Aus dem Institut für Epidemiologie, Helmholtz München und der Ludwig-Maximilians-Universität München
Direktor: Prof. Dr. Annette Peters

Dissertation

zum Erwerb des Doctor of Philosophy (Ph.D.)

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

Air temperature and the risk for cardiometabolic diseases – what are the underlying physiological mechanisms?

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Zaozhuang, China

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2023

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Mit Genehmigung der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

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List of abbreviations

BMI	Body mass index
CI	Confidence interval
CO	Carbon monoxide
CVD	Cardiovascular disease
DLNM	Distributed lag non-linear models
FGF-21	Fibroblast growth factor 21
GAM	Generalized additive models
GEE	Generalized estimating equations
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-6	Interleukin-6
IL-8	Interleukin-8
IQR	Interquartile range
KORA	Cooperative Health Research in the Region of Augsburg
MCP-1	Monocyte chemotactic protein 1
NO_2	Nitrogen dioxide
O_3	Ozone
$PM_{2.5}$	Particulate matter with aerodynamic diameter smaller than 2.5µm
SO_2	Sulfur dioxide
TSP	Total suspended particulate

List of publications

This thesis consists of the following four publications:

1. **Ni W**, Wolf K, Breitner S, Zhang S, Nikolaou N, Ward-Caviness CK, Waldenberger M, Gieger C, Peters A, Schneider A. 2022. Higher Daily Air Temperature Is Associated with Shorter Leukocyte Telomere Length: KORA F3 and KORA F4. *Environmental science & technology*. doi: 10.1021/acs.est.2c04486.
2. **Ni W**, Nikolaou N, Ward-Caviness CK, Breitner S, Wolf K, Zhang S, Wilson R, Waldenberger M, Peters A, Schneider A. 2023. Associations between medium- and long-term exposure to air temperature and epigenetic age acceleration. *Environment International*. doi: 10.1016/j.envint.2023.108109.
3. **Ni W**, Breitner S, Nikolaou N, Wolf K, Zhang S, Peters A, Herder C, Schneider A. 2023. Effects of Short- And Medium-Term Exposures to Lower Air Temperature on 71 Novel Biomarkers of Subclinical Inflammation: Results from the KORA F4 Study. *Environmental science & technology*. doi: 10.1021/acs.est.3c00302.
4. **Ni W**, Schneider A, Wolf K, Zhang S, Chen K, Koenig W, Peters A, Breitner S. 2022. Short-term effects of cold spells on plasma viscosity: Results from the KORA cohort study in Augsburg, Germany. *Environmental pollution*. doi: 10.1016/j.envpol.2022.119071.

Contribution to the publications

Contribution to publication I

The first publication, entitled “Higher Daily Air Temperature Is Associated with Shorter Leukocyte Telomere Length: KORA F3 and KORA F4” explored short-term effects of air temperature on leukocyte telomere length within two independent adult cohorts. We found that higher daily air temperature was associated with shorter leucocyte telomere length. I contributed to the conceptualization, methodology, formal analysis, visualization, writing - original draft, and writing - review & editing.

Contribution to publication II

The second publication, entitled “Associations between medium- and long-term exposure to air temperature and epigenetic age acceleration” explored the medium- and long-term effects of air temperature on epigenetic age acceleration. We found that high air temperature was significantly associated with increased epigenetic age acceleration. I contributed to the conceptualization, methodology, formal analysis, visualization, writing - original draft, and writing - review & editing.

Contribution to publication III

The third publication, entitled “Effects of Short- And Medium-Term Exposures to Lower Air Temperature on 71 Novel Biomarkers of Subclinical Inflammation: Results from the KORA F4 Study” explored the short- and medium-term effects of air temperature on 71 novel biomarkers of subclinical inflammation. We found that low air temperature was significantly associated with an increase in 64 biomarkers of subclinical inflammation. I

contributed to the conceptualization, methodology, formal analysis, visualization, writing - original draft, and writing - review & editing.

Contribution to publication IV

The fourth publication, entitled “Short-term effects of cold spells on plasma viscosity: results from the KORA cohort study in Augsburg, Germany” explored the associations between short-term exposure to cold spells and plasma viscosity. We found that cold spells had significant short-term effects on increased plasma viscosity. I contributed to the methodology, formal analysis, visualization, writing - original draft, and writing - review & editing.

Introductory summary

1. Background

1.1 Air temperature and cardiometabolic disease

Climate change is a growing global public health issue of great importance. The Lancet Countdown on health and climate change report for 2022 stated that there was a 68% increase in deaths caused by heat-related factors between 2000-04 and 2017-21[1]. Studies have often indicated the presence of a J-, V-, or U-shaped association between air temperature and mortality at a city- or regional level[2-4]. Extreme high or low temperatures have shown adverse impacts on morbidity and mortality[2,3,5-8]. However, not only extreme temperatures such as heat waves or cold spells, but also changes in moderate temperature ranges are linked to temperature-associated mortality[8,9]. In addition, vulnerability to non-optimal air temperature effects seems to be influenced by factors like age or sex[3,9]. Also, people with underlying cardiometabolic diseases and other disorders may be more susceptible[2]. This suggests that patients with specific chronic diseases might benefit from potential precautions.

The burden of cardiometabolic disease has increased over recent decades in many parts of the world. For example, the prevalent cases of total cardiovascular disease (CVD) were 271 million in 1990, but by 2019 they had doubled to 523 million, and there were 18.6 million CVD deaths in 2019, up from 12.1 million in 1990[10]. More importantly, increasing evidence shows that exposure to non-optimal air temperatures (both low and high temperatures) is significantly associated with increased risks of cardiometabolic diseases, such as CVD and diabetes[2,7,9,11,12]. A time-stratified case-crossover investigation utilizing a registry of all myocardial infarction and coronary death cases throughout 28

years (from 1987 to 2014) reported that the relative risk of heat-related myocardial infarction demonstrated a significant rise from 0.93 (95% CI: 0.78 to 1.12) during 1987-2000 to 1.14 (95% CI: 1.00 to 1.29) during 2001-2014 in Augsburg, Germany[13]. Furthermore, an extensive review found significant impacts of both low and high air temperatures on increased cardiovascular disease risk[7].

1.2 Potential mechanisms of air temperature on cardiometabolic disease

While it has been noted that low and high air temperatures are associated with increased risks of cardiometabolic disease, the underlying pathophysiological mechanisms are still poorly understood. Previous studies have proposed several biological mechanism pathways that could elucidate the detrimental impacts of non-optimal air temperature on the cardiometabolic system (Figure 1)[7,14,15]. Exposure to high temperatures can cause peripheral vasodilatation and sweating, which may result in dehydration, electrolyte imbalance, sympathetic activation, and tachycardia, enhancing the probability of ischemia or plaque rupture[7,14,15]. Exposure to high temperatures may also facilitate a prothrombotic state characterized by increased blood coagulation, reduced plasma volume from excessive sweating, and elevated levels of circulating red and white blood cells and platelets[7,14,15]. Furthermore, exposure to high temperatures can trigger the secretion of interleukins such as interleukin-1 (IL-1), which can regulate inflammatory responses and consequently cause endothelial cell harm and exaggerated activation[7,15]. Conversely, exposure to low temperatures can trigger sympathetic activation, peripheral vasoconstriction, and increased muscle tone, leading to a rise in blood pressure and heart rate, and haemoconcentration[7,14,15]. Exposure to low temperatures can also be a contributory factor in the deposition of cholesterol crystals in atherosclerotic plaques[7]. Moreover, exposure to low temperatures may activate platelets and stimulate increased

concentrations of inflammatory markers such as fibrinogen, which may explain the delayed reaction to cold events[14,15].

Recently, a set of eight hallmarks of environmental insults, such as oxidative stress and inflammation, epigenetic alterations, and mitochondrial dysfunction, has been put forward to explain the cellular and molecular processes connecting environmental exposures to chronic diseases such as cardiovascular and metabolic diseases[16]. This assortment of hallmarks provides an organizational framework to comprehend why multifaceted combinations of environmental exposures can bring about serious health repercussions. The present framework proposes that epigenetics serves as a key indicator of environmental insults, and substantial evidence confirms that environmental exposures elicit modifications in gene regulation by means of changes in DNA methylation and histone modifications and stimulate age-related epigenetic modifications, such as the hastening of epigenetic age[16]. Furthermore, mitochondria are essential subcellular organelles for cellular energy production, and are of great importance in the cellular response to environmental stressors[16]. A direct association between telomeres and mitochondria has been identified, which has resulted in the telomere-mitochondrial aging hypothesis[17]. Additionally, the significant impact of environmental exposures on the telomere-mitochondrial aging axis has been demonstrated[16,17]. Therefore, this suggests that epigenetic and telomere processes might serve as a possible novel mechanism, or act as a novel biosensor, between non-optimal temperature exposure and cardiometabolic disease.

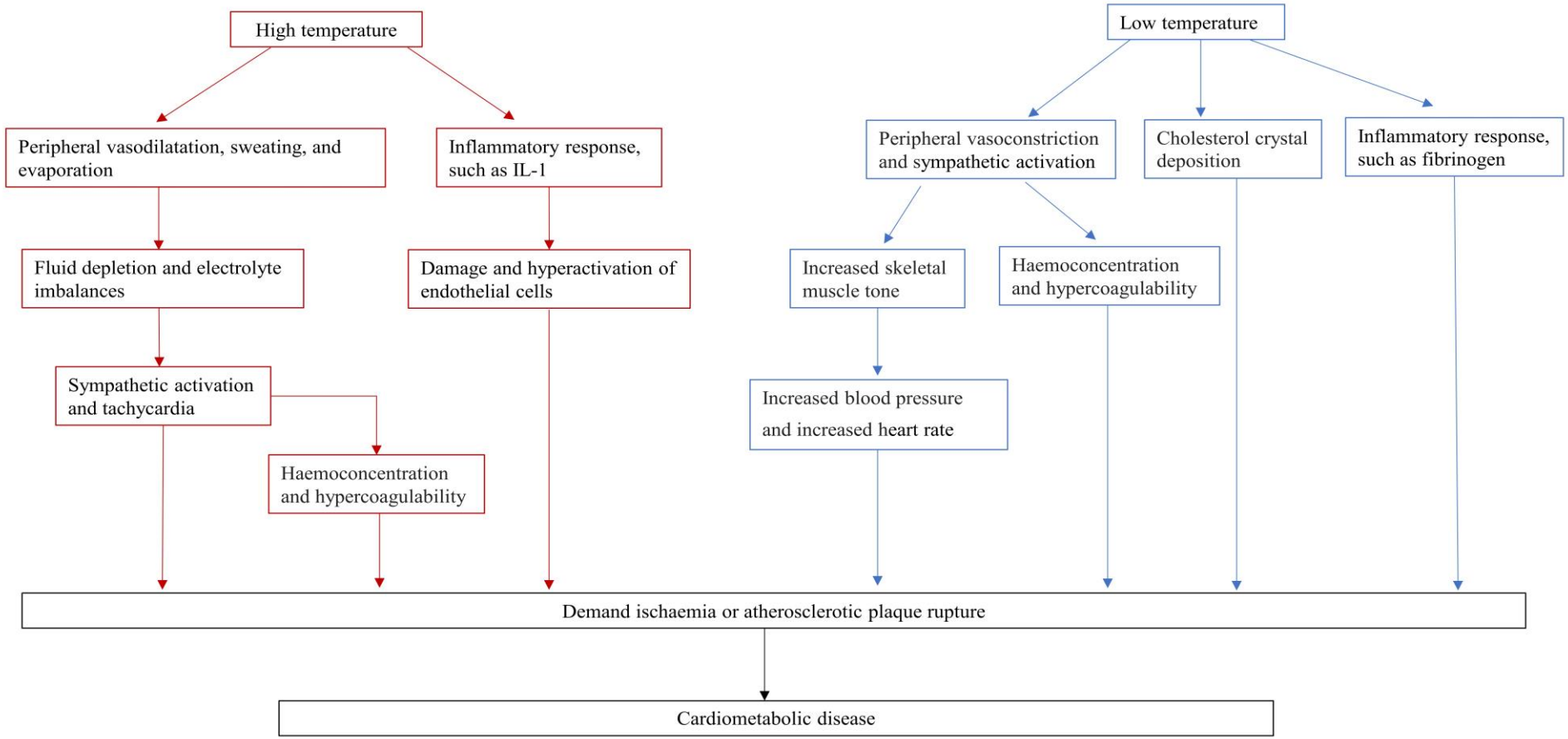

Figure 1. Pathophysiological mechanisms of air temperature-mediated cardiometabolic diseases. Adapted from “Climate change and cardiovascular disease: implications for global health” (Khraishah, H., Alahmad, B., Ostergard, R.L. et al. 2022, Nat Rev Cardiol 19, 798–812).

Therefore, examining the impacts of air temperature on leucocyte telomere length (section 1.2.1), epigenetic age acceleration (section 1.2.2), biomarkers of subclinical inflammation (section 1.2.3), and plasma viscosity (section 1.2.4) can facilitate the comprehension of the biological mechanisms of cardiometabolic diseases associated with exposure to non-optimal air temperature. Moreover, this can aid in recognizing key pathways and potential intervention targets for ameliorating the detrimental health impacts of non-optimal air temperature exposures.

1.2.1 Leucocyte telomere length

Telomeres perform a critical function in cell division, and their length diminishes after each cycle of division[18]. Telomere shortening has been studied as a possible indicator for biological aging and is linked to a range of age-associated disorders, including CVD[19-22]. In addition, evidence indicates that air temperature or heat stress could be associated with increased inflammatory biomarkers and oxidative stress levels[23-26]. Since inflammation and oxidative stress are known to accelerate telomere shortening, this implies that air temperature might have an effect on telomere length[27,28]. A birth study found an association between exposure to non-optimal air temperature in the prenatal period and shorter telomere length in cord blood[29]. However, to date, no research has been done to determine the link between air temperature and leukocyte telomere length in adults.

1.2.2 Epigenetic age acceleration

Epigenetic age predictors, which rely on methylation status of distinct CpG sites across the genome and use regression models to estimate biological age, have been proposed as novel, reliable biomarkers for the aging process in humans[30,31]. The deviation of epigenetic age from chronological age is termed epigenetic age acceleration. Studies have demonstrated that epigenetic age acceleration is linked to an augmented risk of chronic

conditions, such as CVD, or diabetes, as well as a higher risk of mortality[32-35]. Also, it has been noted by past studies that environmental factors and lifestyle impact epigenetic age acceleration[35]. Thus, it appears that epigenetic age acceleration may capture disruptions in biological activities brought on by environmental factors, leading to undesirable health effects. However, no prior research has yet explored the impact of air temperature on epigenetic age acceleration.

1.2.3 Biomarkers of subclinical inflammation

Increasing evidence demonstrated the intricate and multifaceted role of inflammation in developing chronic diseases such as CVD and type 2 diabetes[36,37]. Nevertheless, the associations between air temperature and biomarkers of inflammation still need to be fully comprehended. Previous studies found significant effects of lower air temperature on increased levels of biomarkers of inflammation[38-44]. However, these studies were restricted to only a few biomarkers, specifically selected demographic subgroups, or used an experimental or panel study design with a limited number of individuals. Gaining further understanding on how air temperature can lead to detrimental health consequences through systemic inflammatory pathways is of great significance. Due to the development of proteomic technologies, identifying a variety of inflammatory markers has become more accessible, however, to date, there has been no high-dimensional analysis conducted to explore the link between air temperature and these markers.

1.2.4 Plasma viscosity

Plasma viscosity can affect the development and occurrence of atherosclerosis and ischemic heart disease[45-51]. Elevated plasma viscosity can cause platelet aggregation by increasing the shear force of blood vessel walls[52]. Studies have suggested that high plasma or blood viscosity are linked to an increased risk for cardiovascular events[48,51,53,54]. Our

group has previously shown that plasma viscosity was elevated during a rigorous air pollution event[55]. Nonetheless, until this point, the impacts of air temperature and plasma viscosity had not been investigated more deeply. Previous studies have suggested significant effects of air temperature or seasonal variations on plasma viscosity levels, but the results have been inconsistent due to limitations in sample size, study design, or study population[56-62]. Further investigation is needed to clarify the link between air temperature and plasma viscosity.

2. Aims and hypotheses of the dissertation

The objective of this dissertation is to investigate the underlying physiological mechanisms behind the observed impact of air temperatures on cardiometabolic diseases by assessing biomarkers of leucocyte telomere length, epigenetic age acceleration, subclinical inflammation, and plasma viscosity with data from the Cooperative Health Research in the Region of Augsburg (KORA) cohort. Based on literature search and biological plausibility, we hypothesized:

(1) Shorter leukocyte telomere length in association with non-optimal daily air temperature exposure.

(2) Epigenetic age acceleration in association with exposure to non-optimal medium- and long-term air temperature.

(3) Short- and medium-term effects of non-optimal air temperature on biomarkers of subclinical inflammation.

(4) Increased plasma viscosity in association with low air temperature and/or cold spells.

3. Methods

3.1 Study population

These analyses were based on the KORA study, a population-based cohort that involved cluster-random sampling of German citizens between the ages of 25 and 74 in Augsburg, Germany[63]. In order to gather information about demographics, lifestyle, socioeconomic status, medical history, medication, as well as physiological parameters, participants completed self-administered questionnaires, computer-assisted personal interviews, physical examinations, and underwent a collection of biological samples. Blood samples were taken with minimal stasis and stored at temperatures ranging from 4-8°C. They were then transported in refrigerated packaging to the laboratory of Augsburg Central Hospital within 2-4 hours and preserved at -80°C.

Figure 2 presents an overview of the KORA baseline surveys (S) and follow-up examinations (F). Four independent cross-sectional surveys (KORA S1 - KORA S4) were conducted at five-year intervals (1984-2001). A follow-up examination of S3 (KORA F3) was performed in 2004-2005. In addition, three follow-up examinations of S4 (KORA F4, KORA FF4, and KORA FFF4) were performed in 2006-2008, 2013-2014, and 2021-2022, respectively.

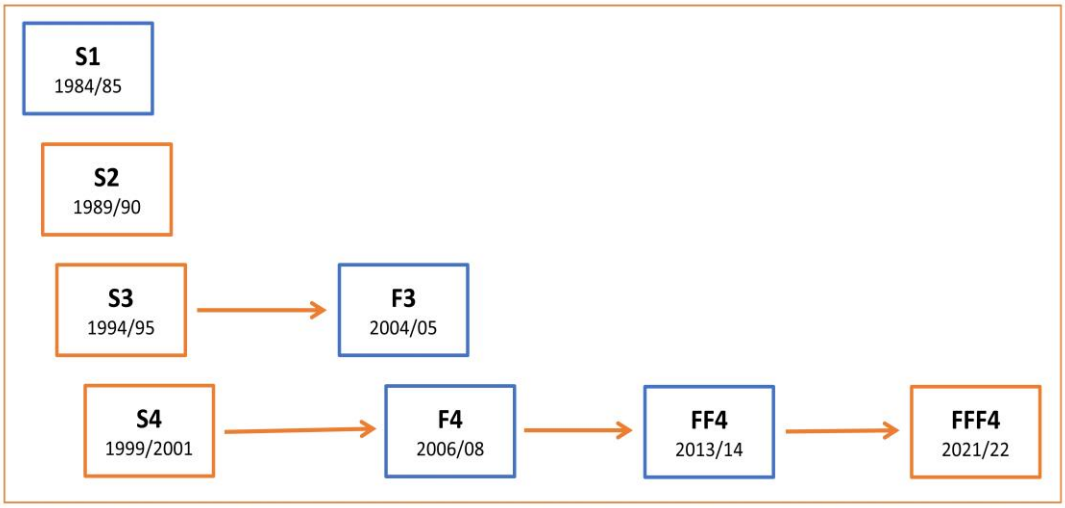

Figure 2. Overview of KORA baseline surveys (S) and follow-up examinations (F).
Note: The studies used in this thesis are denoted by blue boxes.

Table 1 presents a summary of the KORA cohorts and participants included in the analyses. The analyses of the impacts of air temperature/cold spells on leukocyte telomere length, epigenetic age acceleration, biomarkers of subclinical inflammation, and plasma viscosity were conducted using data from KORA F3 (2,865 participants) and F4 studies (2,999 participants), KORA F4 (1,725 participants) and FF4 studies (1,877 participants), KORA F4 study (1,115 participants), and KORA S1 study (3,622 participants), respectively.

Table 1. Summary of KORA cohorts and participants included in the analyses

Analyses	KORA cohorts	Participants (N)
Impact of air temperature on leukocyte telomere length	F3 and F4	2,865 in F3 and 2,999 in F4
Impact of air temperature on epigenetic age acceleration	F4 and FF4	1,725 in F4 and 1,877 in FF4
Impact of air temperature on biomarkers of subclinical inflammation	F4	1,115
Impact of cold spells on plasma viscosity	S1	3,622

3.2 Exposure assessment

3.2.1 Multi-stage regression-based model for air temperature

A multi-stage regression-based modeling approach was employed to compute the daily mean, minimum, and maximum air temperature with a high resolution of 1km × 1 km for the years 2000 to 2020[64]. Multiple data sources, including satellite-based land surface temperature data, ground-based air temperature measurements, and spatial predictors

datasets, were incorporated into the modeling process. The three-stage models were trained to accomplish predictions of air temperature with comprehensive coverage in terms of time and space. The analyses of the impacts of mean air temperature on leukocyte telomere length, epigenetic age acceleration, and biomarkers of subclinical inflammation were conducted using this exposure dataset.

3.2.2 Air temperature monitoring station in Augsburg

Assessment of air temperature was also conducted at a monitoring station in Augsburg, Germany that monitors urban background and is managed by the Bavarian State Office for the Environment[13]. The location for monitoring was situated to the south of the urban center, with a distance of 7 km. Daily 24-hour average air temperature values were assessed by obtaining a minimum of 75% of the hourly measurements.

The impacts of cold spells on plasma viscosity were analyzed using this exposure dataset. The criteria for *cold spells* were two or more continuous days with daily air temperature beneath the third, fifth or tenth percentile of the temperature range during the research period, showcasing subnormal temperatures lower than the average of this area.

3.3 Outcome measurement

3.3.1 Leucocyte telomere length

A quantitative PCR-based approach was employed to evaluate the leucocyte telomere length, which was shown in the form of the proportion of telomere repeat copy number to that of a single-copy gene (T/S ratio)[65,66]. The T/S ratio was assessed with reference to a standard DNA applied in each identification (genomic DNA from the K562 cell line) to guarantee a standardized measurement across PCR plates.

