p -value < 0.0025) are indicated by asterisks. Moving average concentrations of air pollutants over the last 24 hours prior to each participant's clinic visit are presented as 1 MA day, 1 to 2 days as 2 MA days, 1 to 3 days as 3 MA days and up to 7 MA days. Abbreviations: IQR, interquartile range; MA, moving average; PM_{2.5}, fine particulate matter less than 2.5 μm ; PNC_X, number concentration of particulate in given size ranges (nm); PSC_X, surface area concentration of particulate in given size ranges (nm); BC, black carbon; CO, carbon monoxide; NO₂, nitrogen dioxide; NO_x, oxides of nitrogen; CNTF, ciliary neurotrophic factor; HGF, hepatocyte growth factor; BTC, betacellulin.

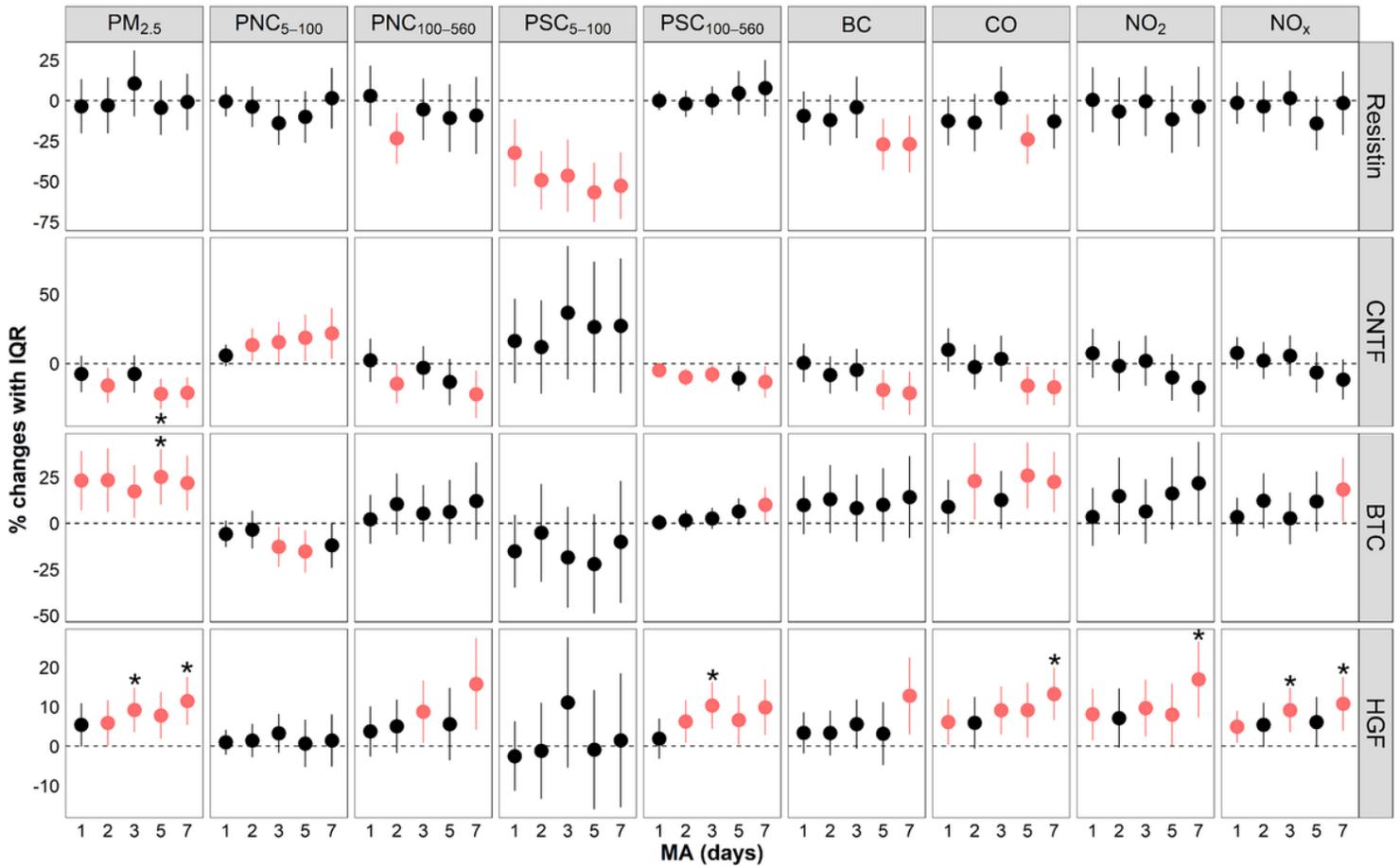


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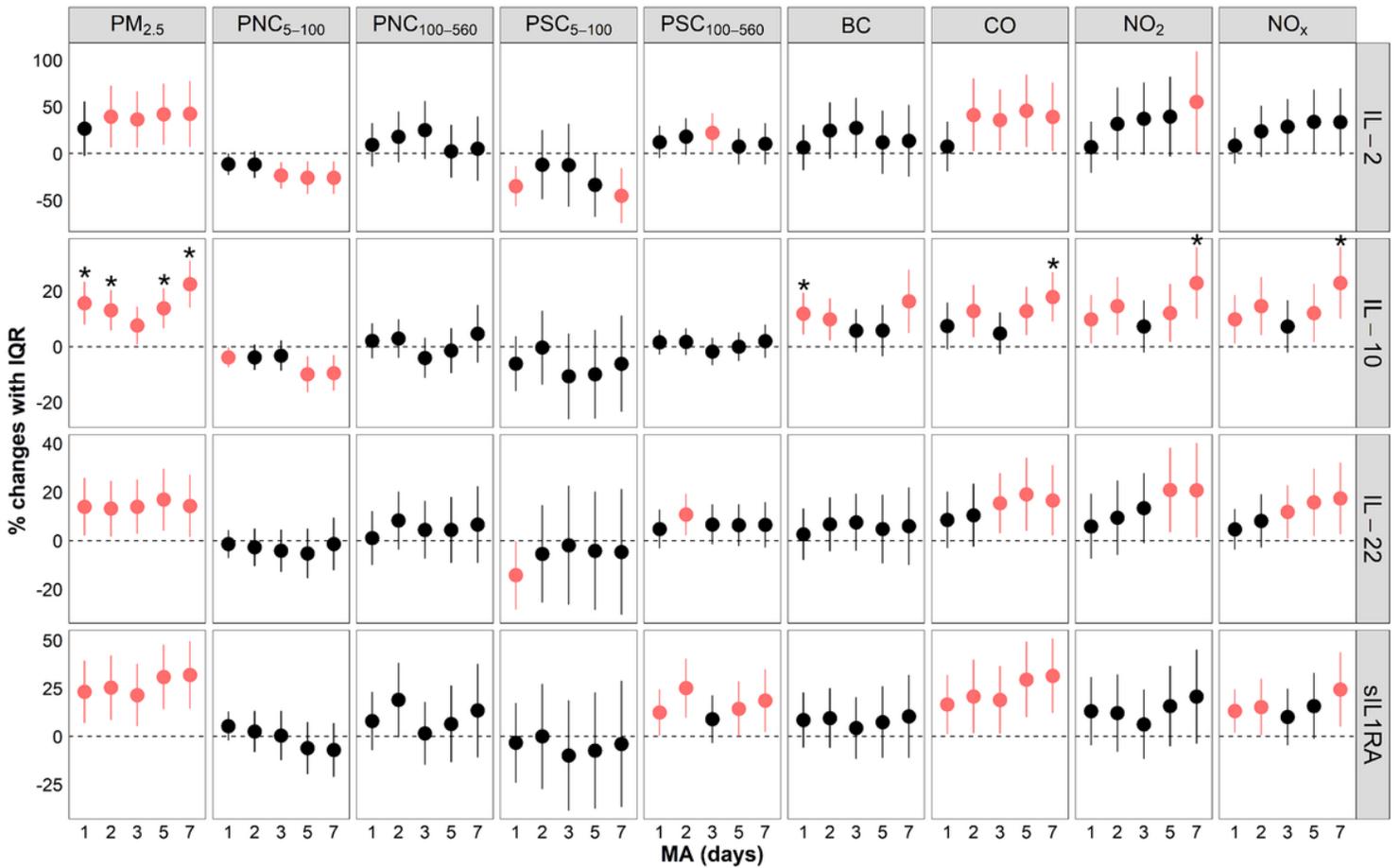


Figure 4

Percent changes in biomarkers of immune inflammation associated with IQR increases in pollutant concentrations. Error bars indicate 95% confidence intervals. Significant associations (p -value < 0.05) are shown in red; Bonferroni corrections with significance (p -value < 0.0025) are indicated by asterisks. Moving average concentrations of air pollutants over the last 24 hours prior to each participant's clinic visit are presented as 1 MA day, 1 to 2 days as 2 MA days, 1 to 3 days as 3 MA days and up to 7 MA days. Abbreviations: IQR, interquartile range; MA, moving average; PM_{2.5}, particulate matter in diameter $< 2.5 \mu\text{m}$; PNC_X, number concentration of particulate in given size ranges (nm); PSC_X, surface area concentration of particulate in given size ranges (nm); BC, black carbon; CO, carbon monoxide; NO₂, nitrogen dioxide; NO_x, oxides of nitrogen; IL, interleukin; sIL1RA, soluble IL-1 receptor antagonist.