3.3.2 Epigenetic age acceleration

The calculation of epigenetic age involved the use of the online New DNA Methylation Age Calculator (https://dnamage.genetics.ucla.edu/new), with normalized DNA methylation data. Epigenetic age acceleration pertains to the dissimilarity between chronological age and epigenetic age biomarkers, and five biomarkers of epigenetic age acceleration were identified: Horvath's epigenetic age acceleration (HorvathAA), Hannum's epigenetic age acceleration (HannumAA), PhenoAge acceleration (PhenoAA), GrimAge acceleration (GrimAA), and Epigenetic Skin and Blood Age acceleration (SkinBloodAA).

3.3.3 Biomarkers of subclinical inflammation

This study applied the OLINK Inflammation multiplex immunoassay (OLINK Proteomics, Uppsala, Sweden) to measure ninety-two inflammation-related protein biomarkers from serum samples[67]. After quality control (twenty-one biomarkers were excluded because ≥ 25% of the samples being below the limit of detection or showing an interassay coefficient of variation > 20%), 71 biomarkers were included in this study.

3.3.4 Plasma viscosity

Plasma viscosity was assessed with a Coulter-Harkness capillary viscometer (Coulter Electronics, Luton, UK) at 37℃, and the experiment was replicated thrice. The process of measuring plasma viscosity is simple, fast, and reliable, and it demonstrates low intra-individual variability[49].

3.4 Statistical method

3.4.1 Short-term effects of air temperature on leukocyte telomere length

We explored the relationships between short-term exposure to air temperature and leukocyte telomere length at lags of 0-1, 2-6, 0-6, and 0-13-days using cohort-specific generalized additive models (GAM). Air temperature was included as a linear term in the model since no exposure-response functions showed significant deviations from linearity. We controlled for age, sex, body mass index (BMI), education, alcohol consumption, day of the week, smoking status, physical activity, season, time trend, and relative humidity. A fixed-effects meta-analysis approach was subsequently employed to combine the results from the individual cohorts.

3.4.2 Medium- and long-term effects of air temperature on epigenetic age acceleration

Generalized estimating equations (GEE) with distributed lag non-linear models (DLNM) and GEE were applied to examine the associations between medium- and long-term exposure to air temperature and epigenetic age acceleration, respectively. Four-week and eight-week moving averages of daily air temperatures prior to blood draw were applied as a measure of medium-term exposure to air temperature. Additionally, the 365-day moving average of daily air temperatures (annual average temperature) prior to blood draw was applied as a measure of long-term exposure to air temperature. According to the exposure-response relationship functions, we included the medium-term exposure parameters non-linearly in the GEE with DLNM models. Annual average temperature was included linearly in the GEE models. In the GEE with DLNM models, median temperature was taken as the reference value. The high temperature effect was determined as the 97.5^{th} percentile of the air temperature distribution with respect to the median temperature, and the low temperature effect was determined as the 2.5^{th} percentile of the

air temperature distribution with respect to the median temperature. We adjusted for age, education, alcohol consumption, physical activity, sex, smoking status, BMI, estimated cell types, batch effect, and season at blood draw. We additionally controlled for time trend, relative humidity, and day of the week when assessing the medium-term effects of temperature.

3.4.3 Short- and medium-term effects of air temperature on biomarkers of subclinical inflammation

We applied GAM to examine the relationship between short- and medium-term exposure to air temperature and subclinical inflammation biomarkers at cumulative lags of 0-1 (2-day average), 0-6 (7-day average), and 0-13 (14-day average) days for short-term effects and at lags of 0-27 and 0-55 days for medium-term effects. Air temperature was included as a linear term in the model since none of the exposure-response functions showed significant deviations from linearity. We adjusted for sex, age, physical activity, height, waist circumference, alcohol consumption, education, systolic blood pressure, diastolic blood pressure, albumin, hematocrit, smoking status, relative humidity, season at blood draw, time trend, and day of the week.

3.4.4 Short-term effects of cold spells on plasma viscosity

We applied GAM with DLNM to investigate the short-term impacts of cold spells on plasma viscosity at lags of 0-1, 0-6, 0-13, 0-20, and 0-27 day. We controlled for sex, age, physical activity, smoking status, day of the week, BMI, education, season at blood draw, alcohol consumption, sulfur dioxide (SO_2), carbon monoxide (CO), total suspended particulate (TSP), time trend, barometric pressure, and relative humidity.

The **effect modification** on associations between air temperature/cold spell and leucocyte telomere length, epigenetic age acceleration, biomarkers of subclinical inflammation, and plasma viscosity by sociodemographic, lifestyles, and health conditions were explored.

We conducted a number of **sensitivity analyses** to evaluate how robust the results are, e.g., additionally controlling for air pollutants or using minimum and maximum air temperature as alternative exposure metrics.

4. Results

4.1 Short-term effects of air temperature on leukocyte telomere length (hypothesis 1)

An interquartile range (IQR) increment in air temperature (10.77°C, 10.11°C, 10.15°C, and 9.54°C, respectively) was linked with shorter leucocyte telomere length at lags of 0-1, 2-6, 0-6 and 0-13 days (percent change [95% CI]: -2.96 [-4.46 to -1.43], -2.79 [-4.49 to -1.07], -4.18 [-6.08 to -2.25], and -6.69 [-9.04 to -4.27], respectively). In general, we did not find significant effect modification. Furthermore, the results were robust across a range of sensitivity analyses.

4.2 Medium- and long-term effects of air temperature on epigenetic age acceleration (hypothesis 2)

We observed significant links between medium-term exposure to high air temperature and increased HorvathAA, HannumAA, GrimAA, and SkinBloodAA. At the same time, we did not find significant links between medium-term exposure to low air temperature and epigenetic age acceleration. For long-term exposure to air temperature, an increase in the annual average temperature at residence was found to be linked to an increase in HorvathAA, HannumAA, PhenoAA, GrimAA, and SkinBloodAA. Furthermore, we

observed that the annual average temperature had a more profound effect on women and on participants with obesity or diabetes, compared to men and participants without obesity or diabetes. In general, the results proofed to be robust through a series of sensitivity analyses.

4.3 Short- and medium-term effects of air temperature on biomarkers of subclinical inflammation (hypothesis 3)

Exposure to lower air temperatures, both short- and medium-term, showed an increase in 64 biomarkers of subclinical inflammation. Of these, 40 biomarkers were significantly associated with short-term exposure, and 60 biomarkers with medium-term exposure. Moreover, elderly participants, those with cardiovascular disease or prediabetes/diabetes or exposed to higher air pollution levels (particulate matter with aerodynamic diameter smaller than 2.5μm [$PM_{2.5}$], nitrogen dioxide [NO_2], and ozone [O_3]) demonstrated stronger associations between lower air temperature exposure and higher biomarker levels. Finally, a number of sensitivity analyses demonstrated the robustness of the findings.

4.4 Short-term effects of cold spells on plasma viscosity (hypothesis 4)

Cold spells (air temperature beneath the third, fifth or tenth percentile) demonstrated a link with higher plasma viscosity levels at lags of 0 to 1 days (percent change [95% CI]: 1.35 [0.06 to 2.68], 1.35 [0.06 to 2.68], and 2.49 [0.34 to 4.69], respectively), and lags of 0 to 27 days (percent change [95% CI]: 18.81 [8.97 to 29.54], 17.85 [8.29 to 28.25], and 7.41 [3.35 to 11.00], respectively). In addition, there were significant associations between cold spells (air temperature beneath the third or tenth percentile) and higher plasma viscosity at lags of 0 to 20 days (percent change [95% CI]: 8.34 [0.43 to 16.88], and 4.96 [1.68 to 8.35], respectively). Our study did not reveal a noteworthy effect

modification. In general, the results were robust as investigated by a series of sensitivity analyses.

5. Discussion

5.1 Summary of key findings

Research on the effects of air temperature on various biological indicators has been ongoing for several years. In this context, this doctoral thesis contributes to this endeavor by providing new insights into the short-term effects of elevated air temperature on leukocyte telomere length (section 5.2) and the medium- and long-term effects on epigenetic age acceleration (section 5.3). Additionally, we identified multiple novel biomarkers of subclinical inflammation (section 5.4) and plasma viscosity (section 5.5) that are associated with exposure to lower air temperature/cold spells. These findings suggest that age-related epigenetic alterations and the mitochondrial-telomere aging axis may represent potential novel pathophysiological reactions of the cardiometabolic system in response to exposure to high air temperature. Conversely, inflammatory response and high plasma viscosity may act as intermediaries for cardiometabolic diseases associated with exposure to low air temperature.

5.2 Short-term effects of air temperature on leukocyte telomere length

The present study was the first to reveal a significant relationship between short-term exposure to elevated air temperature and shorter leucocyte telomere length among a population of adults. Only a single birth study has so far shown that exposures to temperature extremes, both high and low, during the prenatal period were linked to shortened telomere length in cord blood[29]. Our findings showed that air temperature had an immediate effect on leucocyte telomere length, as well as delayed effects at lags of 0

to 1 days, 2 to 6 days, 0 to 6 days, and 0 to 13 days. Additionally, the lagged effects were more prominent than the immediate effects.

The evaluation of leucocyte telomere length is being more widely acknowledged as a clinical marker to gauge the risk associated with age-related health conditions[68]. Our investigation showed that exposure to high air temperatures may contribute to an acceleration of the telomere shortening process. This discovery lends further credence to the notion that climate change and heightened temperatures can have a detrimental impact on human health. Importantly, our results point to telomere length partly mediating the often observed relationships between high daily air temperature and age-related diseases, such as CVD. Furthermore, since telomeres are directly connected to mitochondria, and telomere damage leads to mitochondrial biosynthesis reprogramming and mitochondrial dysfunctions[16,17,69], the mitochondrial-telomere aging axis could be a plausible mechanistic link between high temperature exposures and age-related diseases. In view of these discoveries, it is essential to enact public policies that would curtail the pace of global warming and forestall heat-triggered health hazards. To a certain degree, such measures may have the effect of extending lifespan and mitigating age-related illnesses.

It is still unclear what explicitly causes air temperature to influence telomere length in leucocytes. Telomeres can be shortened as a result of oxidative stress and inflammation[27,28]. Moreover, it has been demonstrated that exposure to high air temperature or heat stress is related to higher levels of inflammation and oxidative stress[23-26]. As such, it is plausible that oxidative stress and inflammation are the pathways through which exposure to higher temperatures leads to shorter telomere length.

5.3 Medium- and long-term effects of air temperature on epigenetic age acceleration

We observed the first evidence that medium- and long-term exposure to high air temperatures is linked with increased epigenetic age acceleration. We observed that

HannumAA and SkinBloodAA were the most sensitive biomarkers to high temperature exposures. Additionally, HorvathAA, PhenoAA, and GrimAA were also affected by high temperatures.

Epigenetic age accelerations, which is a molecular marker of biological aging derived from DNA methylation, is a valuable tool for evaluating the aging process from a molecular standpoint[30,70]. On numerous occasions, studies in environmental health have reported that exposure to adverse environmental conditions accelerates the epigenetic clocks[30,71]. Furthermore, there is increasing evidence linking epigenetic age acceleration to mortality, morbidity, and a variety of clinical traits[30,34,70,72]. Our novel finding of elevated epigenetic age acceleration due to high air temperature exposure suggests that this mechanism may underlie the well-established links between heightened air temperature and increased cardiometabolic morbidity and mortality, thereby uncovering a possible new pathophysiological route in the realm of climate change and health research. Moreover, offering this novel pathophysiological pathway may be a significant stride in averting the health repercussions of heat, particularly those in vulnerable population subgroups. This is especially pertinent given the anticipated rise in the number of hot days and more intense heat waves due to climate change.

Our study further discovered that medium-term exposure to high air temperatures (four- and eight-week averages) was related to an augmented epigenetic age acceleration, which may not be permanent but could serve as an intermediary between high air temperature exposures and cardiovascular and cerebrovascular diseases[73]. Moreover, our findings of significant relationships between long-term exposure to air temperature and all five epigenetic age acceleration metrics suggest that the annual average temperature exerts a powerful influence on epigenetic age acceleration. Significantly, these findings are likely

to be more clinically relevant, as the prolonged exposure of individuals to suboptimal temperatures at their homes.

Our results also indicated that women and participants with obesity or diabetes experienced stronger effects from annual average temperature exposures than men and participants without obesity or diabetes. Diabetes or obesity could potentially lead to disruption of the ability to regulate body temperature[74,75]. This implies that particular subpopulations may be more vulnerable to high temperatures, and thus require greater protection than others.

5.4 Short- and medium-term effects of air temperature on biomarkers of subclinical inflammation

After correcting for multiple testing and controlling for potential confounding factors, 64 out of 71 biomarkers showed significant associations with lower air temperature. We discovered that the medium-term effects of air temperature surpassed the short-term effects, featuring more significant associations of biomarkers and stronger effect estimates. This indicates that lower air temperatures can have a delayed effect on biomarkers of subclinical inflammation, and that the cumulative effects of low temperature exposure appear to be partly responsible for the larger effect size of medium-term exposure.

Inflammatory processes of a complex nature are likely to be implicated in the pathogenesis of a range of chronic diseases, including those related to cardiometabolism[36,37]. An inflammatory response to non-optimal temperature exposure has been hypothesized as one of the key mechanisms that may explain air temperature effects on cardiometabolic diseases[7,14,15]. In our study, sixty-four biomarkers of subclinical inflammation were identified to have significant associations with lower air temperature exposures, with sixty-one of these biomarkers reported for the first time.

Previous investigations have demonstrated that augmented pro- and anti-inflammatory biomarkers (Interleukin-6 [IL-6], Protein S100-A12, and Interleukin-10 [IL-10]) correlated with amplified risks of diabetes, cardiovascular disease, and mortality[76-84]. The analysis of a range of other novel biomarkers of subclinical inflammation from this study needs to be interpreted with caution due to it is rather exploratory nature. However, they point to roles in immune response and neural processes, as well as towards intercellular communication. Importantly, our study revealed novel possible biomarkers for subclinical inflammation that could be used to measure the health consequences of low temperatures and its relationship with increased morbidity and mortality. Notably, these results imply lower air temperature exposures to be associated with multiple chronic diseases (e.g. cardiometabolic disease) as well as mortality, in part attributed to its effect on subclinical inflammation.

Out of the 71 biomarkers of subclinical inflammation discussed here, only five (namely, IL-6, Interleukin-8 [IL-8], IL-10, Fibroblast growth factor 21 [FGF-21], and Monocyte chemotactic protein 1 [MCP-1]) have been previously linked with air temperature in the past, mainly focusing on short-term effects[38-44,85]. Our findings showed significant effects of lower air temperature on increased IL-6, IL-10, and MCP-1 for different cumulative lag windows until 0-55 days. Conversely, no significant effect of air temperature on IL-8 or FGF-21 was found. Previous studies showed that decreased air temperature was linked to higher IL-6, IL-8, IL-10, or MCP-1 levels[38-41]. Another study reported an inverse association between air temperature and IL-8, in contrast to MCP-1[42]. Experimental studies reported that FGF-21 levels dropped significantly after cold exposure among healthy individuals or individuals with brown adipose tissue, but not among those without brown adipose tissue[43,44]. However, a study only examining men found no associations between air temperature and IL-6 or IL-8[85].

Our study also found that older participants showed stronger effects of lower air temperature on higher levels of biomarkers of subclinical inflammation, possibly due to the fact that elderly individuals experience a deterioration of their body's functions and the capacity to regulate body temperature[86,87]. Furthermore, those with underlying health conditions, e.g. diabetes and cardiovascular disease, were more likely to be adversely affected by lower temperatures. Interestingly, we observed a synergistic link between lower air temperature and air pollution, which was associated with increased biomarkers of subclinical inflammation. Crafting integrated climate and air quality policies is essential to properly tackle the concurrent threats to public health by temperature and air pollution.

5.5 Short-term effects of cold spells on plasma viscosity

This research was the first to estimate the association between cold spells, characterized by different criteria, and plasma viscosity across a range of lag times. There was a notable relationship between cold spells exposure and high plasma viscosity.

Studies have shown that plasma viscosity is a contributing factor in the development of CVD, with higher levels being associated with an increase in platelet adhesion and aggregation[45-51,88]. Our results showed that cold spells had a dual impact on plasma viscosity, with effects manifesting immediately (lag of 0-1 days) as well as persisting for an extended period (up to 27 days). The study also showed plasma viscosity was more strongly affected when the cold spells were more extreme. These findings demonstrated that there are various intricate ways in which cold spells can harm health and increase the risk of CVD. It is worth noting that previous research has showed that low temperatures have a fairly long-lagged effect on health[89-91], and disregarding longer lag days may underestimate the impacts of cold spells on health and the risk of CVD. Thus, delayed health impacts of cold spells are also noteworthy and should be taken into account. Taken

together, our findings provided support for the proposition that higher plasma viscosity could be a mediator in the biological mechanisms linking low temperature/cold spells to cardiometabolic disorders.

Prior studies have observed that exposure to low temperatures caused a significant enhancement in plasma or blood viscosity[59,60]. Furthermore, seasonal analyses also showed that plasma or blood viscosity was increased in winter[56,62]. However, research conducted in a population of males aged between 50 and 65 years showed no difference in the level of plasma viscosity between the coldest and warmest months[92]. Results from animal studies with rats demonstrated that a decrease in temperature caused a significant rise in blood viscosity [57,58,61]. Though, a different study with hamsters showed that plasma viscosity was lower after exposure to low temperatures for four weeks[93]. Overall, our results are generally in line with most of the prior studies, and thus further validate the impact of air temperature on plasma viscosity.

5.6 Strengths and limitations

The availability of comprehensive participant characteristics in the KORA cohort facilitated adjustments for multiple potential confounding variables and the exploration of multiple effect modifiers. Furthermore, KORA has a unique advantage over larger cohorts in that it has a wide range of interesting health parameters available as outcome measures. This makes KORA a valuable resource for studying the health outcomes of interest, despite its relatively small size. Additionally, by using an advanced statistical modelling technique, we were able to accurately estimate air temperature at the residential addresses of participants within a 1km x 1km grid. This approach outperformed the use of fixed monitoring sites, resulting in sufficient variability in air temperature measurements across both time and space and lowering the risk of exposure misclassifications. However, the studies of this thesis also have some limitations that need

to be acknowledged. First, because they were observational studies, there may be residual confounding that cannot be entirely eliminated, and causality cannot be inferred. Second, our results were derived from a cohort study conducted in a single center situated in Augsburg, Germany and the results may not be representative of populations with diverse ethnic compositions or climatic conditions. Additionally, the studies of this thesis only assessed outdoor temperature at a regional level, without accounting for individual-level exposure variability, which could introduce exposure misclassification bias.

6. Conclusions and Outlook

In conclusion, this thesis provides evidence that higher air temperatures were associated with shorter leucocyte telomere length and higher epigenetic age acceleration. On the other hand, lower air temperature or cold spells were associated with higher biomarkers of subclinical inflammation and higher plasma viscosity. These findings shed light on the complexity of multiple mechanisms underlying the adverse cardiometabolic effects of high and low air temperatures and expand our understanding of how high or low air temperature affects the human body. Furthermore, the findings from this thesis may support the design of potential interventions that can diminish the risk of cardiometabolic illness and death in either high- or low-temperature settings.

This thesis is limited by the single-center observational design, which may affect the generalizability and causality of our findings. Therefore, we suggest further research to validate our findings and explore the causal mechanisms linking non-optimal air temperature and pathophysiological reactions within the cardiometabolic system. Moreover, similar studies should be conducted in different climate zones with lower or higher temperatures or ethnic populations to evaluate the applicability and robustness of our findings. By expanding research to include a wider range of participants and environments, one can gain a more comprehensive understanding of the impact of non-

optimal air temperature on the cardiometabolic system. Additionally, we suggest that future studies should also explore the potential interventions that can mitigate the adverse effects of high or low air temperature on cardiometabolic health.

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Publication I

Title:	Higher Daily Air Temperature Is Associated with Shorter Leukocyte Telomere Length: KORA F3 and KORA F4
Authors:	Wenli Ni, Kathrin Wolf, Susanne Breitner, Siqi Zhang, Nikolaos Nikolaou, Cavin K Ward-Caviness, Melanie Waldenberger, Christian Gieger, Annette Peters, Alexandra Schneider
Journal:	Environmental Science & Technology
Status:	Published
Volume:	56
Page:	17815-17824
Year:	2022
doi:	10.1021/acs.est.2c04486

Environmental Science & Technology

pubs.acs.org/est

Article

Higher Daily Air Temperature Is Associated with Shorter Leukocyte Telomere Length: KORA F3 and KORA F4

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Cite This: *Environ. Sci. Technol.* 2022, 56, 17815−17824

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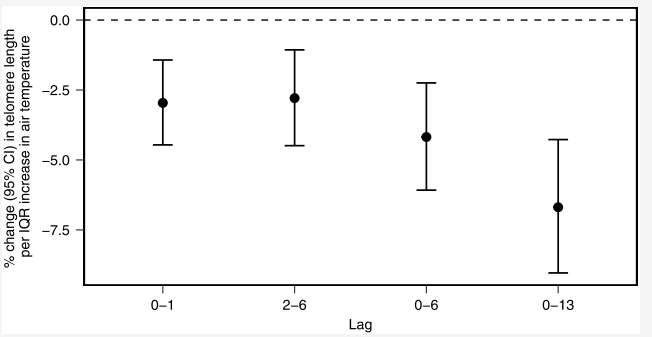
ABSTRACT: Higher air temperature is associated with increased age-related morbidity and mortality. To date, short-term effects of air temperature on leukocyte telomere length have not been investigated in an adult population. We aimed to examine the short-term associations between air temperature and leukocyte telomere length in an adult population-based setting, including two independent cohorts. This population-based study involved 5864 participants from the KORA F3 (2004−2005) and F4 (2006−2008) cohort studies conducted in Augsburg, Germany. Leukocyte telomere length was assessed by a quantitative PCR-based method. We estimated air temperature at each participant's residential address through a highly resolved spatiotemporal model. We conducted cohort-specific generalized additive models to explore the short-term effects of air temperature on leukocyte telomere length at lags 0−1, 2−6, 0−6, and 0−13 days separately and pooled the estimates by fixed-effects meta-analysis. Our study found that between individuals, an interquartile range (IQR) increase in daily air temperature was associated with shorter leukocyte telomere length at lags 0−1, 2−6, 0−6, and 0−13 days (%change: −2.96 [−4.46; −1.43], −2.79 [−4.49; −1.07], −4.18 [−6.08; −2.25], and −6.69 [−9.04; −4.27], respectively). This meta-analysis of two cohort studies showed that between individuals, higher daily air temperature was associated with shorter leukocyte telomere length.



KEYWORDS: *short-term effects, air temperature, telomere length*

1. INTRODUCTION

Climate change is a major public health concern that is becoming increasingly important worldwide. The global surface temperature for July 2021 was the highest for July in the 142-year record of the National Centers for Environmental Information of National Oceanic and Atmospheric Administration (NOAA), which dates back to 1880.[1] The climate is warming quickly in the World Health Organization European Region, which is experiencing accelerated rates of temperature increase and increased frequency and intensity of heat waves.[2] Previous studies have shown that the frequency and severity of extreme weather events are increasing as a consequence of climate change.[3] Prominent examples are the heat waves in America and Canada and the floods in Germany and China in 2021. Importantly, accumulating evidence shows that increases in air temperature endanger human health and well-being in numerous ways and are associated with increased morbidity and mortality, especially in age-related vulnerable population subgroups, such as the elderly.[4−10] A recent worldwide study based on data from 750 locations in 43 countries showed that the global heat-related excess death ratio increased by 0.21% between 2000−2003 and 2016−2019.[7]

The global population is aging rapidly and the prevalence of age-related diseases is quickly increasing.[11] Telomeres are highly conserved tandem repetitive nucleotide sequences (TTAGGG), which provide a protective cap at the ends of chromosome to maintain genome stability.[12,13] Telomeres are fundamental for cell division and shorten after each round of cell division.[14] Consequently, leukocyte telomere length shortening is evaluated as a potential biomarker for biologic aging, and telomere shortening has been associated with increasing numbers of age-related diseases such as stroke, cardiovascular disease, or cancer.[15−18] A recent large cohort study from the United Kingdom Biobank found that shortened leukocyte telomere length was associated with increased overall cardiovascular, respiratory, digestive, musculoskeletal, and COVID-19 mortality.[19] A further review study showed that telomere attrition was influenced by genetic and environmental

Received: June 22, 2022
Revised: November 1, 2022
Accepted: November 2, 2022
Published: November 28, 2022

factors.[12] Evidence is rapidly growing that telomere shortening can be accelerated by exposure to nonoptimal environmental factors like air pollution,[20,21] and pesticides.[22]

Air temperature is an important environmental factor, especially in the context of climate change. A study in the three largest English cities (Greater London, Greater Manchester, and West Midlands) found that heat and cold exposure increased the risk of mortality and years of life lost.[23] In addition, high air temperature or heat stress has been associated with higher levels of oxidative stress and inflammatory biomarkers.[24−27] As oxidative stress and inflammation can speed up telomere attrition,[28,29] these findings suggest that air temperature might affect telomere length. However, only one birth study has reported that prenatal high and low air temperature exposures were associated with shorter cord blood telomere length.[30] So far, the effect of air temperature on leukocyte telomere length has not been investigated among an adult population.

Therefore, we aimed to examine the short-term associations between air temperature and leukocyte telomere length in the region of Augsburg, Germany, within two independent adult cohorts.

2. MATERIALS AND METHODS

2.1. Study Design. Data were from the Cooperative Health Research in the Region of Augsburg (KORA) F3 study (February 9, 2004−May 13, 2005) and F4 study (October 9, 2006−May 31, 2008), which were follow-up studies of the population-based KORA S3 and KORA S4 survey conducted in 1994−1995 and 1999−2001. The study area is located in the city of Augsburg and two surrounding counties in Southern Germany.[31] The study design, standardized sampling method, and data collection have been described in detail elsewhere.[31−33] Of 2974 and 3080 individuals who participated in KORA F3 and KORA F4, the current analysis included 2865 F3 and 2999 F4 participants, with no missing information on leukocyte telomere length, air temperature, or covariates of interest.

The study was approved by the ethics board of the Bavarian Chamber of Physicians (Munich, Germany) in adherence with the declaration of Helsinki. All participants gave written informed consent.

2.2. Exposure Assessment. Countrywide high-resolution (1 km × 1 km) minimum (Tmin), mean (Tmean), and maximum (Tmax) daily air temperature data were estimated using hybrid spatiotemporal regression-based models, following the approach from Kloog et al.[34,35] Several data sets from multiple sources were incorporated in the modeling process, including air temperature measurements from weather stations as well as satellite-derived land surface temperature (LST), elevation, vegetation, urban fabric, arable land, pastures, forests, and inland waters, which were harmonized into 366,536 grid cells of 1 km × 1 km based on the European INSPIRE (Infrastructure for Spatial Information in the European Community) standard using the Lambert Azimuthal Equal-Area projection, EPSG: 3035 (GeoBasis-DE/BKG (2021)) before modeling. We trained three-stage models to achieve air temperature predictions with complete temporal and spatial coverage. In the first stage, a linear mixed effects model with daily random intercepts and slopes for LST and adjusted for the aforementioned spatial predictors was trained in the combinations of days and grid cells, where air temperature measurements and satellite LST were both available. In the second stage, the first stage model was used to predict air temperature for grid cells without air temperature measurements but with available LST data. For the remaining days and grid cells with neither LST data nor air temperature measurements available, we regressed the second-stage air temperature predictions against thin plate spline interpolated air temperature values to obtain fully covered air temperature countrywide. We performed internal and external out-of-sample 10-fold cross-validation to quantify the prediction accuracy of our models. All models achieved excellent performance ($0.91 \leq R^2 \leq 0.98$) and low errors (1 °C < root-mean-square error < 2 °C). The individual subject-specific data have been linked to the environmental exposure data with daily resolution over the pseudonymized unique participant ID and over the grid ID of the exposure. The data-linkage processes have been performed using the geocoded participants' residential addresses. Care was taken to remove all spatial information from the data set. Pseudonymized data were provided to the research team. Finally, we matched phenotype data with exposure data by date, according to different lag days.

The daily concentrations of relative humidity (RH), ozone (O_3), nitrogen dioxide (NO_2), particulate matter with an aerodynamic diameter < 2.5 μm ($PM_{2.5}$), and black carbon (BC) were assessed via fixed monitoring sites within Augsburg, Germany.[36−38] O_3 and RH were obtained from an official urban background monitoring site operated by the Bavarian Environment Agency (LfU, Bayerisches Landesamt für Umwelt), which was located about 5 km south of the city center. NO_2 was measured at an urban background measurement station located approximately 2 km north of the city center [also operated by the Bavarian Environment Agency (LfU, Bayerisches Landesamt für Umwelt)]. $PM_{2.5}$ and BC were measured at a single urban background site located 1 km south of the city center and assessed via a tapered element oscillating microbalance (TEOM model 1400A, Thermo Fisher Scientific) equipped with the filter dynamics measurement system (FDMS, model 8500b; Thermo Fisher Scientific) and an aethalometer (model series 8100; Thermo Fisher Scientific), respectively. Daily 24-h average relative humidity, daily maximum 8-h average O_3, daily 24-h average NO_2, daily 24-h average $PM_{2.5}$, and daily 24-h average BC were calculated if at least 75% of the hourly measurements were available.

2.3. Measurement of Leukocyte Telomere Length. Standardized procedures for measuring leukocyte telomere length have been described previously in detail.[39] In brief, blood samples were collected on the same day the questionnaires were completed. Before the KORA study center visit, participants were asked to fast for at least 8 h and avoid exercising and smoking the day before and the morning before blood sampling. Blood samples were taken in a sitting position. Samples for measurement of leukocyte telomere length were stored at −80 °C until analysis.

Telomere length in samples of F3 and F4 was assayed at the same time and normalized together. Leukocytes from peripheral blood samples were used to extract genomic DNA, and the telomere length was assessed using a quantitative PCR-based method and expressed as the ratio between the telomere repeat copy number (T) and a single-copy gene: 36B4 (S) (T/S ratio).[39,40] We ran DNA samples in duplicate in 25 μL reactions on a Rotorgene-Q real-time thermal cycler and CAS-1200 liquid handling system (Qiagen, U.K.). A no-template control and a calibrator sample (genomic

https://doi.org/10.1021/acs.est.2c04486
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DNA from the K562 cell line) in duplicate were included in each run to standardize the measurement across PCR plates. The PCR output was analyzed using comparative quantification (Qiagen Rotorgene analysis software, Qiagen, U.K.), and quantification was relative to calibrator DNA. For quality control, all samples were checked for concordance between duplicate values and to ensure that the experiment ran within the linear range of the assay established. Furthermore, to ensure the reproducibility of the assay, samples were regularly re-run at random on a different day and/or machine. All samples reproduced well, and the inter-run coefficient of variability (CV) for the T/S ratio was 2.63% in KORA F3 and 3.08% in KORA F4, and the intra-run CV for the T/S ratio was 2.84% in KORA F3 and 3.07% in KORA F4. Correlation coefficients (r^2) of the measurements from different days were 0.85 for KORA F3 and 0.93 for KORA F4.

2.4. Assessment of Covariates. Participants completed a computer-assisted personal interview and a self-administered questionnaire with information regarding demographic characteristics, including age (year), sex (male, female), education (years), and body mass index (BMI [kg/m^2]); lifestyle characteristics, including smoking status, alcohol consumption (g/day), and physical activity; medical history of the participants, including hypertension (no/yes), angina pectoris (no/yes), myocardial infarction (no/yes), and stroke (no/yes); and current medications, including antihypertensive (no/yes), lipid-lowering (no/yes), and antidiabetic medication (no/yes). Body weight and height were obtained during physical examinations. BMI was calculated as weight in kilograms divided by height squared in meters.

Four categories of smokers were defined in the questionnaire: never smokers, former smokers, occasional smokers, and regular smokers. Participants were divided into three categories for the present analysis: never-smokers, former smokers, and current smokers (including occasional and regular smokers). Alcohol consumption was rated by asking participants how many alcoholic drinks (beer, wine, or spirits) they consumed during the previous weekday and the previous weekend. Physical activity levels were estimated by asking subjects how much time each week they spent engaging in physical activity during their leisure time in summer and winter (almost no or no physical activity, irregularly about 1 h per week, regularly about 1 h per week, and regularly more than 2 h per week). For this analysis, participants were categorized into three categories: low (almost no exercise), medium (regularly or irregularly approx. 1 h per week), and high (regularly more than 2 h per week). Seasons at blood draw were defined as spring (March−May), summer (June−August), autumn (September−November), and winter (December−February).

Participants with a history of hypertension were defined by measured blood pressure (over 140/90 mm Hg) or reported use of antihypertensive medications. Participant history of myocardial infarction or stroke was based on self-reported physician diagnoses treated in hospitals. The history of angina pectoris was based on self-reported physician diagnoses.

Data on the use of medications were collected using the standardized software IDOM (an instrument for database-supported online medication registration).[41] The following classes were considered as antihypertensive medication: antihypertensive medication, diuretics, calcium antagonists, β-blockers, ACE inhibitors, and angiotensin antagonists; lipid-lowering medication: lipid-lowering, including herbal substances, statins, and fibrates; and antidiabetic medication, including antidiabetics, insulin, and oral antidiabetics.

Serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides were measured using the CHOL Flex, AHDL Flex, ALDL Flex, and TGL Flex (Dade Behring, Germany), respectively.

2.5. Statistical Analysis. Characteristics of the study population were given as mean and standard deviation (SD) for continuous variables and frequency and percentage for categorical variables. We compared the demographic characteristics of two cohort study populations using chi-squared tests for categorical variables and *t*-tests for continuous variables. The levels of meteorological variables and air pollutants were presented as mean, SD, 5, 25%, median, 75, 95%, and interquartile range (IQR), and Spearman's correlation was used to evaluate their correlations.

We applied cohort-specific generalized additive models (GAMs) to explore the short-term effects of mean air temperature on leukocyte telomere length. To explore the potential cumulative effects of mean temperature, we investigated the moving averages of daily mean temperature at lags 0−1, 2−6, 0−6, and 0−13 days before blood draw. In preliminary analyses, we investigated the shape of the association by including the temperature term as a nonlinear function (spline with three degrees of freedom). Since none of the curves indicated significant deviations from linearity (Supporting Information, Figure S1), we included air temperature as a linear term for the main analyses. Leukocyte telomere length was natural log-transformed to increase normality of residuals. We controlled for age, sex, BMI, education, smoking status, alcohol consumption, physical activity, day of the week, season, time trend (cubic spline with ten degrees of freedom), and relative humidity with the same lag period as the air temperature. The time trend was calculated as the day of the year. We included the time trend in our model to adjust for unmeasured confounding, such as variables varying over time, even in this short period.

We conducted effect modification analysis by including an interaction term between air temperature and the potential effect modifier: sex (male vs female), age (<65 years vs ≥ 65 years), physical activity (low vs medium or high), obesity (BMI <30 kg/m^2 vs ≥30 kg/m^2), smoking status (current vs former or never smoker), hypertension (yes vs no), cardiovascular disease (defined as a history of angina pectoris, myocardial infarction, or stroke [yes vs no]), season (warm: April−September vs cold: October−March), and O_3 (low [<median O_3] vs high [≥median O_3]).

To assess the robustness of the results, we performed several sensitivity analyses. First, we applied four different confounder models. Model 1 only adjusted for age, sex, BMI, day of the week, time trend, and relative humidity. Model 2 included all covariates of the main model and additionally adjusted for total cholesterol, triglyceride, LDL, and HDL. Model 3 extended Model 2 by additionally adjusting for medication intake (antihypertensive, lipid-lowering, or antidiabetic medication). In Model 4, we further included air pollutants (O_3, $PM_{2.5}$, NO_2, and BC) with the same lag period as the air temperature. These four pollutants were additionally included in the model separately to avoid collinearity. Further, we only additionally adjusted for BC only in KORA F4 because there was no BC data available in KORA F3. Second, instead of Tmean, we used Tmin and Tmax as alternative exposure metrics. Third, we excluded outliers in leukocyte telomere length values less than

https://doi.org/10.1021/acs.est.2c04486
Environ. Sci. Technol. 2022, 56, 17815−17824

the first quartile of the data (Q1) − 1.5 × IQR or more than the third quartile of the data (Q3) + 1.5 × IQR to avoid overestimating the effects induced by extreme values. Fourth, to avoid the effects of extreme air temperature and the influence of elevated environmental temperature on the quality of blood samples, we excluded subjects exposed to air temperature greater than 95% of temperature. Finally, we extended the lag days and investigated the effect of 4-week moving air temperature averages (lags 0−27 days) on leukocyte telomere length.

All models were first fitted separately for each cohort, and the results from the individual cohorts were then pooled using fixed-effect meta-analysis. Heterogeneity was examined using the *I*-squared (I^2) statistic. A *P*-value > 0.05 and/or I^2 <50% was considered homogeneous.

To compare the effect estimates across different exposure windows, effect estimates were presented as percent changes of the geometric outcome mean with 95% confidence intervals (CIs) per IQR increase in air temperature. $P < 0.05$ was considered to be statistically significant for all statistical tests. All statistical analyses were performed using R (Version 4.1.2) with "mgcv" and "metafor" packages.

3. RESULT

3.1. Study Population and Exposure Data. The characteristics of the study population and level of leukocyte telomere length are described in Table 1. The overall geometric mean level of leukocyte telomere length was lower in KORA F3 with 1.7 T/S compared to 1.8 T/S in KORA F4. The KORA F3 population was older, had a lower level of education, higher proportions of current smokers and hypertension, and higher levels of triglycerides, HDL, and total cholesterol than the KORA F4 population. The KORA F4 population had a higher level of LDL and a higher proportion of antihypertensive medication intake than the KORA F3 population.

Mean daily mean temperature was 7.2 °C in KORA F3 and 7.8 °C in KORA F4 (Table 2). Spearman's correlation coefficients (r) between meteorological variables and air pollutants (O_3, $PM_{2.5}$, NO_2, and BC) were similar for both studies (Supporting Information, Figure S2). The correlations between the different temperature variables and $PM_{2.5}$, NO_2, and BC were generally high ($r \geq 0.72$), whereas the correlations between temperature variables, RH, and air pollutants were only weak to moderate.

3.2. Short-Term Effects of Air Temperature on Leukocyte Telomere Length. Our meta-analyses showed that between individuals, higher air temperature was associated with shorter leukocyte telomere length for all investigated lags (Figure 1). We found that between individuals, an IQR increase in daily mean air temperature (10.77, 10.11, 10.15, and 9.54 °C, respectively) was significantly associated with shorter leukocyte telomere length at lags 0−1, 2−6, 0−6, and 0−13 days (%change: −2.96 [−4.46; −1.43], −2.79 [−4.49; −1.07], −4.18 [−6.08; −2.25], and −6.69 [−9.04; −4.27], respectively). The cohort-specific associations (KORA F3 and KORA F4) showed similar results with slightly more pronounced estimates for F4 and lags 2−6 days for F3 being the only weaker association (Figure 2). Effect estimates expressed as absolute changes between individuals with 95% CIs per IQR increase in air temperature are shown in Figure S3 (Supporting Information).

Table 1. Descriptive Statistics of Participant Characteristics and Leukocyte Telomere Length in KORA F3 (2004−05) and KORA F4 (2006−08)

	mean ± SD or N (%)		
	KORA F3 (n = 2865)	KORA F4 (n = 2999)	*P*-value
leukocyte telomere length (T/S)[a]	1.7 ± 0.3/1.7	1.9 ± 0.3/1.8	<0.001
age (years)	57.1 ± 12.8	56.1 ± 13.2	0.004
sex (male)	1391(48.6)	1447 (48.2)	0.837
body mass index (kg/m^2)	27.7 ± 4.6	27.6 ± 4.8	0.727
education (years)	11.4 ± 2.6	11.7 ± 2.7	<0.001
	Smoking Status		
never smoker	1276 (44.5)	1248 (41.6)	0.016
former smoker	1055 (36.8)	1213 (40.4)	
current smoker	534 (18.6)	538 (17.9)	
	Physical Activity		
low (none or <1 h per week)	941 (32.8)	967 (32.2)	0.240
medium (∼1 h per week)	1279 (44.6)	1301 (43.4)	
high (∼2 h per week or more)	645 (22.5)	731 (24.4)	
alcohol consumption (g/day)	15.3 ± 19.6	14.4 ± 19.6	0.063
triglycerides (mmol/L)	1.9 ±1.5	1.4 ± 1.0	<0.001
HDL (mmol/L)	1.5 ± 0.4	1.4 ± 0.4	<0.001
LDL (mmol/L)	3.3 ± 0.8	3.5 ± 0.9	<0.001
total cholesterol (mmol/L)	5.64 ± 1.0	5.58 ± 1.0	0.011
	History of Diseases		
hypertension (yes)	1432 (50.0)	1153 (38.4)	<0.001
cardiovascular disease (yes)	319 (11.1)	308 (10.3)	0.297
	Medication Intake		
antihypertensive medication (yes)	746 (26.0)	954 (31.8)	<0.001
lipid-lowering medication (yes)	312 (10.9)	377 (12.6)	0.05
antidiabetic medication (yes)	181 (6.3)	173 (5.8)	0.41
	Season		
spring	939 (32.8)	864 (28.8)	<0.001
summer	479 (16.7)	407 (13.6)	
autumn	776 (27.1)	804 (26.8)	
winter	671 (23.4)	924 (30.8)	

[a]Geometric mean, additionally shown for telomere length. Data are mean (SD)/geometric mean. LDL, low-density lipoproteins; HDL, high-density lipoproteins.

Our study also showed that between individuals, a 1 °C increase in daily mean air temperature was significantly associated with shorter leukocyte telomere length at lags 0−1, 2−6, 0−6, and 0−13 days (%change: −0.28 [−0.42; −0.13], −0.28 [−0.45; −0.11], −0.42 [−0.62; −0.22], and −0.72 [−0.99; −0.46], respectively) (Supporting Information, Figure S4).

3.3. Effect Modification. Our analysis mainly indicated no significant effect modification by the investigated potential effect modifiers (Figure 3). Only participants examined in the cold season showed significantly shorter leukocyte telomere length for air temperature at lags 0−1 days. In contrast, no association was observed for participants examined in the warm season.

Effect estimates were presented as percent changes of the geometric outcome mean with 95% CIs per IQR increase in air

https://doi.org/10.1021/acs.est.2c04486
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Table 2. Descriptive Statistics of Meteorological Variables and Air Pollutants[a]

	mean	SD	5%	25%	median	75%	95%	IQR
KORA F3 (09th February, 2004−13th May, 2005)								
Tmean (°C)	7.2	7.6	−4.8	0.8	8.1	13.3	18.5	12.5
Tmin (°C)	2.9	7	−9.9	−1.8	3.7	8.6	12.5	10.4
Tmax (°C)	11.9	9	−1.5	3.4	12	19.3	26.1	15.9
RH (%)	72.6	13	51.2	61.5	73.4	83.7	92.1	22.2
O_3 ($\mu g/m^3$)	45.5	23.1	5.6	27.2	48.3	63	82	35.8
$PM_{2.5}$ ($\mu g/m^3$)	22.2	12.3	7.6	13.6	19.7	27.8	46.3	14.2
NO_2 ($\mu g/m^3$)	38.6	15.9	18.3	27	36.7	45.5	69.5	18.5
KORA F4 (09th October, 2006−31st May, 2008)								
Tmean (°C)	7.8	6.1	−0.9	3	7.1	11.9	19.1	8.9
Tmin (°C)	3.7	5.3	−4	0	2.8	7.1	13.2	7.1
Tmax (°C)	12.5	7.5	2.3	7.1	11.6	17.7	26.3	10.6
RH (%)	76.5	9.9	58.6	70	77.7	84	91.1	14
O_3 ($\mu g/m^3$)	39.8	22.6	6.5	20.1	38.5	57	78.1	36.9
$PM_{2.5}$ ($\mu g/m^3$)	15.1	11.1	2.6	6.9	12.8	19.6	36.1	12.7
NO_2 ($\mu g/m^3$)	32.8	11.7	16.4	23.3	31.8	41.1	53.3	17.8
BC ($\mu g/m^3$)	1.8	1.1	0.7	1	1.5	2.1	4.3	1.1

[a]Tmean: daily mean temperature; Tmin: daily minimum temperature; Tmax: daily maximum temperature; RH: relative humidity; O_3: ozone; $PM_{2.5}$: particulate matter with an aerodynamic diameter of ≤2.5 μm; NO_2: nitrogen dioxide; and BC: black carbon. IQR: interquartile range.