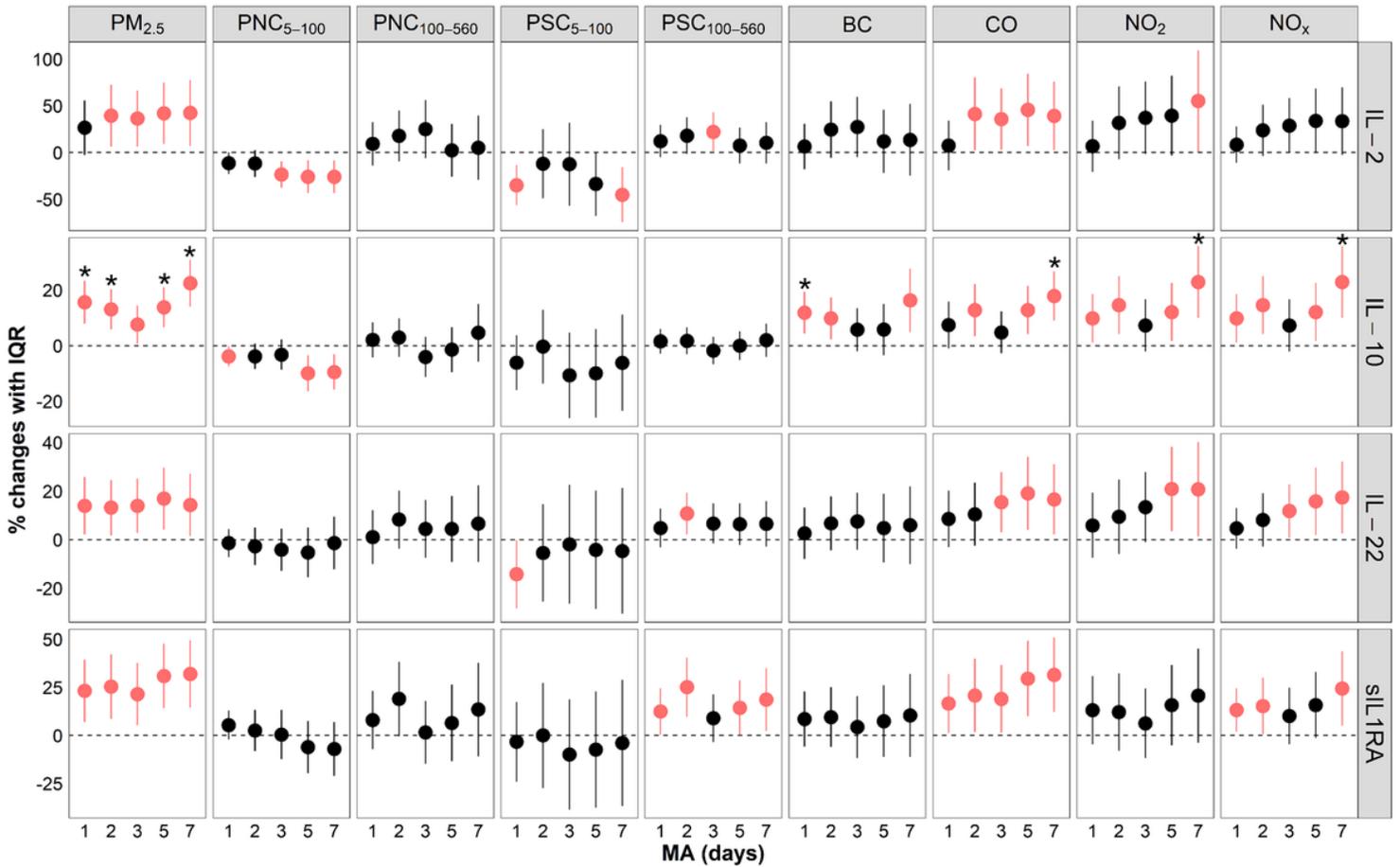


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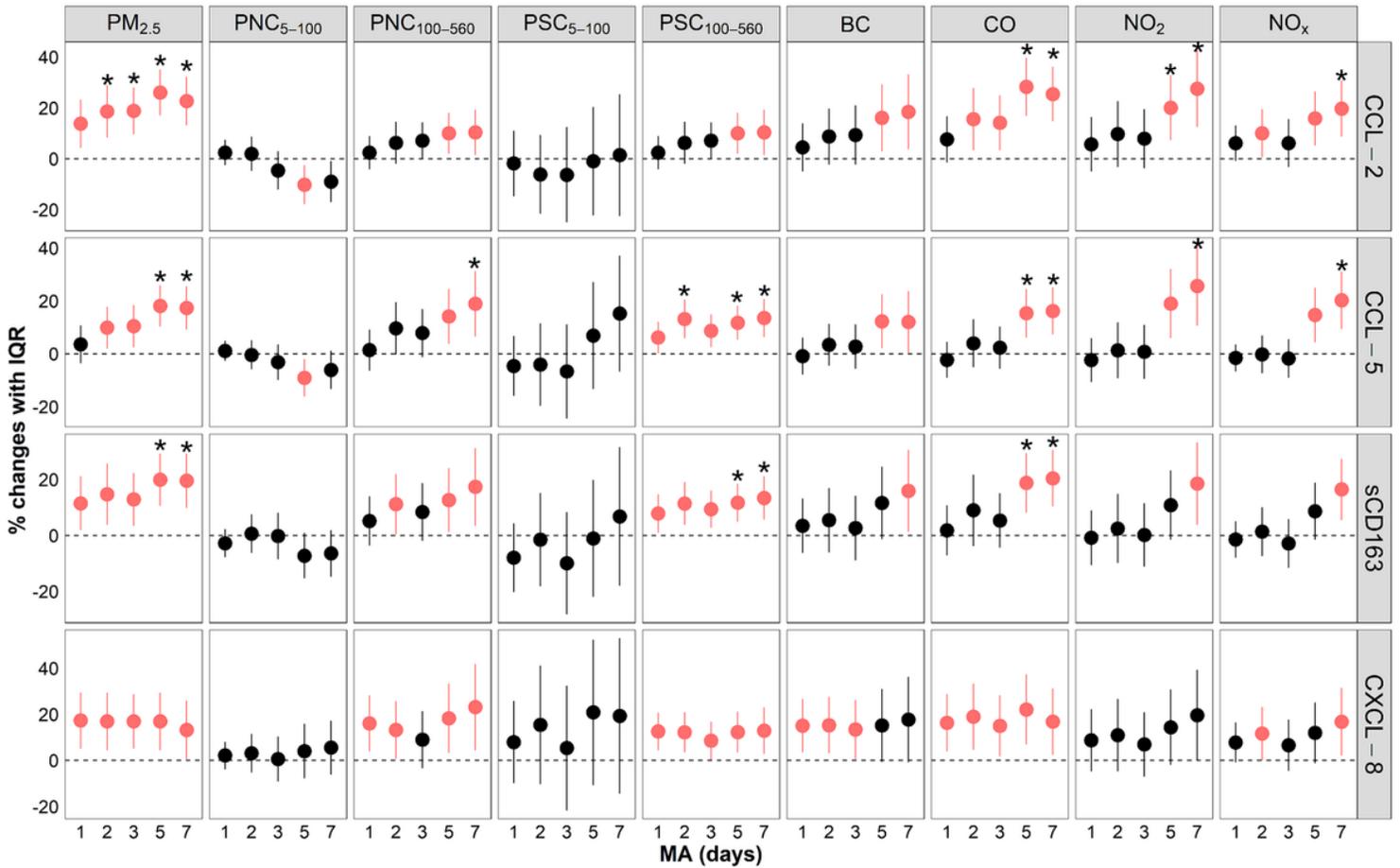


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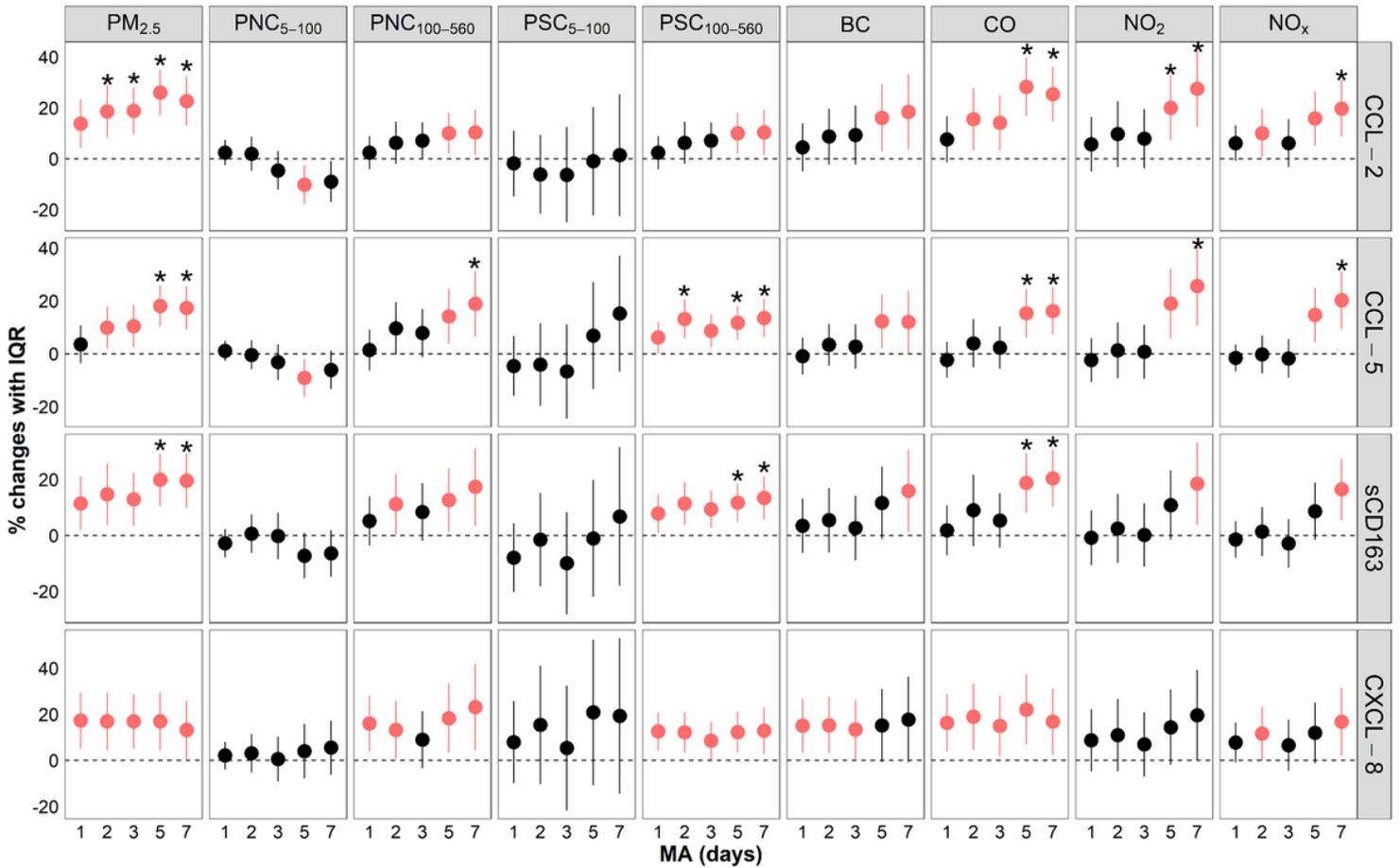