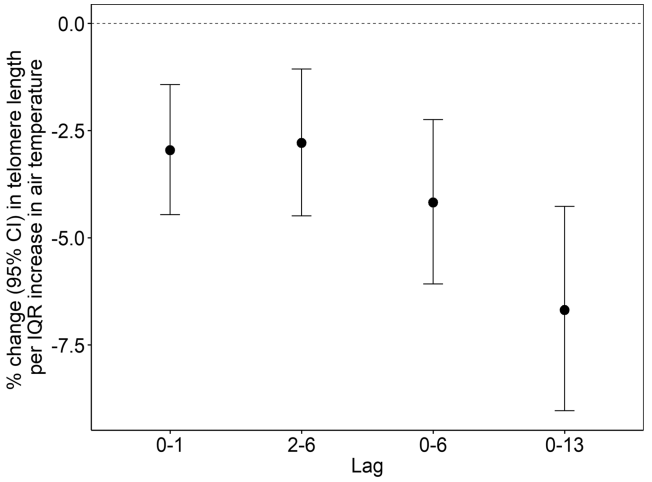

Figure 1. Estimated effects (percent change [95% CI] of geometric mean) of air temperature on leukocyte telomere length between individuals at lags 0−1, 2−6, 0−6, and 0−13 days per IQR increase in air temperature. Generalized additive models were first fitted separately for KORA F3 and KORA F4 cohorts. The results from the individual cohorts were then pooled using fixed-effect meta-analysis. Heterogeneity testing across KORA F3 and KORA F4: lag 0−1, $I^2 = 0$, $P = 0.71$; lag 2−6, $I^2 = 0$, $P = 0.44$; lag 0−6, $I^2 = 0$, $P = 0.42$; lag 0−13, $I^2 = 13.46$, $P = 0.28$. All models were adjusted for age, sex, BMI, education, smoking status, alcohol consumption, physical activity, day of the week, season, time trend (cubic spline with ten degrees of freedom), and relative humidity with the same lag period as the air temperature. Effect estimates were presented as percent changes of the geometric outcome mean with 95% CIs per IQR increase in air temperature. The respective IQR increases were 10.77 °C for lags 0−1 days, 10.11 °C for lags 2−6 days, 10.15 °C for lags 0−6 days, and 9.54 °C for lags 0−13 days.

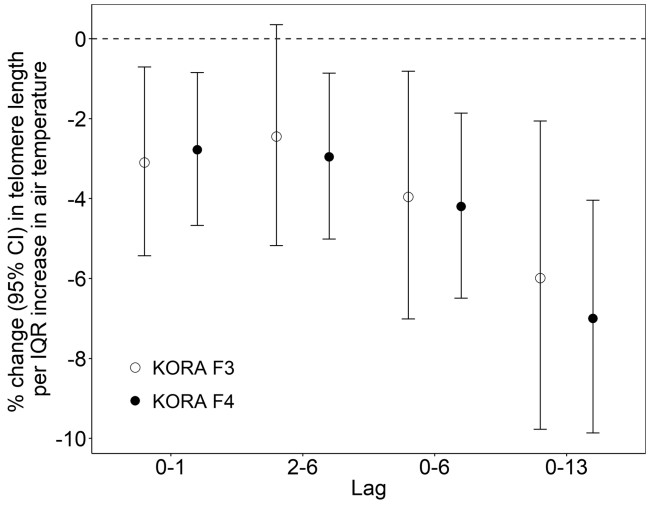

Figure 2. Estimated effects (percent change [95% CI] of geometric mean) of air temperature on leukocyte telomere length between individuals at lags 0−1, 2−6, 0−6, and 0−13 days per IQR increase in air temperature in KORA F3 and KORA F4. All models were adjusted for age, sex, BMI, education, smoking status, alcohol consumption, physical activity, day of the week, season, time trend (cubic spline with ten degrees of freedom), and relative humidity with the same lag period as the air temperature. Effect estimates were presented as percent changes of the geometric outcome mean with 95% CIs per IQR increase in air temperature. KORA F3: the respective IQR increases were 12.42 °C for lags 0−1 days, 11.62 °C for lags 2−6 days, 11.80 °C for lags 0−6 days, and 10.74 °C for lags 0−13 days; KORA F4: the respective IQR increases were 9.12 °C for lags 0−1 days, 8.60 °C for lags 2−6 days, 8.50 °C for lags 0−6 days, and 8.35 °C for lags 0−13 days.

temperature. Error bars in red indicate significant differences in effect estimates between subgroups (P-value for the interaction term <0.05). The respective IQR increases were 10.77 °C for lags 0−1 days, 10.11 °C for lags 2−6 days, 10.15 °C for lags 0−6 days, and 9.54 °C for lags 0−13 days.

3.4. Sensitivity Analysis. In general, the associations between air temperature and leukocyte telomere length between individuals remained robust in the sensitivity analyses (Supporting Information, Figure S5). We observed similar associations when using different sets of confounder adjustment. Also, alternative exposure metrics Tmin and Tmax showed comparable effects as Tmean. Moreover, the exclusion of outliers and the exclusion of subjects exposed to air temperatures greater than 95% of temperature did not affect the results. Finally, we still found a significant effect of daily air

https://doi.org/10.1021/acs.est.2c04486
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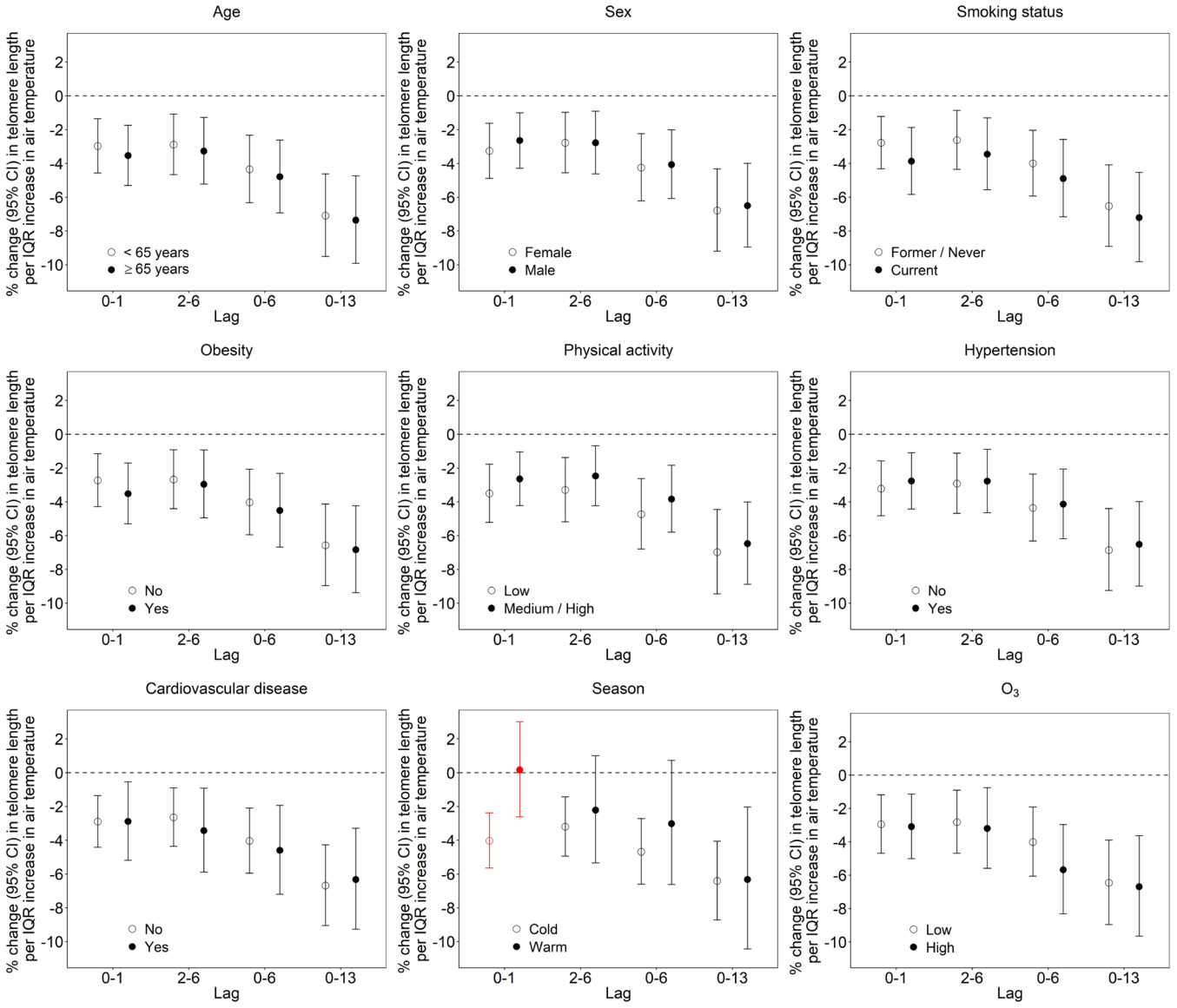

Figure 3. Estimated effects (percent change [95% CI] of geometric mean) of air temperature on leukocyte telomere length between individuals at lags 0−1, 2−6, 0−6, and 0−13 days per IQR increase in air temperature modified by age, sex, smoking status, obesity, physical activity, hypertension, cardiovascular disease, season, and O_3.

temperature on leukocyte telomere length at lags 0−27 days (−11.77%, 95% CI: −14.91; −8.51%).

4. DISCUSSION

To our knowledge, this is the first study to explore the short-term effects of air temperature on leukocyte telomere length within an adult population using data from two cohort studies. Pooling the results of the two cohort studies showed that between individuals, higher daily air temperature was significantly associated with shorter leukocyte telomere length.

Leukocyte telomere length measurement is increasingly recognized as a clinical indicator of the risk of age-related disease.[42] Our study is the first to show significant short-term associations between higher air temperature and shorter leukocyte telomere length between individuals in an adult population. To date, only one birth study on prenatal temperature exposure was able to demonstrate an association, finding that the strongest effect of a 1 °C increase in air temperature above the heat threshold (19.5 °C) took place at week 36 of gestation, resulting in a significantly associated 3.29% (95% CI: 1.88, 4.67%) T/S shorter cord blood telomere length. However, the association with a 1 °C decrease in air temperature below the cold threshold (5.0 °C) was strongest at week 10 of gestation, with a 0.72% (95% CI: 0.46, 0.97%) T/S longer cord blood telomere length.[30] Another study explored the association between a normal body temperature range (35.0−37.5 °C) and leukocyte telomere length in middle-aged and older adults, reporting a significant negative correlation between baseline body temperature and telomere length. However, there was no association between body temperature and the follow-up leukocyte telomere length and the 6-year longitudinal differences in telomere length.[43]

We found both significant immediate and lagged effects of air temperature on leukocyte telomere length at lags 0−1, 2−6, 0−6, and 0−13 days. The results indicate that high air temperatures over the preceding 2 weeks are associated with shorter telomere length. A recent epidemiological study based on 1792 participants with overweight/obesity reported a

https://doi.org/10.1021/acs.est.2c04486
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decreasing trend in leukocyte telomere length in association with increasing PM_{10}-levels, which was observed with a lag of up to 2 weeks.[44] These findings suggest that short-term effects of environmental factors on leukocyte telomere length should also be considered as adverse effects with potential health implications. Moreover, this study indicates that the more delayed effects (lags 0−13 days: −6.69% [−9.04%; −4.27%]) are larger than the immediate effects (lags 0−1 days: −2.96% [−4.46%; −1.43%]). It is conceivable that the immediate effects on telomere length on a day-to-day basis are at least partly compensated. However, the findings of this study suggest that repeated exposure to high temperatures may have compounding effects on telomere length. The reversibility of these effects still needs to be explored, in particular, to determine if there is an exposure level or frequency at which effects become partially or totally irreversible.

Except for season with lags 0−1 days, no significantly different effects were found across investigated population subgroups concerning age, sex, smoking status, obesity, physical activity, hypertension, cardiovascular disease, or different O_3-levels. Furthermore, it is noteworthy that we found between individuals significant short-term effects of higher air temperature on shorter leukocyte telomere length in all of these subgroups. This means that the significant effects of air temperature on leukocyte telomere length are robust across all population subgroups. Moreover, we found similar results for different temperature metrics (Tmean, Tmin, and Tmax), again suggesting the stability of our results.

Global warming is an increasingly critical global challenge. The Intergovernmental Panel on Climate Change (IPCC) Working Group II Sixth Assessment Report 2022 reported that there is at least a greater than 50% probability that global warming will reach or exceed 1.5 °C in the near term, even for the very low greenhouse gas emissions scenario.[45] Furthermore, leukocyte telomere length shortening is a clinical gauge for biological aging and age-related disease risk.[15−18] Our results showed that between individuals, an IQR/1 °C increased daily air temperature was associated with shorter leukocyte telomere length. The findings of our study support the growing evidence that climate change and subsequent temperature increases can lead to adverse human health effects. In light of these findings, public policies should be implemented to decrease the rate of global warming and prevent heat-induced health risks, which, to a certain extent, may help increase lifespan as well as delay or reduce age-related diseases.

The exact underlying mechanisms by which air temperature impacts leukocyte telomere length are still far from being understood. Telomere lengths tend to shorten with increasing age, and telomere attrition can be accelerated by oxidative stress and inflammation.[28,29] Because of rich guanine content in the 5-TTAGGG-3 repeat sequence, telomeres are highly sensitive to oxidative stress, which causes telomere DNA to be deficient in the repair of single-strand breaks.[46] A recent study among bakery workers found that heat stress can increase the level of malondialdehyde (oxidative stress biomarker).[24] Heat stress also has been shown to induce the production of reactive oxygen species and the increased expression of the interleukin (IL)-8 and IL-8 receptor genes in human dental pulp cells.[25] Increasing short-term air temperature was significantly associated with hypomethylation *TLR-2*, which may activate the expression of *TLR-2* gene and lead to biological responses activating the C-reactive protein expression.[26,27] Another previous study showed that short-term apparent temperature increase was associated with an increase in the acute inflammation factor high-sensitivity C-reactive protein.[47] A significant decrease in leukocyte telomere length was found as high-sensitivity C-reactive protein levels increased.[48,49] Thus, oxidative stress and inflammation may be among the mechanisms by which exposure to increasing temperature could lead to telomere shortening. Furthermore, telomerase, an enzymatic activity that plays a pivotal role in stabilizing telomeres by which telomeric repeats are added to the ends of chromosomes,[50] has been shown to be temperature-dependent, peaking at 37 °C under experimental conditions, followed by a decrease in the human telomerase's processivity with increasing temperature, primer concentration, and potassium ion (K^+).[51]

This study has several strengths. We estimated the air temperature at each participant's residential address for each calendar day by a sophisticated statistical modeling method using satellite, meteorological, and land-use data, which introduced sufficient spatial and temporal air temperature variability and reduced exposure misclassifications compared to a fixed monitoring site. However, no personal temperature exposure measurements have been considered. Second, this study combined two independent cohort studies with a large sample size (total N = 5,864), which should be considered more representative and generalizable than a single cohort study. Third, the KORA cohort (F3 and F4) provided extensive information regarding subject characteristics, which allowed us to adjust for a large number of confounders and explore several potential effect-modifying factors. Fourth, this study gives insight into the short-term effect of air temperature on shorter leukocyte telomere length among an adult population, something that has not been previously studied. However, our study also has some limitations. First, since this is an observational study, we cannot ignore the influence of potential residual confounding. However, we applied several different confounders to our model, and the results stayed robust. Second, our results are based on single-center cohort studies in Augsburg, Germany, and may not be generalizable to other populations with different ethnic, climatic, or demographic conditions. Furthermore, we only used ambient area level air temperature, but we do not know the extent to which individuals were exposed to outdoor temperatures, which increases the likelihood of misclassification of exposures. Finally, due to the lack of data in the present study, the association between air temperature and leukocyte telomere length was not investigated for different leukocyte subtypes, which may be characterized by different telomere lengths.

In conclusion, we found that between individuals, higher daily air temperature was associated with shorter leukocyte telomere length. Our findings add to the burgeoning evidence regarding how increased air temperature can adversely impact human health.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c04486.

Exposure−response functions of air temperature and leukocyte telomere length at lags 0−1, 2−6, 0−6, and 0−13 days (Figure S1); Spearman's correlation coefficients (r) between meteorological variables and air

https://doi.org/10.1021/acs.est.2c04486
Environ. Sci. Technol. 2022, 56, 17815−17824

pollutants (Figure S2); estimated effects (absolute change [95% CI]) of air temperature on leukocyte telomere length between individuals at lags 0−1, 2−6, 0−6, and 0−13 days per IQR increase in air temperature (Figure S3); estimated effects (percent change [95% CI] of geometric mean) of air temperature on leukocyte telomere length between individuals at lags 0−1, 2−6, 0−6, and 0−13 days per 1 °C increase in air temperature (Figure S4); sensitivity analysis: estimated effects (percent change [95% CI] of geometric mean) of air temperature on leukocyte telomere length between individuals at lags 0−1, 2−6, 0−6, and 0−13 days per IQR increase in air temperature (Figure S5) (PDF)

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Notes

The authors declare no competing financial interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

This work was supported by a scholarship under the State Scholarship Fund by the China Scholarship Council (File No. 201906010316). The KORA Study was initiated and financed by the Helmholtz Zentrum München−German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ.

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Higher daily air temperature is associated with shorter leukocyte telomere length: KORA F3 and KORA F4

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Pages: 9

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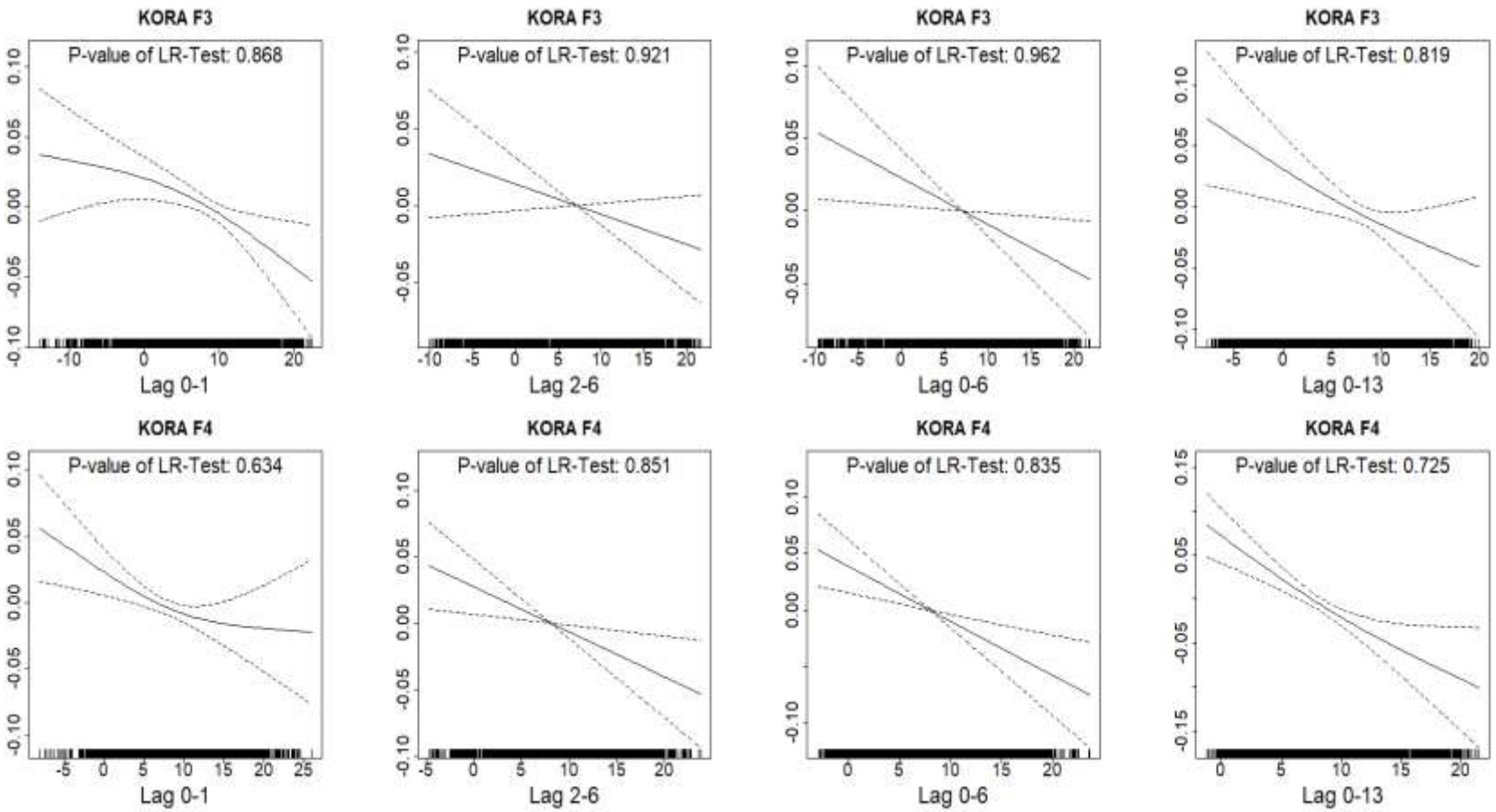

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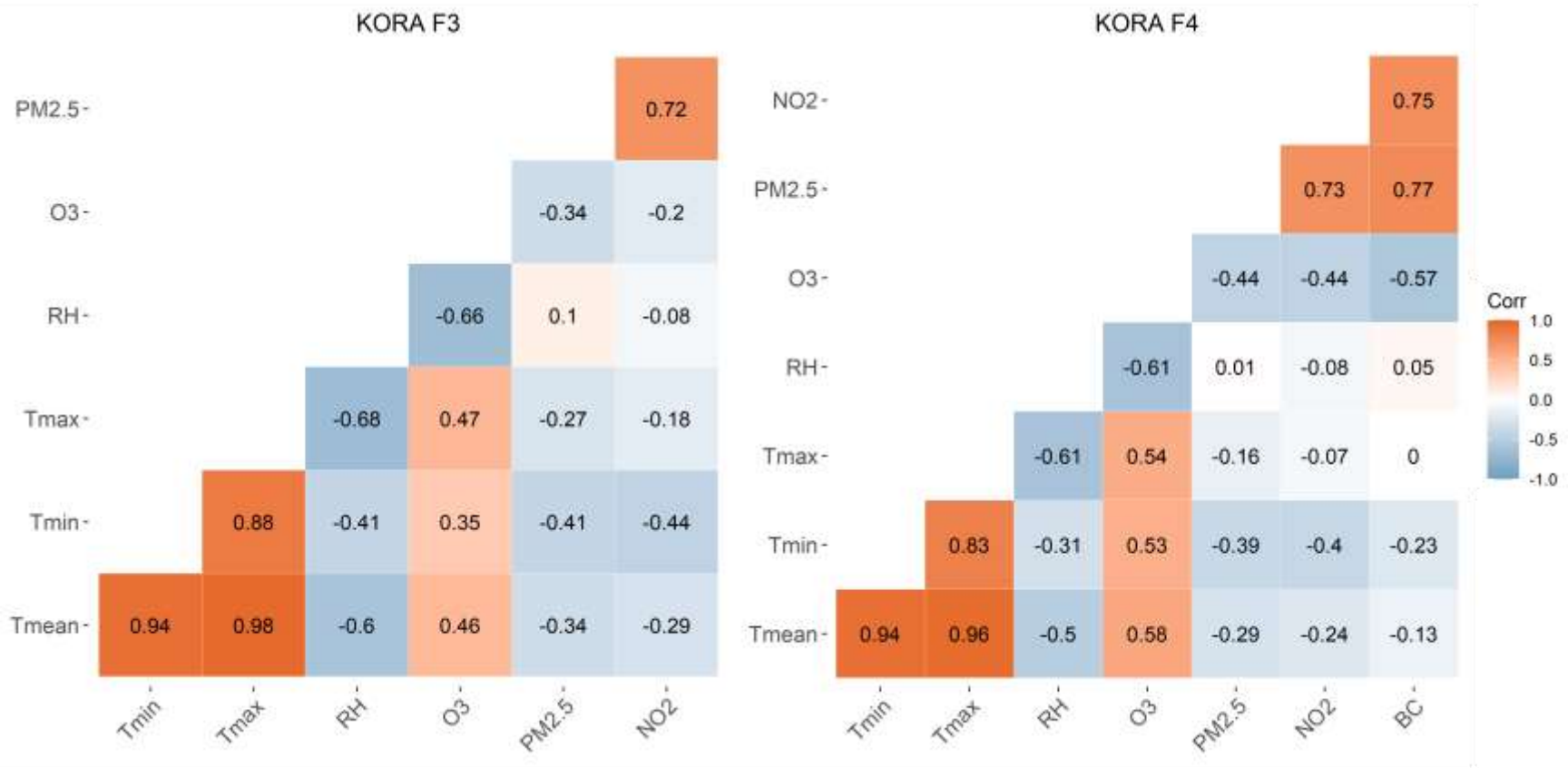


Figure S2. Spearman correlation coefficients (r) between meteorological variables and air pollutants.

*Tmean***:** daily mean temperature; *Tmin*: daily minimum temperature; *Tmax*: daily maximum temperature; *RH*: relative humidity; O_3*:* ozone; $PM_{2.5:}$ particulate matter with an aerodynamic diameter of ≤2.5 µm; NO_2: nitrogen dioxide; *BC*: black carbon.

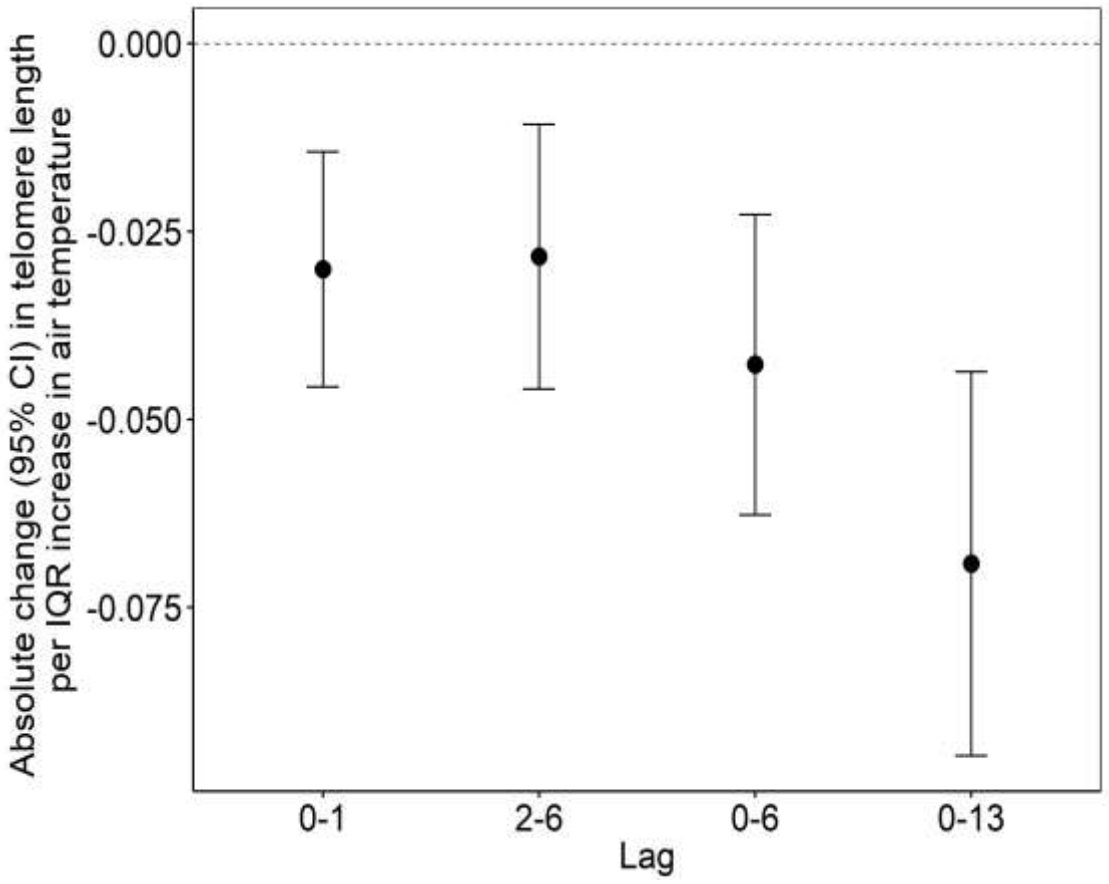
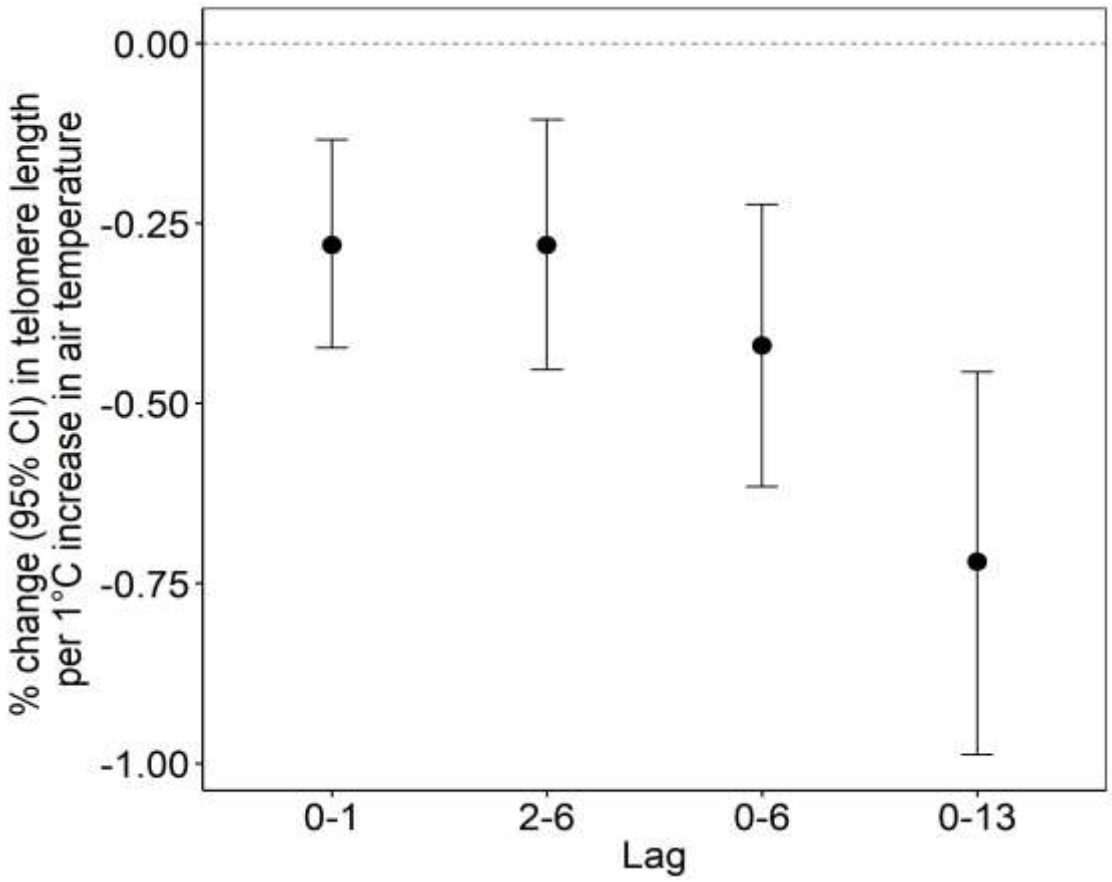


Figure S3. Estimated effects (absolute change [95% CI]) of air temperature on leukocyte telomere length between individuals at lags 0-1, 2-6, 0-6, and 0-13 days per IQR increase in air temperature.

Generalized additive models were adjusted for age, sex, BMI, education, smoking status, alcohol consumption, physical activity, day of the week, season, time trend (cubic spline with ten degrees of freedom), and relative humidity with the same lag period as the air temperature. Effect estimates were presented as absolute changes with 95% CIs per IQR increase in air temperature. The respective IQR increases were 10.77°C for lags 0-1 days, 10.11°C for lags 2-6 days, 10.15°C for lags 0-6 days, and 9.54°C for lags 0-13 days.



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Generalized additive models were adjusted for age, sex, BMI, education, smoking status, alcohol consumption, physical activity, day of the week, season, time trend (cubic spline with ten degrees of freedom), and relative humidity with the same lag period as the air temperature. Effect estimates were presented as percent changes of the geometric outcome mean with 95% CIs per 1°C increase in air temperature. Heterogeneity testing across KORA F3 and KORA F4: lag 0-1, $I^2 = 0$, P = 0.71; lag 2-6, $I^2 = 0$, P = 0.44; lag 0-6, $I^2 = 0$, P = 0.42; lag 0-13, $I^2 = 13.46$, P = 0.28.

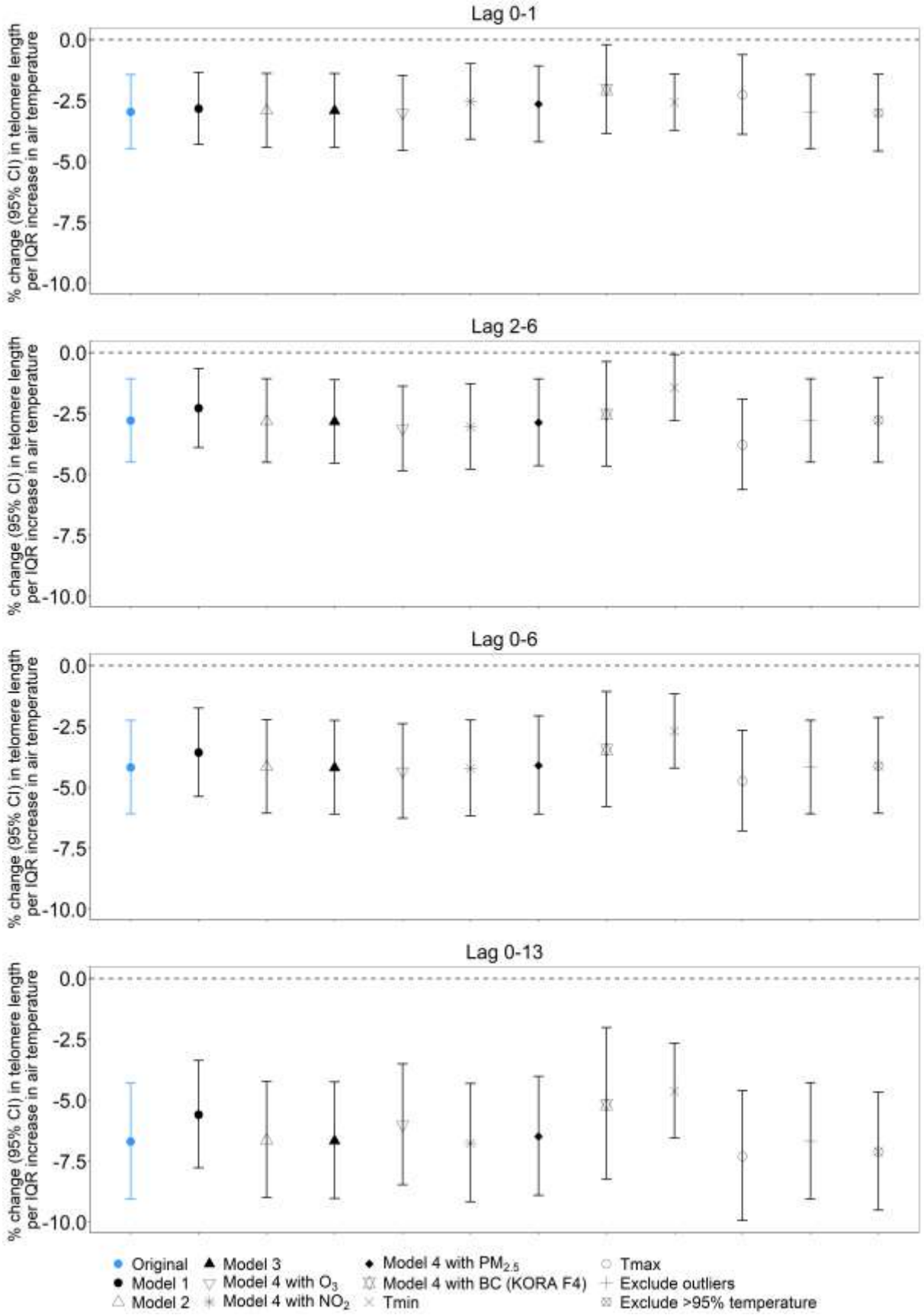
Lag 0-1
Lag 2-6
Lag 0-6
Lag 0-13
% change (95% CI) in telomere length per IQR increase in air temperature
0.0
-2.5
-5.0
-7.5
-10.0
Original
Model 1
Model 2
Model 3
Model 4 with O_3
Model 4 with NO_2
Model 4 with $PM_{2.5}$
Model 4 with BC (KORA F4)
Tmin
Tmax
Exclude outliers
Exclude >95% temperature

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Figure S5. Sensitivity analysis: Estimated effects (percent change [95% CI] of geometric mean) of air temperature on leukocyte telomere length between individuals at lags 0-1, 2-6, 0-6, and 0-13 days per IQR increase in air temperature.

Effect estimates were presented as percent changes of the geometric outcome mean with 95% CIs per IQR increase in air temperature. *Original*: main model; *Model 1*: only adjusted for age, sex, BMI, day of the week, time trend and relative humidity; *Model 2:* Main model additionally adjusted for total cholesterol, triglyceride, LDL, and HDL. *Model3:* Model 2 additionally adjusted for medication intake (antihypertensive, lipid-lowering, or antidiabetic medication); *Model 4 with* O_3: Model 3 additionally adjusted for O_3 with the same lag period as the air temperature; *Model 4 with* NO_2: Model 3 additionally adjusted for NO_2 with the same lag period as the air temperature; *Model 4 with* $PM_{2.5}$: Model 3 additionally adjusted for $PM_{2.5}$ with the same lag period as the air temperature; *Model 4 with BC*: Model 3 additionally adjusted for BC with the same lag period as the air temperature (KORA F4); *Tmin*: daily minimum temperature; *Tmax*: daily maximum temperature. *Exclude outliers:* leukocyte telomere length values less than the first quartile of the data (Q1) - 1.5 × IQR or more than the third quartile of the data (Q3) + 1.5 × IQR were excluded. *Exclude >95% temperature:* subjects exposed to air temperature greater than 95% of temperature were excluded.

Publication II

Title: Associations between medium- and long-term exposure to air temperature and epigenetic age acceleration

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Journal: Environment International

Status: Published

Volume: 178

Page: 108109

Year: 2023

doi: 10.1016/j.envint.2023.108109

Environment International 178 (2023) 108109

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

Associations between medium- and long-term exposure to air temperature and epigenetic age acceleration

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ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Air temperature
Medium- and long-term effects
Spatial and temporal variation
Epigenetic age acceleration

ABSTRACT

Climate change poses a serious threat to human health worldwide, while aging populations increase. However, no study has ever investigated the effects of air temperature on epigenetic age acceleration. This study involved 1,725 and 1,877 participants from the population-based KORA F4 (2006–2008) and follow-up FF4 (2013–2014) studies, respectively, conducted in Augsburg, Germany. The difference between epigenetic age and chronological age was referred to as epigenetic age acceleration and reflected by Horvath's epigenetic age acceleration (HorvathAA), Hannum's epigenetic age acceleration (HannumAA), PhenoAge acceleration (PhenoAA), GrimAge acceleration (GrimAA), and Epigenetic Skin and Blood Age acceleration (SkinBloodAA). Daily air temperature was estimated using hybrid spatiotemporal regression-based models. To explore the medium- and long-term effects of air temperature modeled in time and space on epigenetic age acceleration, we applied generalized estimating equations (GEE) with distributed lag non-linear models, and GEE, respectively. We found that high temperature exposure based on the 8-week moving average air temperature (97.5th percentile of temperature compared to median temperature) was associated with increased HorvathAA, HannumAA, GrimAA, and SkinBloodAA: 1.83 (95% CI: 0.29–3.37), 11.71 (95% CI: 8.91–14.50), 2.26 (95% CI: 1.03–3.50), and 5.02 (95% CI: 3.42–6.63) years, respectively. Additionally, we found consistent results with high temperature exposure based on the 4-week moving average air temperature was associated with increased HannumAA, GrimAA, and SkinBloodAA: 9.18 (95% CI: 6.60–11.76), 1.78 (95% CI: 0.66–2.90), and 4.07 (95% CI: 2.56–5.57) years, respectively. For the spatial variation in annual average temperature, a 1 °C increase was associated with an increase in all five measures of epigenetic age acceleration (HorvathAA: 0.41 [95% CI: 0.24–0.57], HannumAA: 2.24 [95% CI: 1.95–2.53], PhenoAA: 0.32 [95% CI: 0.05–0.60], GrimAA: 0.24 [95%: 0.11–0.37], and SkinBloodAA: 1.17 [95% CI: 1.00–1.35] years). In conclusion, our results provide first evidence that medium- and long-term exposures to high air temperature affect increases in epigenetic age acceleration.

1. Introduction

Climate change poses a serious threat to human health worldwide. There is accumulating evidence that higher air temperatures are associated with increased risks for many diseases, especially age-related disease (Chen et al., 2018; Khraishah et al., 2022). The 2021 report of the Lancet Countdown on health and climate change showed that compared to the annual averages of the 1986–2005 baseline, the record temperatures in 2020 were related to a new high of 3.1 billion more person-days of heatwave exposure among people older than 65 years

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https://doi.org/10.1016/j.envint.2023.108109
Received 6 March 2023; Received in revised form 12 July 2023; Accepted 20 July 2023
Available online 23 July 2023

(Romanello et al., 2021). A global study found that 37.0% (global range from 20.5% to 76.3%) of warm-season heat-related deaths can be attributed to man-made climate change from 1991 to 2018 based on data from 732 locations in 43 different countries (Vicedo-Cabrera et al., 2021). Furthermore, exposure to high temperature can lead to physiologic dysfunction across some pathways, which possibly includes ageing ones, e.g. cholesterol (Madaniyazi et al., 2020); although this has not been extensively explored.

A further aspect to consider is that the average age of the population is increasing globally. The world's population aged 60 years and more is estimated to increase to more than 2 billion by 2050 (Chatterji et al., 2015); which brings unprecedented challenges. Therefore, a better understanding of the effects of non-optimal environmental conditions on aging is crucial (Peters et al., 2021). Epigenetic age predictors, also defined as epigenetic clocks or DNA methylation ages, are emerging robust biomarkers of biological aging and have been suggested as novel DNA methylation-based biomarkers for the aging process in humans (Simpson and Chandra, 2021). Epigenetic age predictors are based on methylation levels of genome-wide selected CpG sites and are coupled with regression models to compute an estimate of biological age (Noroozi et al., 2021). There are two generations of epigenetic age predictors. The first-generation clocks, Horvath's epigenetic age and Hannum's epigenetic age, were developed to predict chronological age in 2013 and are the most widely used clocks (Horvath, 2013; Hannum et al., 2013). The next-generation clocks, which evolved to combine aging-related features (physiological or cellular aging) to define composite epigenetic age metrics, including PhenoAge and GrimAge, were subsequently developed to better capture changes in biological aging, and predict lifespan and healthspan (Levine et al., 2018; Lu et al., 2019). Furthermore, Horvath et al. developed another novel epigenetic age predictor, the Epigenetic Skin and Blood Age, which has been shown to provide more accurate estimates of chronological age when applied to blood-derived samples compared to the original Horvath' and Hannum' epigenetic age predictors (Horvath et al., 2018).

Epigenetic age acceleration is defined as the discrepancy between DNA methylation-based biological age and chronological age. A positive epigenetic age acceleration value indicates accelerated epigenetic aging, while a negative value of epigenetic age acceleration indicates decelerated epigenetic aging (Noroozi et al., 2021). Epigenetic age acceleration has been associated with increased risks of chronic diseases, such cardiovascular disease, diabetes, and mortality (Wang et al., 2021; Perna et al., 2016; Simpson and Chandra, 2021; Oblak et al., 2021). Previous studies have shown that epigenetic age acceleration is influenced by lifestyle and environmental factors (Oblak et al., 2021); including tobacco smoking (Wu et al., 2019); and air pollution (Ward-Caviness et al., 2020). These suggest that epigenetic age acceleration may capture perturbations in biological processes triggered by environmental exposures, which can then lead to adverse health outcomes. So far, no study has yet investigated the effects of air temperature on epigenetic age acceleration.

Therefore, the goal of this population-based study was to investigate the impact of medium- and long-term air temperature exposure (temporal-spatial variation of 4-week and 8-week averages of air temperature and the spatial variation of annual average air temperature) on epigenetic age acceleration using a longitudinal study design. The 4-week and 8-week exposure windows allow for the examination of temperature exposures over a period of several weeks, which aligns with the timescales of physiological and biological responses to changing temperature conditions. By focusing on these medium-term exposures, we aimed to investigate the delayed responses and adaptation mechanisms of individuals to shifting temperature patterns, which are often manifested over weeks rather than days or years. Moreover, the annual average temperature provides a representative measure of the long-term climatic conditions experienced by individuals. This enables us to assess the sustained exposure to temperature over an extended period, which may have a more pronounced impact on human health and aging processes. Importantly, it also captures sustained changes in temperature associated with climate change over time.