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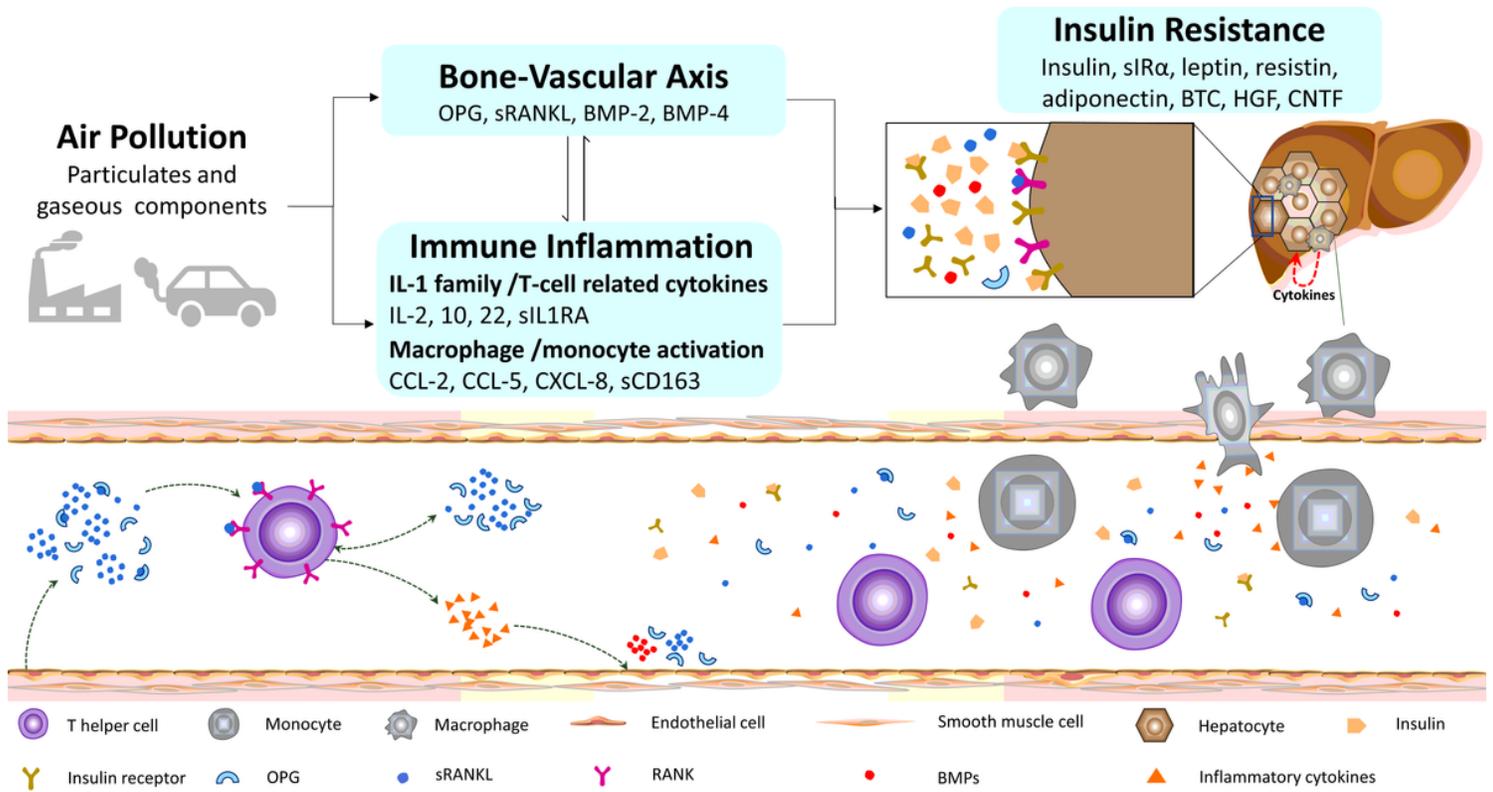


Figure 6

Schematic illustration showing the proposed mechanisms with regard to the role of bone-vascular axis on the effects of air pollution exposure on insulin resistance. Abbreviations: OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor- κ B ligand; BMP, bone morphogenetic protein; IL, interleukin; sIL1RA, soluble IL-1 receptor antagonist; CCL, C-C chemokine ligand; CXCL-8, C-X-C chemokine motif ligand-8; sCD163, soluble CD163; sIR α , soluble insulin receptor ectodomain; BTC, betacellulin; HGF, hepatocyte growth factor; CNTF, ciliary neurotrophic factor.