2. Materials and methods

2.1. Study population

This study was based on data from the Cooperative Health Research in the Region of Augsburg (KORA) studies F4 (2006–2008) and FF4 (2013–2014). Both studies were follow-up examinations of the population-based KORA S4 study (1999–2001) conducted in the city of Augsburg, Southern Germany, and two adjacent counties (Holle et al., 2005). Participants underwent physical examinations and standardized interviews assessing sociodemographic characteristics and medical history. Detailed information on the KORA cohort design, measurement, and data collection have been described elsewhere (Holle et al., 2005; Rathmann et al., 2009; Wawro et al., 2020). The present study included 1,725 participants from KORA F4 and 1,877 participants from KORA FF4, who underwent genome-wide DNA methylation measurements using the Illumina 450 K Infinium Methylation BeadChip for KORA F4 and Infinium MethylationEPIC BeadChip for KORA FF4.

The study was approved by the ethics board of the Bavarian Chamber of Physicians (Munich, Germany) in adherence to the declaration of Helsinki. All participants gave written informed consent.

2.2. Exposure assessment

High-resolution (1 × 1 km) daily mean air temperature data were derived for the whole country of Germany using hybrid spatiotemporal regression-based models (Young Virtual Conference and Basel, 2021). These included satellite-based land surface temperature data, ground-based air temperature measurements, and spatial predictors datasets. Three-stage models were trained to achieve air temperature predictions with comprehensive temporal and spatial coverage. The satellite-derived land surface temperature and spatial predictors were modeled as linear mixed effects models with daily random intercepts and slopes using the combinations of days and grid cells where air temperature measurements and satellite-derived land surface temperature were available. A second stage was carried out using the first-stage model to predict air temperature for grid cells with no air temperature measurements but with available satellite-derived land surface temperature data. In cases where neither satellite-derived land surface temperature data nor air temperature measurements were available, the second stage air temperature predictions were regressed against thin-plate spline interpolated air temperature values to achieve full air temperature coverage in the country. The prediction accuracy of our models was quantified using internal and external 10-fold cross-validation. All models performed well ($0.91 \leq R^2 \leq 0.98$), and all models had low errors (1 °C < Root Mean Square Error < 2 °C). In this study, the residential address of each participant was effectively linked to the nearest exposure grid centroid, facilitating the integration of individual participant data with daily exposure data characterized by high spatial resolution (1 × 1 km). This linkage was established through the utilization of pseudonymized participant IDs and exposure grid IDs, thereby ensuring privacy and confidentiality.

We measured the daily concentrations of relative humidity (RH), ozone (O_3), particulate matter with an aerodynamic diameter of ≤ 2.5 μm ($PM_{2.5}$) and nitrogen dioxide (NO_2) by fixed monitoring sites in Augsburg, Germany (Wolf et al., 2015; Chen et al., 2019). RH and O_3 were measured at the official urban background monitoring site located approximately 5 km south of the city center. $PM_{2.5}$ and NO_2 were measured at an urban background monitoring site located approximately 1 km south of the city center, and approximately 2 km north of the city center, respectively. Annual average concentrations of O_3, $PM_{2.5}$ and NO_2 were estimated using land-use regression (LUR) models (Wolf et al., 2017). Briefly, three 2-week measurement campaigns were

conducted at 20 locations in the KORA study area between March 6, 2014, and April 7, 2015. These measurements were taken at each location during the warm, cold, and intermediate seasons. LUR models were then developed by regressing the annual average concentrations measurement at monitoring sites against spatial predictors derived from geographic information systems. Based on the fitted models, we calculated the level of residential exposure for KORA study participants.

2.3. Epigenetic age acceleration

We uploaded normalized DNA methylation data to the online New DNA Methylation Age Calculator (https://dnamage.genetics.ucla.edu/new) according to the recommended guidelines to estimate epigenetic age. Horvath's epigenetic age and Hannum's epigenetic age were calculated using 353 age-related CpGs and 71 age-related CpGs, respectively (Horvath, 2013; Hannum et al., 2013). PhenoAge was developed using 513 phenotypic age-related CpGs; which were DNA methylation surrogate of nine clinical biomarker (alkaline phosphatase, albumin, C-reactive protein, red cell distribution width, creatinine, lymphocyte percent, glucose, mean cell volume, and white blood cell count) (Levine et al., 2018). GrimAge was developed as a function of mortality risk by combining chronological age, sex, and 1030 unique CpGs sites, which were DNA methylation surrogate of cigarette pack-years and DNA methylation surrogates for seven plasma protein markers (growth differentiation factor-15, adrenomedullin, plasminogen activator inhibitor-1, cystatin C, leptin, beta-2-microglobulin, and tissue inhibitor metalloproteinases 1) (Lu et al., 2019). Epigenetic Skin and Blood Age was developed using 391 age-related CpGs (Horvath et al., 2018). In this study, differences between these epigenetic age biomarkers and chronological age (epigenetic age – chronological age) were referred to as epigenetic age acceleration. Therefore, we obtained five epigenetic age acceleration biomarkers: Horvath's epigenetic age acceleration (HorvathAA), Hannum's epigenetic age acceleration (HannumAA), PhenoAge acceleration (PhenoAA), GrimAge acceleration (GrimAA), and Epigenetic Skin and Blood Age acceleration (SkinBloodAA). Details of measures of genome-wide DNA methylation are given in Supplementary material.

2.4. Statistical analysis

We reported the characteristics of participants as mean and standard deviation (SD) for continuous variables and frequencies and percentages for categorical variables.

We applied generalized estimating equations (GEE) with distributed lag non-linear models (DLNMs) (Gasparrini et al., 2010) and GEE to explore the medium- and long-term effects of air temperature on repeatedly assessed epigenetic age acceleration, respectively. In our analysis, we used all available measures of epigenetic age accelerations, including both repeated and non-repeated measures from the KORA F4 and FF4 cohorts, to maximize statistical power. Repeated measures allow for within-person comparisons over time, while non-repeated measures provide additional data points. To calculate the medium-term exposures to air temperature, we used 4-week and 8-week moving averages of daily air temperature before the blood draw. Moreover, as a surrogate for long-term exposure to air temperature, we used the 365-day moving average of daily air temperature (annual average temperature) before the blood draw. First, we conducted generalized additive mixed models with a spline (four degrees of freedom) to assess deviations of the temperature-response relationship from linearity (supplementary, Figure S1). As there were no significant deviations from linearity for annual average temperature on all markers, the annual average temperature was included linearly in the GEE for these outcomes, and effects were estimated as a 1 °C increase in annual average temperature. However, there were non-linear exposure–response functions of 4-week and 8-week moving averages of temperature and epigenetic age acceleration markers, except for PhenoAA. To make the results more comparable between epigenetic age acceleration biomarkers, we included the medium-term exposure parameters non-linearly in the GEE with DLNMs. For these non-linear models (GEE with DLNMs), a natural cubic spline for the exposure–response function with internal knots (30th and 70th percentile of temperature) was selected. In addition, the median temperature value (9.7 °C) was selected as the reference value. We calculated the high temperature effect as the 97.5th percentile of air temperature distribution (18.5 °C and 18.3 °C for 4-week and 8-week moving averages of temperature, respectively) relative to the median temperature, and the low temperature effect as the 2.5th percentile of air temperature distribution (1.5 °C and 1.4 °C for 4-week and 8-week moving averages of temperature, respectively) relative to the median temperature.

All models were adjusted for *a priori* selected covariates according to previous literature (Kresovich et al., 2021; Zhao et al., ; Oblak et al., 2021) and our own experience: chronological age (years), sex (male, female), education (years), body mass index (BMI, kg/m^2), alcohol consumption (g/day), smoking status (never, former, current), physical activity (low: no exercise at all; medium: occasionally or regularly approximately one hour per week; high: at least two hours per week regularly), season of blood draw (warm: April-September vs. cold: October-March), estimated cell types (monocytes, B Cells, CD4 T cells, CD8 T cells, and natural killer cells). Additionally, to account for potential technical effect, we adjusted for the chip as a covariate in all models. For medium-term temperature effects, we additionally adjusted for day of the week, time trend (natural cubic spline with five degrees of freedom per year), and relative humidity (with the same lag period as the air temperature).

We included an interaction term between air temperature and potential effect modifier in the effect modification analysis. The examined modifiers included sex (male vs. female), obesity (BMI < 30 kg/m^2 vs. ≥ 30 kg/m^2), cardiovascular disease (defined as a history of hypertension, myocardial infarction, angina pectoris, or stroke [yes vs. no]), diabetes (yes vs. no), and areas (urban vs. rural).

We performed several sensitivity analyses to assess the robustness of our results. First, to control for potential confounding from air pollution, we additionally adjusted for O_3, $PM_{2.5}$, and NO_2, separately. Second, we excluded participants who moved throughout the study period to reduce exposure misclassification. Third, instead of epigenetic age acceleration, we used epigenetic age and the residuals computed by linearly regressing chronological age on epigenetic age to explore the air temperature effects. Fourth, we excluded values that deviated beyond 1.5 times the interquartile range from either the lower quartile or the upper quartile to avoid the effects of extreme outliers of outcomes. Fifth, we included only participants with repeated measurements of epigenetic age acceleration (985 participants with 1,970 observations) in the analysis. Sixth, to avoid overestimation, for non-linear exposure–response functions (medium-term effects), the effects were estimated for high temperature as 97.5th percentile of air temperature distribution compared to the 75th percentile (14.5 °C and 14.3 °C for 4-week and 8-week moving averages of temperature, respectively) and for low temperature as 2.5th percentile of air temperature distribution compared to the 25th percentile (4.1 °C and 4.2 °C for 4-week and 8-week moving averages of temperature, respectively). Seventh, we incorporated season into the model as a four-factor category consisting of spring (March - May), summer (June - August), fall (September - November), and winter (December - February), rather than treating season as a dichotomous variable. Finally, 4- and 8-week moving averages of temperature were included as a linear term in the GEE model for PhenoAA.

For the long-term effects (linear associations), we quantified the effect estimates as the change in epigenetic age acceleration per 1 °C increase in air temperature with corresponding 95% confidence intervals (CIs). For the medium-term effects (non-linear associations), the effect estimates indicate changes in epigenetic age acceleration per increase or decrease in air temperature from the median to the 97.5th and 2.5th percentiles, respectively, with 95% (CIs). Additionally, to facilitate

comparisons of the effects across different epigenetic age acceleration markers, we expressed the effect estimates as percent changes relative to the standard deviation (SD). The multiple tests were adjusted using Benjamin-Hochberg false discovery rate (FDR) methods, and adjusted $p < 0.05$ was considered for the significance. All statistical analyses were done with R (version 4.1.2).

3. Results

3.1. Study population, epigenetic age acceleration, and exposure data

There were 3,602 observations from 2,617 participants of KORA F4 (1,725) and FF4 (1,877) included in this analysis. Of these 2,617 participants, 985 (37.6%) completed two examinations. Table 1 presents the characteristics of the study population in KORA F4 and KORA FF4. The mean chronological age was 61.0 years in KORA F4 and 58.6 years in KORA FF4. The mean BMI in KORA F4 and FF4 was 28.1 kg/m^2 and 27.8 kg/m^2, respectively. 48.9% and 47.7% of the participants were male in KORA F4 and FF4, respectively. Of the participants, 31.4% and 27.2% had low physical activity in KORA F4 and FF4, respectively.

The levels of epigenetic age acceleration are presented in Table 2. The mean was −2.3 years, 0.6 years, −9.7 years, −0.2 years, and −2.7 years for HorvathAA, HannumAA, PhenoAA, GrimAA, SkinBloodAA, respectively. There were weak correlations between epigenetic age acceleration biomarkers (r: 0.34–0.44), except for HannumAA and SkinBloodAA (r = 0.81). The levels of epigenetic age acceleration at each examination are presented in Supplementary Table S1.

Table 3 shows the distributions of meteorological variables and air pollutants. The mean of the 4-week moving average temperature was 9.4 °C, the 8-week moving average temperature was 9.5 °C, and the annual average temperature was 9.3 °C for all 3,602 observations. The distributions of meteorological variables and air pollutants at each examination are presented in Supplementary Table S2.

Table 1
Descriptive statistics of participant characteristics and epigenetic age acceleration at each examination.

	KORA F4 (n = 1,725)	KORA FF4 (n = 1,877)
Chronological age (years)	61.0 (8.9)	58.6 (11.6)
Sex (male)	843 (48.9%)	895 (47.7%)
Body mass index (kg/m^2)	28.1 (4.78)	27.8 (5.12)
Education (years)	11.5 (2.64)	12.0 (2.66)
Smoking status		
Never	721 (41.8%)	770 (41.0%)
Former smoker	754 (43.7%)	796 (42.4%)
Current smoker	248 (14.4%)	311 (16.6%)
Physical activity		
Low	542 (31.4%)	511 (27.2%)
Medium	736 (42.7%)	863 (46.0%)
High	445 (25.8%)	503 (26.8%)
Alcohol consumption (g/day)	15.5 (20.5)	14.6 (19.4)
History of diseases		
Cardiovascular diseases (yes)	814 (47.2%)	701 (37.3%)
Diabetes (yes)	158 (9.2%)	162 (8.6%)
Season		
Cold	1147 (66.5%)	785 (41.8%)
Warm	578 (33.5%)	1092 (58.2%)
Areas		
Urban	763 (44.2%)	734 (39.1%)
Rural	959 (55.6%)	1139 (60.7%)
Estimated cell types, %		
CD8 + T lymphocytes	5.9 (6.3)	5.0 (3.8)
CD4 + T lymphocytes	16.3 (6.7)	18.6 (5.9)
Natural killer cells	4.3 (3.1)	6.5 (3.8)
B cells	5.0 (3.0)	5.4 (3.0)
Monocytes	14 (2.8)	7.0 (2.2)

Note: Data are reported as mean (SD) or n (%). *KORA*: Cooperative Health Research in the Region of Augsburg. *F4*: first follow-up examination of KORA S4. *FF4*: second follow-up examination of KORA S4.

3.2. Effects of air temperature on epigenetic age acceleration

We found significant medium-term effects of high temperature for the 4-week (Fig. 1. A) and the 8-week moving average of air temperature (Fig. 1. B) on HorvathAA, HannumAA, GrimAA, and SkinBloodAA. For an increment in the 4-week moving average air temperature from the median (9.7 °C) to the 97.5th percentile (18.5 °C, high temperature exposure), HannumAA, GrimAA, and SkinBloodAA significantly increased by 9.18 (95% CI: 6.60–11.76), 1.78 (95% CI: 0.66–2.90), and 4.07 (95% CI: 2.56–5.57) years, respectively. HorvathAA showed a borderline significant association (1.36 years, 95% CI: −0.005–2.73). In addition, for an increment in the 8-week moving average air temperature from the median (9.7 °C) to the 97.5th percentile (18.3 °C, high temperature exposure), HorvathAA, HannumAA, GrimAA, and SkinBloodAA significantly increased by 1.83 (95% CI: 0.29–3.37), 11.71 (95% CI: 8.91–14.50), 2.26 (95% CI: 1.03–3.50), and 5.02 (95% CI: 3.42–6.63) years, respectively. No significant effects were found for low temperature for the 4-week or 8-week moving averages.

Regarding the long-term effects of annual average temperature (Fig. 2), we found a 1 °C increase in annual average temperature to be significantly associated with an increase in HorvathAA, HannumAA, PhenoAA, GrimAA, and SkinBloodAA (0.41 [95% CI: 0.24–0.57], 2.24 [95% CI: 1.95–2.53], 0.32 [95% CI: 0.05–0.60], 0.24 [95% CI: 0.11–0.37], and 1.17 [95% CI: 1.00–1.35] years, respectively).

Furthermore, effect estimates expressed as percent changes of the SD of outcomes with 95% CIs are shown in Figure S2 (Supplementary). The effect estimates for potential demographic and lifestyle confounders are presented in Figure S3 (Supplementary).

3.3. Effect modification

Fig. 3 shows that the long-term effects of annual average temperature were slightly stronger for female participants for most of the biomarkers, with a significant difference between women and men for HovathAA and SkinBloodAA only. Also, obese participants showed slightly stronger effects with a significant difference compared to the non-obese participants only for GrimAA. A slight indication of stronger effects could also be found for participants with cardiovascular disease, but none of the biomarkers showed a significant difference compared to participants without cardiovascular disease. Moreover, participants with diabetes demonstrated significantly stronger effects than those without diabetes for HannumAA, PhenoAA, and SkinBloodAA. There were no significant effect modifications for 4- and 8-week moving averages of air temperature (Supplementary Figure S4 and S5). Finally, there were no effect modifications with urban and rural areas, except that participants living in rural areas showed a significantly stronger effect of annual average temperature on HannumAA (data not shown).

3.4. Sensitivity analysis

In general, associations between medium- and long-term exposure to air temperature and epigenetic age acceleration were robust to a series of sensitivity analyses (Supplementary Figures S6 and S7, and Table S3). We found similar effect estimates when additionally adjusting for air pollutants, except for medium-term effects on HannumAA and SkinBloodAA, which was slightly decreased. Secondly, the observed associations remained robust when restricting the analyses to the subpopulation that did not move throughout the study period and after excluding outliers. Thirdly, alternative outcome metrics (epigenetic age and epigenetic age acceleration: residuals) also showed similar effects. Additionally, the restriction to participants with repeated measurements of epigenetic age acceleration did not affect most of our results, expect for effects of the annual average temperature on GrimAA were decreased. Fifth, when we used the 25th and 75th percentile of the air temperature distribution as reference values, the effect estimates still showed significant associations, although the effect estimates values

Table 2
Descriptive statistics and Spearman correlation coefficients of epigenetic age acceleration across all observations.

	Mean	SD	25%	Median	75%	Correlation coefficients				
						HorvathAA	HannumAA	PhenoAA	GrimAA	SkinBloodAA
HorvathAA (years)	−2.3	5.1	−5.5	−2.1	1.1	–				
HannumAA (years)	0.6	10.1	−7.7	−0.9	8.9	0.34	–			
PhenoAA (years)	−9.7	6.8	−14.3	−10.1	−5.5	0.40	0.41	–		
GrimAA (years)	−0.2	5.2	−3.7	−0.7	2.6	0.41	0.43	0.37	–	
SkinBloodAA (years)	−2.7	5.2	−6.3	−2.9	1.1	0.44	0.81	0.43	0.36	–

Note: *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration. *SD*: standard deviation.

Table 3
Descriptive analysis of meteorological variables and air pollutants.

	Mean	SD	2.5%	25%	Median	75%	97.5%
Medium-term							
4-week moving average of temperature							
Tmean (°C)	9.4	5.6	1.5	4.1	9.7	14.5	18.5
RH (%)	74.8	7.4	59.3	69.2	75.5	80.7	85.9
$PM_{2.5}$ (μg/m^3)	12.7	4.7	6.2	8.9	12.2	16.2	21.5
O_3 (μg/m^3)	41.5	17.2	18	26.4	37	58.4	70.1
NO_2 (μg/m^3)	29.2	5.5	20.6	24.5	28.5	33.6	39.8
8-week moving average of temperature							
Tmean (°C)	9.5	5.3	1.4	4.2	9.7	14.3	18.3
RH (%)	74.8	6.6	63.2	69.1	76.1	80.7	84.3
$PM_{2.5}$ (μg/m^3)	12.9	3.8	7.7	9.5	12.7	15.9	20.1
O_3 (μg/m^3)	41.2	16.2	18.6	26.1	37	57.2	66.8
NO_2 (μg/m^3)	29.3	4.8	20.7	25	29.5	33.7	36.8
Long-term							
Annual average temperature							
Tmean (°C)	9.3	0.8	8	8.6	9.2	9.9	11
$PM_{2.5}$ (μg/m^3)	11.8	1	9.6	11.1	11.9	12.5	13.6
O_3 (μg/m^3)	39.1	2.4	34.6	37.4	39.2	40.9	43.4
NO_2 (μg/m^3)	14.1	4.4	7	10.6	13.7	17.4	23

Note: *Tmean:* mean temperature. *RH:* relative humidity. *$PM_{2.5}$:* particulate matter with an aerodynamic diameter of ≤ 2.5 μm. *O_3*: ozone; *NO_2*: nitrogen dioxide. *SD*: standard deviation.

decreased for the medium-term effects on HannumAA and SkinBloodAA. Sixth, when we incorporated season into the model as a four-factor category consisting of spring, summer, fall and winter, the results were consistent with the main analyses. Finally, there were also no significant medium-term effects of air temperature on PhenoAA when the 4- and 8-week moving averages of temperature were included as a linear term in the GEE model (Supplementary Table S3).

4. Discussion

To our knowledge, this study, for the first time, explores the impact of temporal-spatial variation of 4-week and 8-week -averages of air temperature and the spatial variation of annual average air temperature on epigenetic age acceleration. In this study, we found significant associations between medium-term exposures to high temperature and increased HorvathAA, HannumAA, GrimAA, and SkinBloodAA. Furthermore, higher annual average temperature exposure was significantly associated with an increase in HorvathAA, HannumAA, PhenoAA, GrimAA, and SkinBloodAA.

Aging involves the accumulation of biological changes in an individual over time that increase the risk for disease and death. Epigenetic clocks provide an opportunity to assess the biological age and general health of individuals (Noroozi et al., 2021; Oblak et al., 2021). Importantly, there has been increasing recognition of the association of epigenetic age acceleration with multiple clinical traits, morbidity, and mortality (Noroozi et al., 2021; Simpson and Chandra, 2021; Oblak et al., 2021; Roberts et al., 2021). We firstly found medium- and long-term effects of higher air temperature on increased epigenetic age accelerations. Our study provides new insights that fill an important

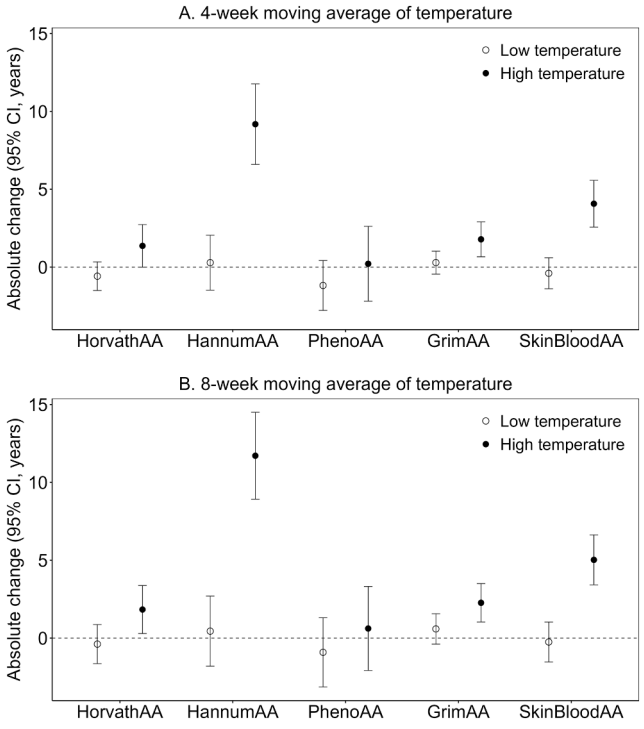

Fig. 1. Medium-term effects of 4- and 8-weeks moving average of air temperature on epigenetic age acceleration.
Note: *Low temperature*: effects of low temperature exposure, 2.5th percentile of temperature compared to median temperature. *High temperature*: effects of high temperature exposure, 97.5th percentile of temperature compared to median temperature. The median temperature was 9.7 °C; 2.5th percentile of temperature was 1.5 °C and 1.4 °C for 4-week and 8-week moving averages of temperature, respectively; 97.5th percentile of temperature was 18.5 °C and 18.3 °C for 4-week and 8-week moving averages of temperature, respectively. *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration.

knowledge gap in the context of a changing climate and, simultaneously, a worldwide aging population (Peters et al., 2021). Furthermore, our findings suggest that implementing policies to slow the rate of climate change may contribute in part to prolonging lifespans and decreasing or delaying health risks associated with aging.

We observed that HannumAA and SkinBloodAA appeared to be the most sensitive biomarkers to high temperature exposures. However, high temperatures also affected three other epigenetic age acceleration metrics: HorvathAA, PhenoAA, and GrimAA. These five clocks seem to capture different aspects of aging based on how they are constructed (Simpson and Chandra, 2021). HannumAA is a first-generation blood-specific age predictor designed to improve the accuracy of blood age estimation (Simpson and Chandra, 2021). Previous studies have shown

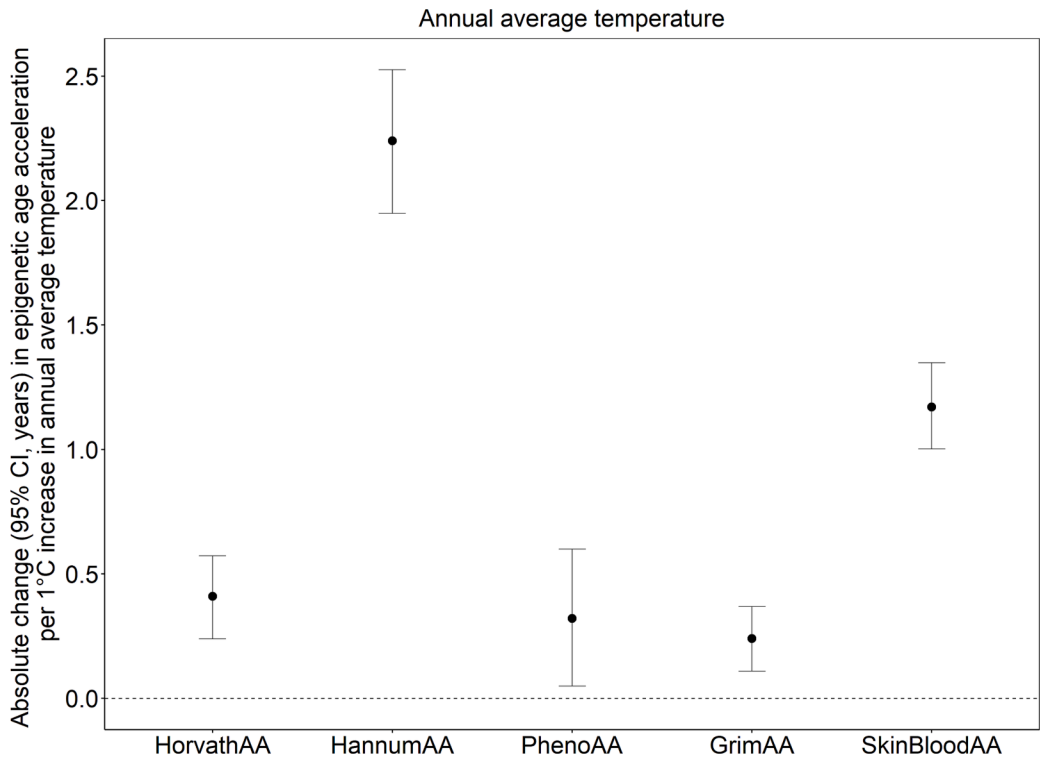

Fig. 2. Long-term effects of annual average temperature per 1 °C increase on epigenetic age acceleration.
Note: *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration.

that Hannum's clock indicates immune system aging (Stevenson et al., 2018; Gibson et al., 2019; Jonkman et al., 2022; Dhingra et al., 2018). For example, a *meta*-analysis of genome-wide association studies of epigenetic age acceleration showed that genes associated with Hannum's clock, which several involved in innate immune system pathways (such as *TRIM46* and MUC1) or with metabolic and immune system functions (*MANBA*, *UBE2D3*) (Gibson et al., 2019). Therefore, this suggests that HannumAA may be particularly associated with environmental exposures, e.g., higher air temperature. SkinBloodAA and HorvathAA are both multi-tissue age predictors, and the novel SkinBloodAA is considered to be a more accurate age estimator of blood methylation data (Horvath, 2013; Horvath et al., 2018). To date, few studies are available about the effect of environmental exposures on SkinBloodAA. A previous study showed that SkinBloodAA was the most sensitive to occupational benzene and trichloroethylene exposure (van der Laan et al., 2022). Given these findings and the higher accuracy of SkinBloodAA, it is suggested that SkinBloodAA, which is not yet widely used, maybe a suitable biomarker to explore the association between environmental exposures and biological aging. PhenoAA is optimized to predict physiological dysregulation across multiple systems and predict physical functioning more accurately than previous epigenetic clocks (Levine et al., 2018). GrimAA is a powerful mortality predictor, and strongly predicts lifespan and healthspan (Lu et al., 2019; McCrory et al., 2021). Our results suggest that epigenetic age acceleration may reflect processes that contribute to the often observed associations between high air temperature and mortality and risk of age-related diseases (Chen et al., 2018; Khraishah et al., 2022; Gasparrini et al., 2022); opening up a potentially new pathophysiological pathway in the field of weather, climate, and health research.

We used two exposure window definitions (4- and 8-week moving averages) to assess the medium-term effects of air temperature modeled in space and time on epigenetic age acceleration. We found heat effects for both exposure windows on epigenetic age acceleration. The results of the two time periods were almost identical, indicating our findings' robustness. The medium-term effects of high air temperature on epigenetic age acceleration may be thought to be short lived, as 4- and 8-week high air temperature exposure may not be expected to permanently accelerate epigenetic aging. However, epigenetic age acceleration may be a potential mechanism between air temperature and cardiovascular disease and cerebrovascular disease, as a previous study has revealed that medium-term high air temperature exposures are associated with increased cardiovascular disease and cerebrovascular disease mortality (Wang et al., 2015). Importantly, by using 4- and 8-week moving average temperatures, we can capture the broader temporal context and identify potential cumulative effects that may be missed when focusing solely on long-term exposures. Furthermore, medium-term temperatures encompass both temporal and spatial variability, diverging from capturing to such an extent day-to-day changes of temperature (short-term effects) as well as the climate-related patterns observed in annual assessments. Instead, it captures the variability resulting from distinct weather classes that bring specific weather types to a region and typically dominate the weather for durations longer than days but shorter than a full year. Additionally, as climate change progresses, individuals and communities implement various adaptation strategies to cope with shifting temperature patterns. Investigating medium-term effects helps us understand the delayed responses and adaptation mechanisms of individuals to changing temperature conditions, which often operate on timescales of weeks to months.

In addition to the medium-term effects, we also found significant long-term effects of air temperature on all five epigenetic age acceleration metrics. This implies that spatial variability in annual average temperature strongly affects epigenetic age acceleration and shows a high stability of our results across the different biomarkers. In other research contexts, it is often observed that results are rather inconsistent between the different epigenetic age acceleration metrics (Xu et al., 2021; van der Laan et al., 2021). Hence our study is affirming to see consistent effects of air temperature across the different biomarkers. More importantly, long-term air temperature exposure may have a more

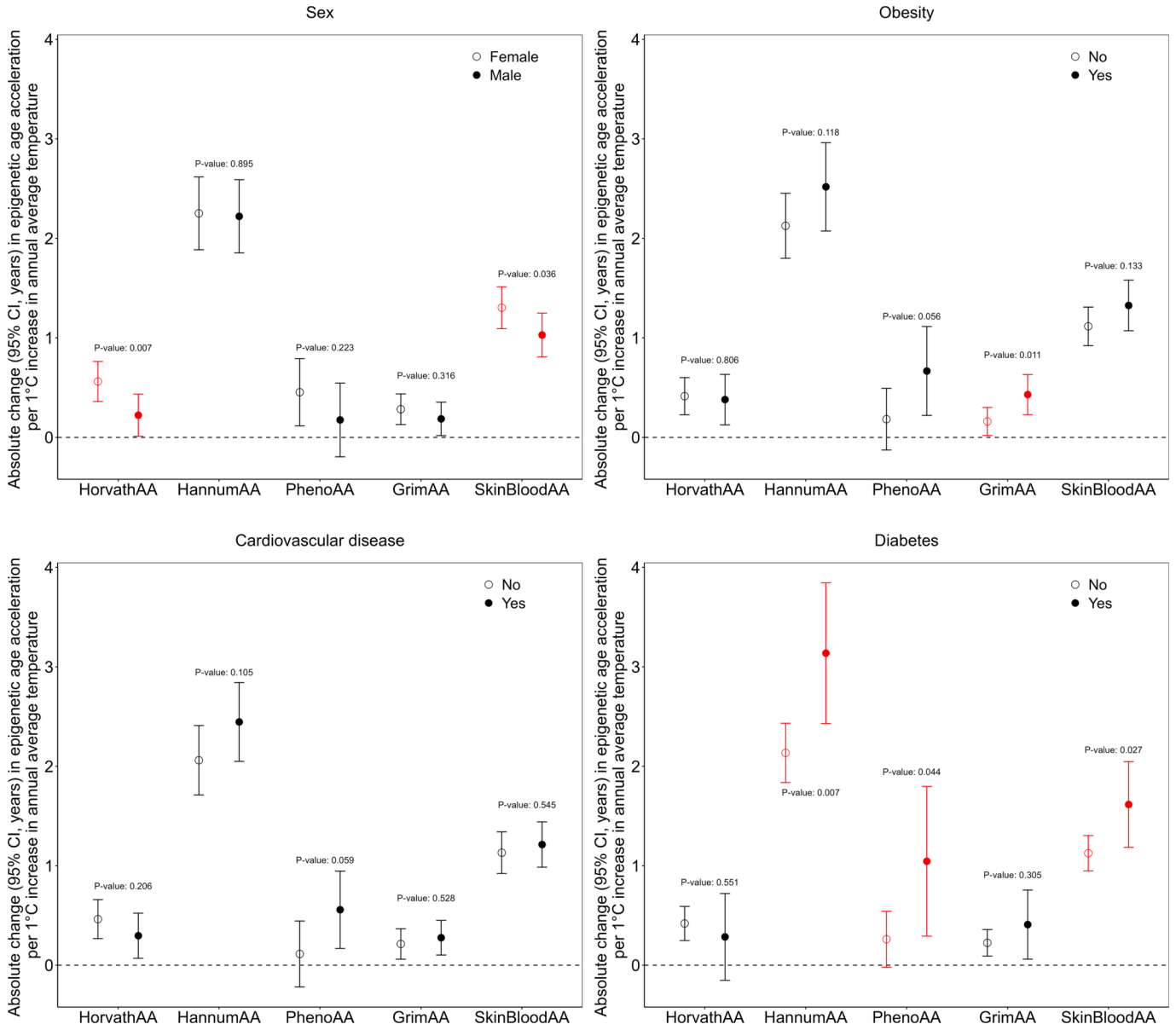

Fig. 3. Long-term effects of annual average temperature per 1 °C increase on epigenetic age acceleration modified by sex, obesity, cardiovascular disease, and diabetes.

Note: Red error bars show significantly different effect estimates between subgroups (*P*-value for the interaction term < 0.05). *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration.

pertinent and clinically relevant health effect, given that individuals are exposed to non-optimal temperatures over longer periods at their residential addresses. And the long-term effects may reflect more long-term stable alterations in accelerated aging as well as physiologic dysfunction that may affect health over an extended period of time. Furthermore, long-term effects help elucidate the relationship between climate-related temperature changes and epigenetic age acceleration. As climate change involves long-term shifts in temperature patterns, studying the long-term effects provides valuable insights into the potential consequences of ongoing climate change on human health and aging.

Spatial variability of annual averages temperature had stronger effects on epigenetic age acceleration in women, participants with obesity or diabetes, compared to men, participants without obesity or diabetes. This suggests that certain subpopulations may be more susceptible to high temperature and need more protection than others. Some studies also found higher mortality risks associated with exposure to higher temperatures among women (Chen et al., 2018; Achebak et al., 2019; Petkova et al., 2021). This may be related to the fact that females have a lower thermosensitivity in their response to temperature stimuli and a lower sweating capacity, which causes a greater increase in body temperature (Gagnon et al., 2013; Gagnon and Kenny, 2011; Gagnon and Kenny, 2012). Further, it has been observed that women have a higher threshold for activating their sweating mechanisms at high temperatures (Bittel et al., 1975). Participants with certain pre-existing health conditions, including obesity or diabetes, may also be more susceptible to high temperature. People with diabetes often have impaired endothelial function or poor blood flow to the skin, which can compromise their thermoregulation and affect the mechanisms of heat dissipation at high temperatures (Petrofsky, 2011). In addition, the increased insulation in obese adults increases thermal resistance between the core and the skin, thereby reducing heat dissipation from the core to the skin (Zhang et al.,

2016).

There are several strengths of the present study. Firstly, this is the first study to explore the association between air temperature and epigenetic age acceleration with a relatively large sample size of 3,602 observations. Secondly, we used satellite, meteorological, and terrestrial data to estimate the temperature at each participant's residence for each calendar day by a novel hybrid spatiotemporal regression-based model. Thereby, we were able to capture the temporal-spatial variability in monthly averages of temperatures. In contrast, temperature data obtained through one or two fixed monitoring sites would only assess the temporal variation and ignore differences within a region. The novel model gave us sufficient spatial and temporal variability of the analyzed air temperature and reduced exposure misclassification. Thirdly, we used multiple biomarkers of epigenetic clocks to define epigenetic age acceleration. The robust associations across all used biomarkers make us confident that our associations were not observed by chance only.

However, our study also has several limitations. First, this study was performed in a single study center in Augsburg, Germany, which limits the generalizability of our findings and may not reflect individuals from other regions due to potential ethnic, climatic, and geographic differences. Secondly, as an observational study, the possibility of residual confounding and/or unmeasured confounders could not be excluded, although we already adjusted for a large set of covariates. Thirdly, the genome-wide DNA methylation in KORA FF4 was analyzed using the Infinium MethylationEPIC BeadChip, which lacked 19 CpG and 6 CpG sites used to calculate the Horvath' and Hannum' epigenetic age. This may lead to inaccurate estimates of these two epigenetic ages, so results from these should be interpreted with caution. Fourth, we used ambient area-level air temperatures rather than personal exposures to air temperature (including, e.g., also indoor temperatures), which may result in exposure misclassification. Finally, a limitation is that different DNA methylation profiling platforms were used for the two cohorts, which could introduce some technical discrepancy despite adjustments. Residual biases likely remain due to the challenges of fully correcting for different array types.

5. Conclusion

In conclusion, our results provide the first evidence that medium- and long-term exposures to high air temperature affect increases in epigenetic age acceleration. Providing this new pathophysiological pathway could be an important step in preventing the health effects of heat, especially for susceptible population subgroups - particularly when considering the predicted future increases in the number of hot days and more intense heat waves in times of climate change.

Funding

This work was supported by a scholarship under the State Scholarship Fund by the China Scholarship Council (File No. 201906010316). The KORA Study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ.

CRediT authorship contribution statement

Wenli Ni: Conceptualization, Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Nikolaos Nikolaou:** Writing – review & editing. **Cavin K. Ward-Caviness:** Methodology, Writing – review & editing. **Susanne Breitner:** Methodology, Writing – review & editing. **Kathrin Wolf:** Methodology, Writing – review & editing. **Siqi Zhang:** Methodology, Writing – review & editing. **Rory Wilson:** Writing – review & editing. **Melanie Waldenberger:** Writing – review & editing. **Annette Peters:** Conceptualization, Supervision, Funding acquisition, Resources, Writing - review & editing. **Alexandra Schneider:** Conceptualization, Resources, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Acknowledgment

Methylation analysis in KORA FF4 was performed at the Core Facility Genotyping (Genome Analysis Center [GAC], Helmholtz Zentrum München) under the supervision of Dr. Sonja Kunze, Dr. Jennifer Kriebel and Dr. Eva Reischl.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108109.

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Supplementary material for

Associations between medium- and long-term exposure to air temperature and epigenetic age acceleration

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Measures of genome-wide DNA methylation

The genome-wide DNA methylation microarray of whole blood was analyzed in KORA F4 (Illumina Inc.; San Diego, CA, USA) using the Illumina 450K Infinium Methylation BeadChip as described earlier[1]. The probes with SNPs (single nucleotide polymorphisms) were removed and the background was adjusted by minfi[2]. Probes were filtered out if the detection *P*-value was more than 0.01, bead count was less than 3, or if more than 5% of probes failed. Samples were excluded if the detection rate was less than 0.95. The probe intensities were normalized using the quantile normalization procedure in KORA F4. The Illumina genome-wide DNA methylation microarray of whole blood was analyzed in KORA FF4 using the Infinium MethylationEPIC BeadChip (Illumina Inc.; San Diego, CA, USA). Probes with SNPs with a minor allele frequency of more than 5% or the single base extension or with more than 5% missing values were excluded by minfi[2]. The samples were excluded if the reported sex did not match the one predicted by minfi[2], the median intensity was below 50% of the experiment-wide mean, or less than 2,000 arbitrary units, or had more than 5% missing values on the autosomes. The probe intensities were normalized using the quantile normalization procedure in KORA FF4.

Table S1. Descriptive statistics of epigenetic age acceleration for each examination.

	Mean	SD	25%	Median	75%
KORA F4 (N=1,725)					
HorvathAA	-1.8	5	-5	-1.8	1.6
HannumAA	9.6	6	5.9	9.2	12.9
PhenoAA	-8	7.3	-12.9	-8.2	-3.3
GrimAA	1.1	5	-2.4	0.4	3.8
SkinBloodAA	1.1	3.8	-1.4	1.1	3.4
KORA FF4 (N=1,877)					
HorvathAA	-2.8	5.1	-5.8	-2.5	0.8
HannumAA	-7.6	4.7	-10.7	-7.4	-4.4
PhenoAA	-11.3	6	-15.2	-11.3	-7.6
GrimAA	-1.4	5.1	-4.9	-1.8	1.3
SkinBloodAA	-6.2	3.6	-8.4	-6	-3.8

Note: *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration. *SD*: standard deviation.

Table S2. Descriptive analysis of meteorological variables and air pollutants at each examination.

	KORA F4 (N=1,725)							KORA FF4 (N=1,877)						
	Mean	SD	2.5%	25%	Median	75%	97.5%	Mean	SD	2.5%	25%	Median	75%	97.5%
Medium-term														
4-week moving average of temperature														
Tmean (°C)	8.2	5.4	-0.4	3.7	6.2	12.9	17.5	10.5	5.5	1.6	5.2	10.4	15.7	19.6
RH (%)	76.7	6.4	61.5	72	78.3	81.5	85.3	73.1	7.9	58.8	67	73.1	78.9	86.5
$PM_{2.5}$ ($\mu g/m^3$)	15	4.8	5.7	11	15.3	18.1	27.7	10.7	3.6	6.2	8	9.5	13	19.7
O_3 ($\mu g/m^3$)	38.8	16.7	18	26	32.1	56.4	67.4	43.9	17.4	18	27	46.3	58.5	71.7
NO_2 ($\mu g/m^3$)	31.3	5.1	21.5	28	31.8	34.9	40.5	27.2	5	20.1	23	26	31.7	38.3
8-week moving average of temperature														
Tmean (°C)	8.4	5.2	0.9	3.9	7.7	13.4	17.2	9.5	5.3	1.4	4.2	9.7	14.3	18.3
RH (%)	76.8	5.6	65	71	79	81.3	84.5	74.8	6.6	63.2	69	76.1	80.7	84.3
$PM_{2.5}$ ($\mu g/m^3$)	15	3.6	8	13	15.2	17.9	21.6	12.9	3.8	7.7	9.5	12.7	15.9	20.1
O_3 ($\mu g/m^3$)	38.5	15.5	21.5	26	32.1	53.9	66.7	41.2	16.2	18.6	26	37	57.2	66.8
NO_2 ($\mu g/m^3$)	31.3	3.8	23.8	29	31.6	34.3	37.1	29.3	4.8	20.7	25	29.5	33.7	36.8
Long-term														
Annual average temperature														
Tmean (°C)	9.7	0.8	8.1	9.1	9.8	10.4	11.2	8.9	0.6	8	8.4	8.8	9.4	10.1
$PM_{2.5}$ ($\mu g/m^3$)	11.8	1	9.7	11	11.9	12.5	13.6	11.8	1	9.6	11	11.9	12.5	13.6
O_3 ($\mu g/m^3$)	39.1	2.4	34.6	38	39.2	40.8	43.5	39.1	2.4	34.5	37	39.2	40.9	43.2
NO_2 ($\mu g/m^3$)	14.3	4.4	7	11	13.9	17.6	23.2	13.9	4.3	7	10	13.5	17.1	22.7

Note*: Tmean:* mean temperature. *RH:* relative humidity. $PM_{2.5:}$ particulate matter with an aerodynamic diameter of ⩽2.5 μm. O_3: ozone. NO_2: nitrogen dioxide. *SD*: standard deviation.

Table S3. Sensitivity analysis: Medium-term effects of air temperature per 1°C increase on PhenoAA (linear model: GEE).