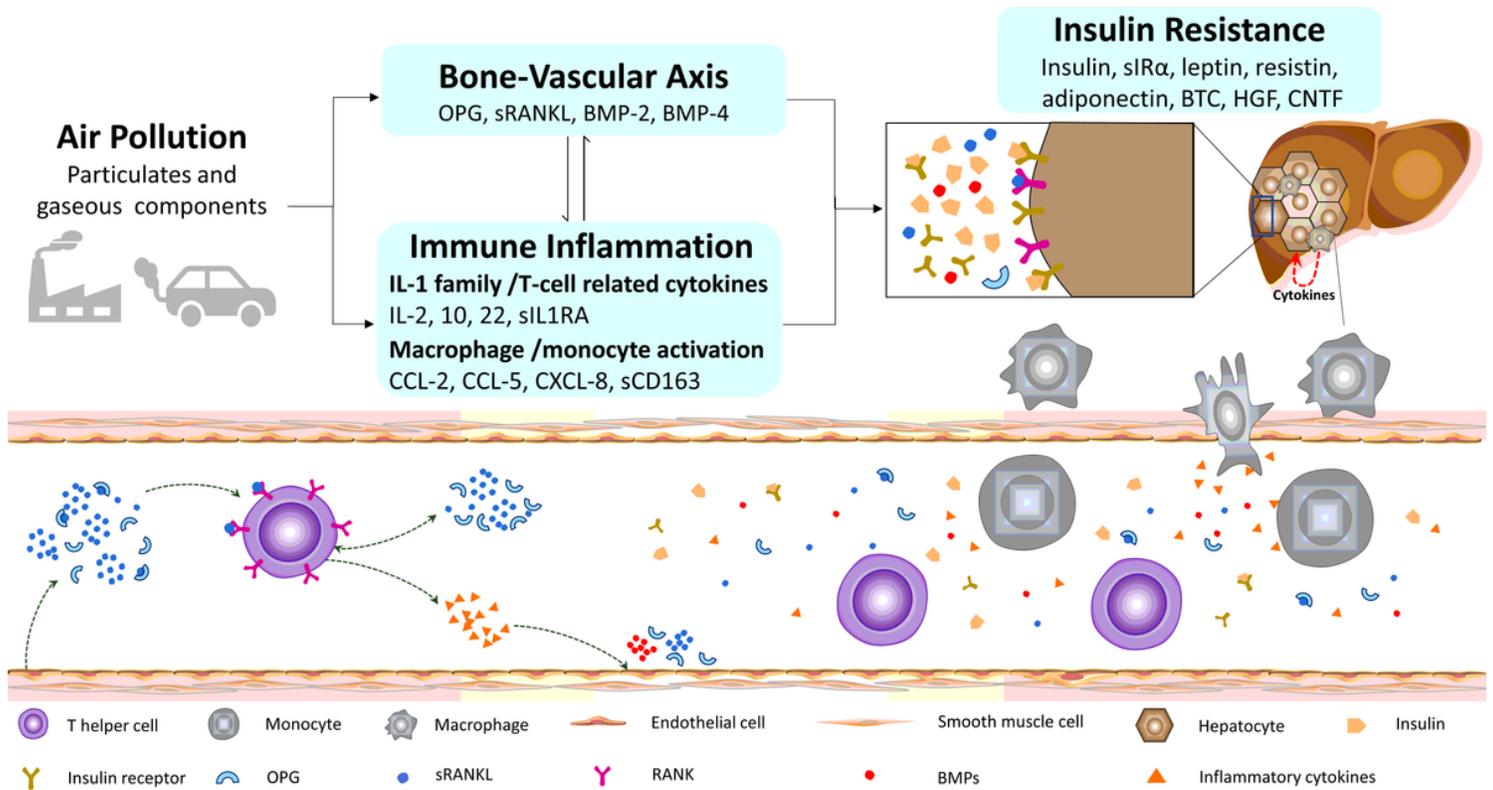


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