Exposure	Absolute change (95% CI, years)
4-week moving average of temperature	0.09 (-0.09. 0.26)
8-week moving average of temperature	0.09 (-0.13. 0.32)

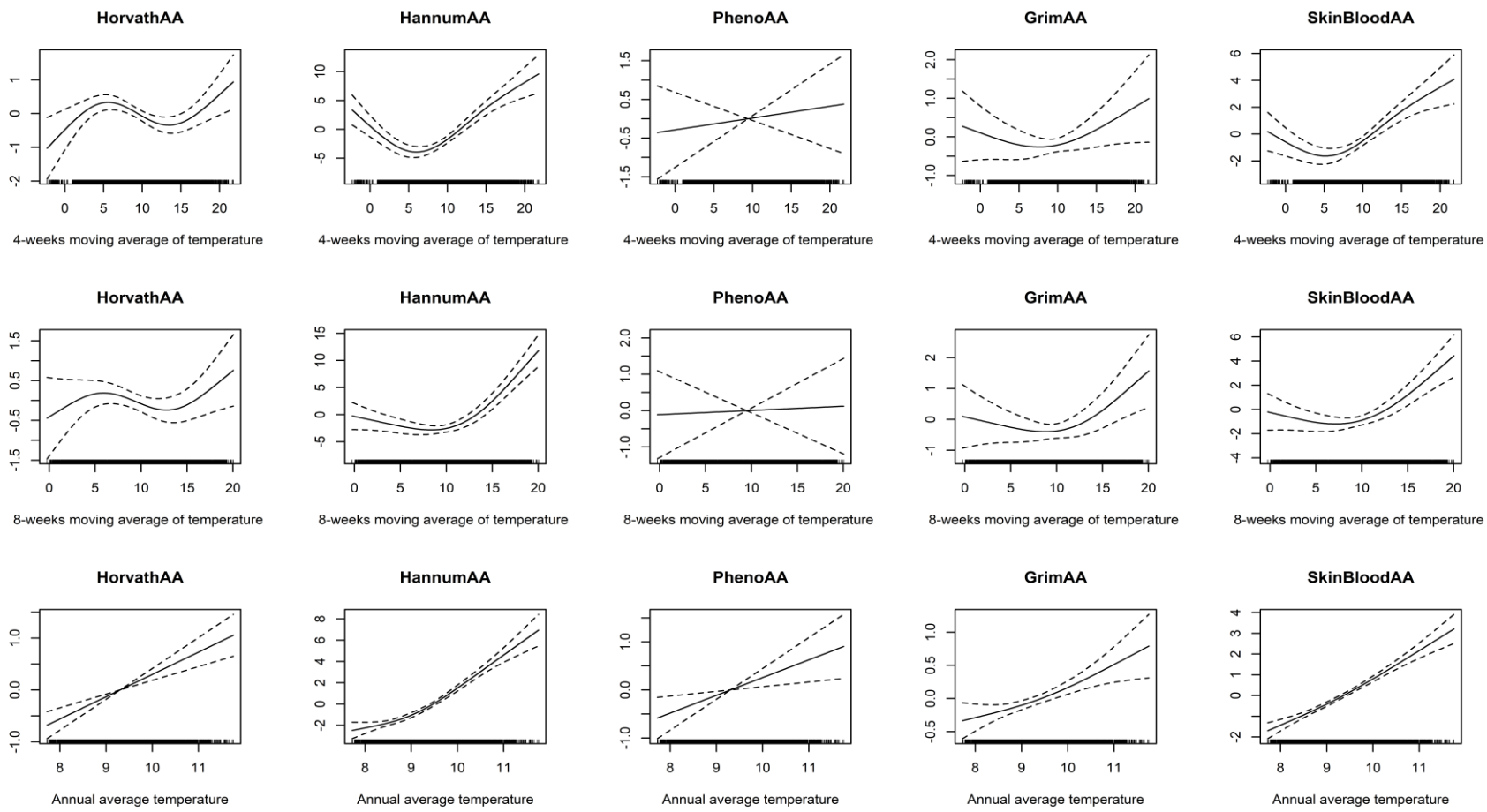
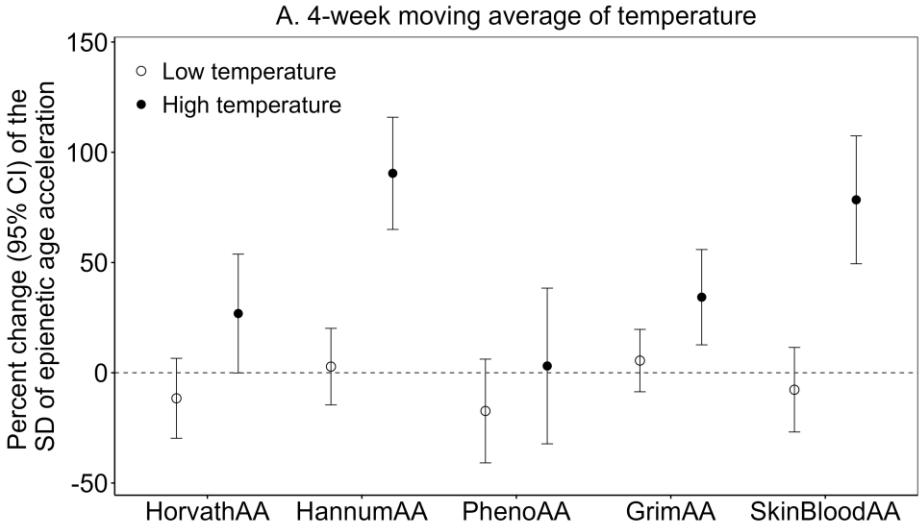
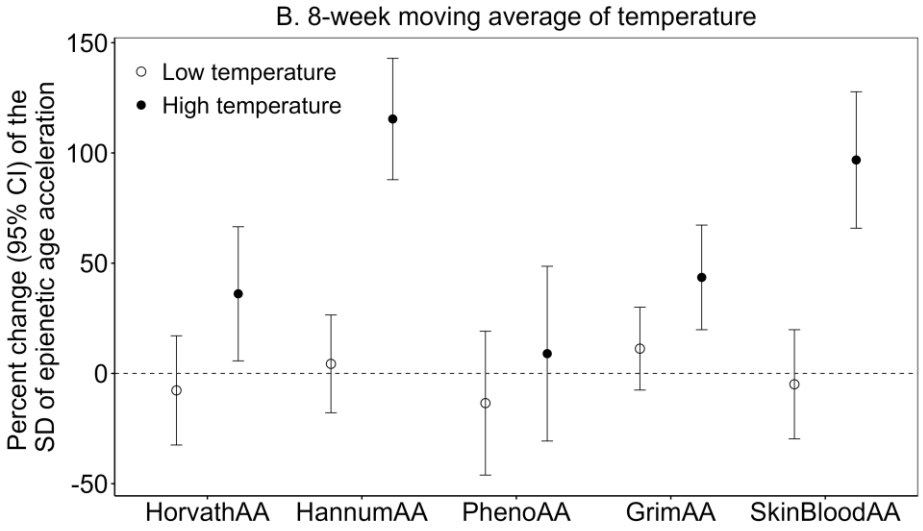


Figure S1. Exposure-response functions of different time windows of air temperature and epigenetic age acceleration.

Note: *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration.


A. 4-week moving average of temperature
Low temperature
High temperature
Percent change (95% CI) of the SD of epienetic age acceleration
150
100
50
0
-50
HorvathAA
HannumAA
PhenoAA
GrimAA
SkinBloodAA


B. 8-week moving average of temperature
Low temperature
High temperature
Percent change (95% CI) of the SD of epienetic age acceleration
150
100
50
0
-50
HorvathAA
HannumAA
PhenoAA
GrimAA
SkinBloodAA

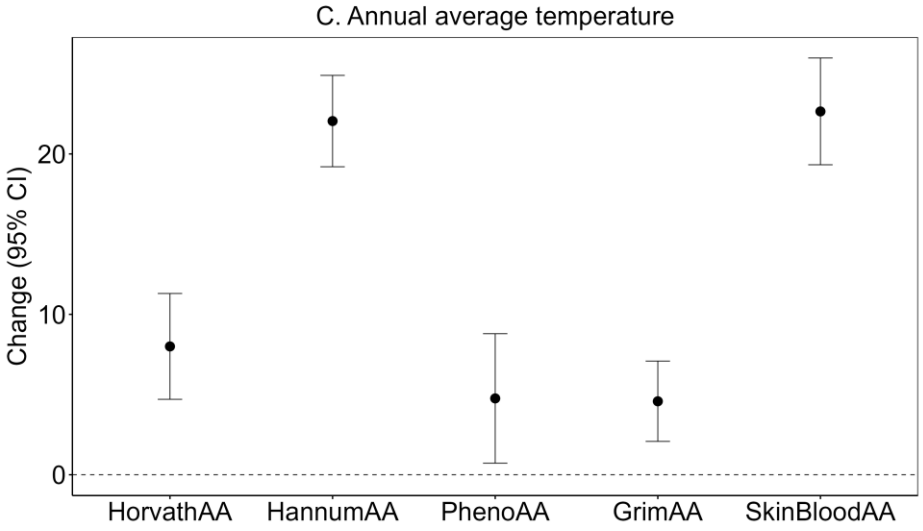

C. Annual average temperature
Change (95% CI)
20
10
0
HorvathAA
HannumAA
PhenoAA
GrimAA
SkinBloodAA

Figure S2. Estimated effects (percent change [95% CI] of SD) of air temperature on epigenetic age acceleration

Note: *Low temperature*: effects of low temperature exposure, 2.5th percentile of temperature compared to median temperature. *High temperature*: effects of high temperature exposure, 97.5th percentile of temperature compared to median temperature. The median temperature was 9.7℃; 2.5th percentile temperature was 1.5℃ and 1.4℃ for 4-week and 8-week moving averages of temperature, respectively; 97.5th percentile temperature was 18.5℃ and 18.3℃ for 4-week and 8-week moving averages of temperature, respectively. *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration.

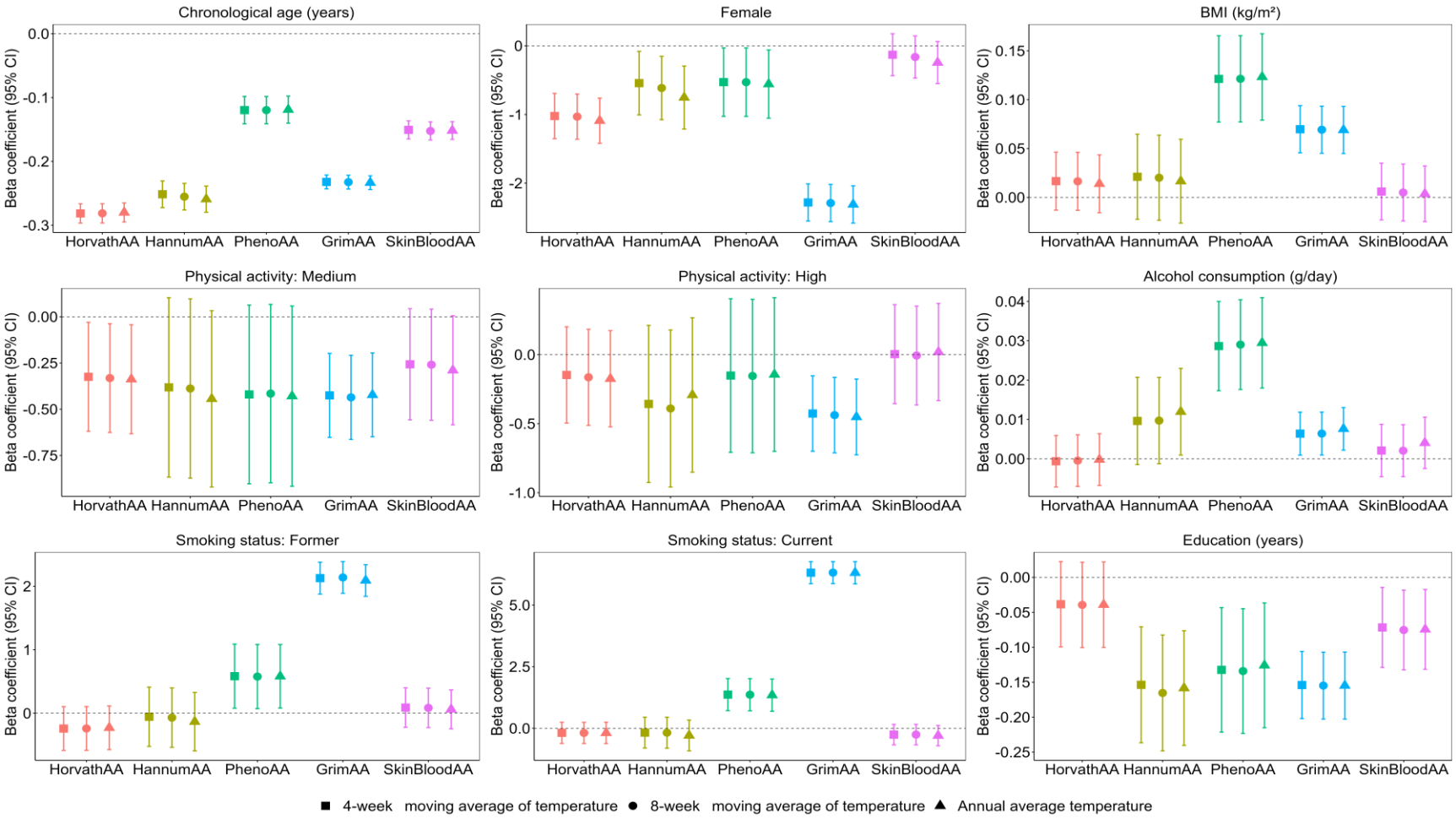


Figure S3: Effect estimates of potential demographic and lifestyle confounders on epigenetic age acceleration.

Note: Effect estimates represent the change in epigenetic age acceleration per unit change in the corresponding confounder, while controlling for other variables in the model.

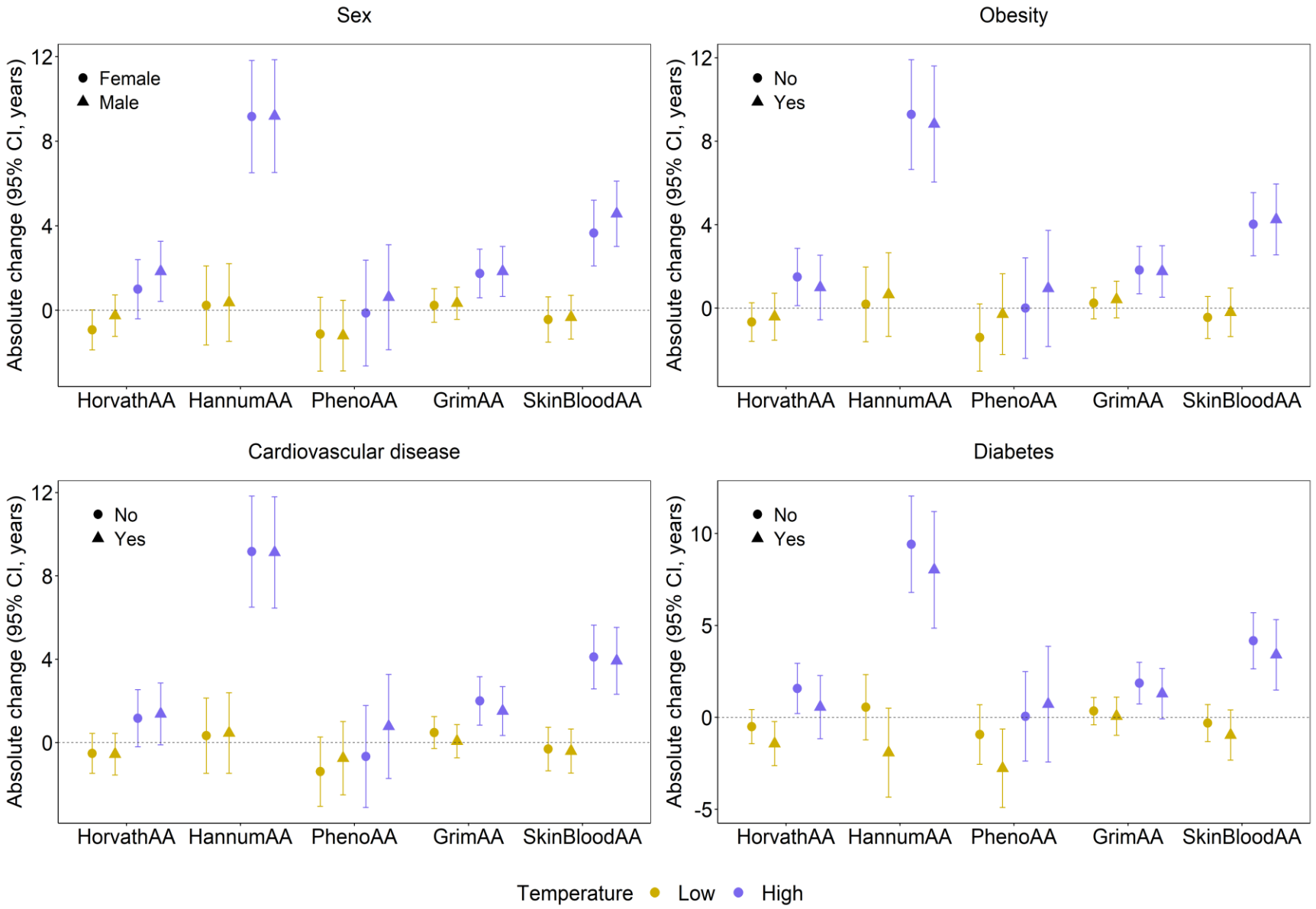

Sex
Female
Male
Obesity
No
Yes
Cardiovascular disease
No
Yes
Diabetes
No
Yes
Absolute change (95% CI, years)
HorvathAA
HannumAA
PhenoAA
GrimAA
SkinBloodAA
Temperature
Low
High

Figure S4. Medium-term effects of 4-week moving average of air temperature on epigenetic age acceleration modified by sex, obesity, cardiovascular disease, and diabetes.

Note: *Low*: effects of low temperature exposure, 2.5th percentile of temperature compared to median temperature. *High*: effects of high temperature exposure, 97.5th percentile of temperature compared to median temperature. The median temperature was 9.7℃; 2.5th percentile temperature was 1.5℃ and 1.4℃ for 4-week and 8-week moving averages of temperature, respectively; 97.5th percentile temperature was 18.5℃ and 18.3℃ for 4-week and 8-week moving averages of temperature, respectively. *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration.

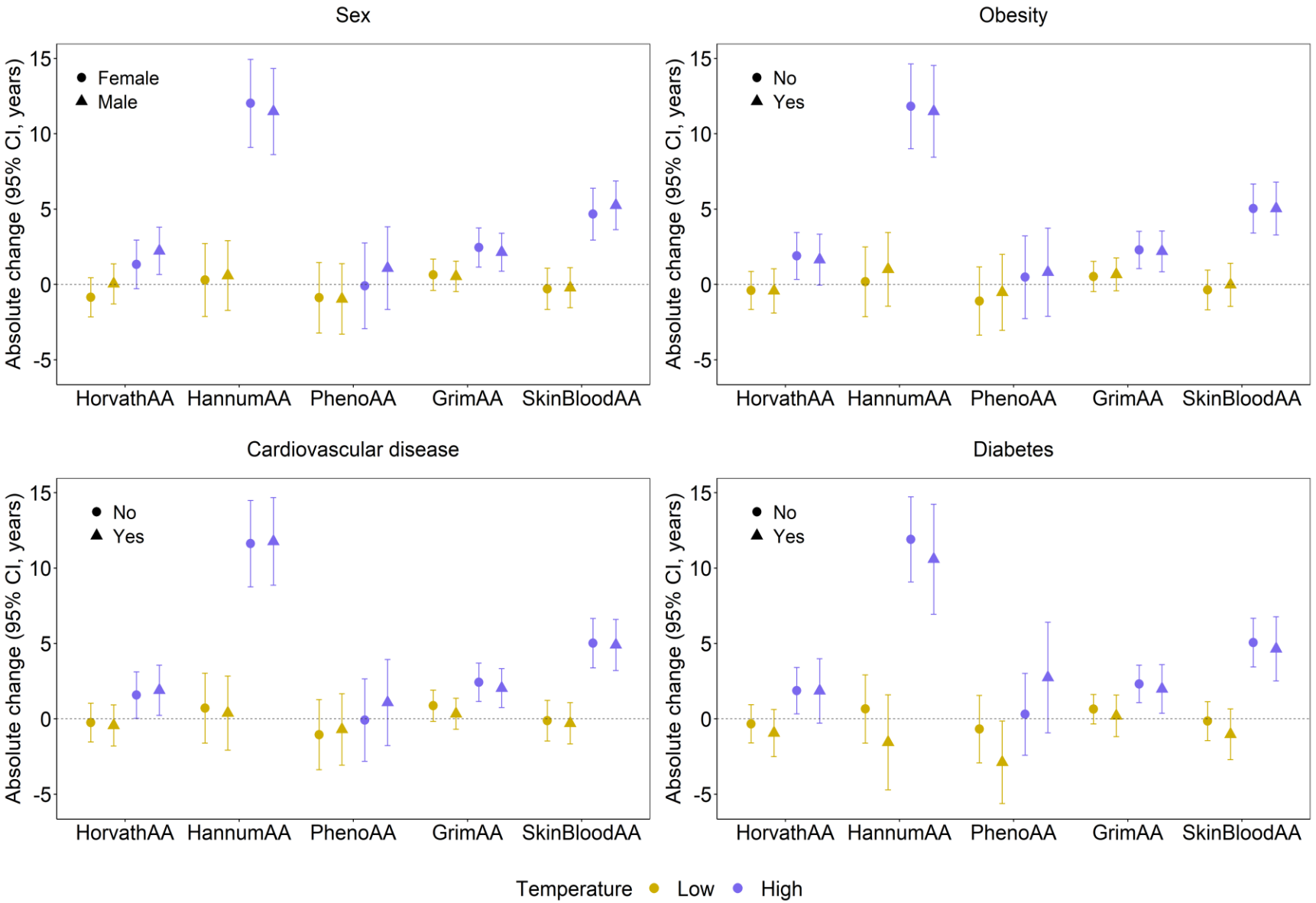

Sex
Female
Male
Obesity
No
Yes
Cardiovascular disease
No
Yes
Diabetes
No
Yes
Absolute change (95% CI, years)
15
10
5
0
-5
HorvathAA
HannumAA
PhenoAA
GrimAA
SkinBloodAA
Temperature
Low
High

Figure S5. Medium-term effects of 8-week moving average of air temperature on epigenetic age acceleration modified by sex, obesity, cardiovascular disease, and diabetes.

Note: *Low*: effects of low temperature exposure, 2.5th percentile of temperature compared to median temperature. *High*: effects of high temperature exposure, 97.5th percentile of temperature compared to median temperature. The median temperature was 9.7℃; 2.5th percentile temperature was 1.5℃ and 1.4℃ for 4-week and 8-week moving averages of temperature, respectively; 97.5th percentile temperature was 18.5℃ and 18.3℃ for 4-week and 8-week moving averages of temperature, respectively. *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration.

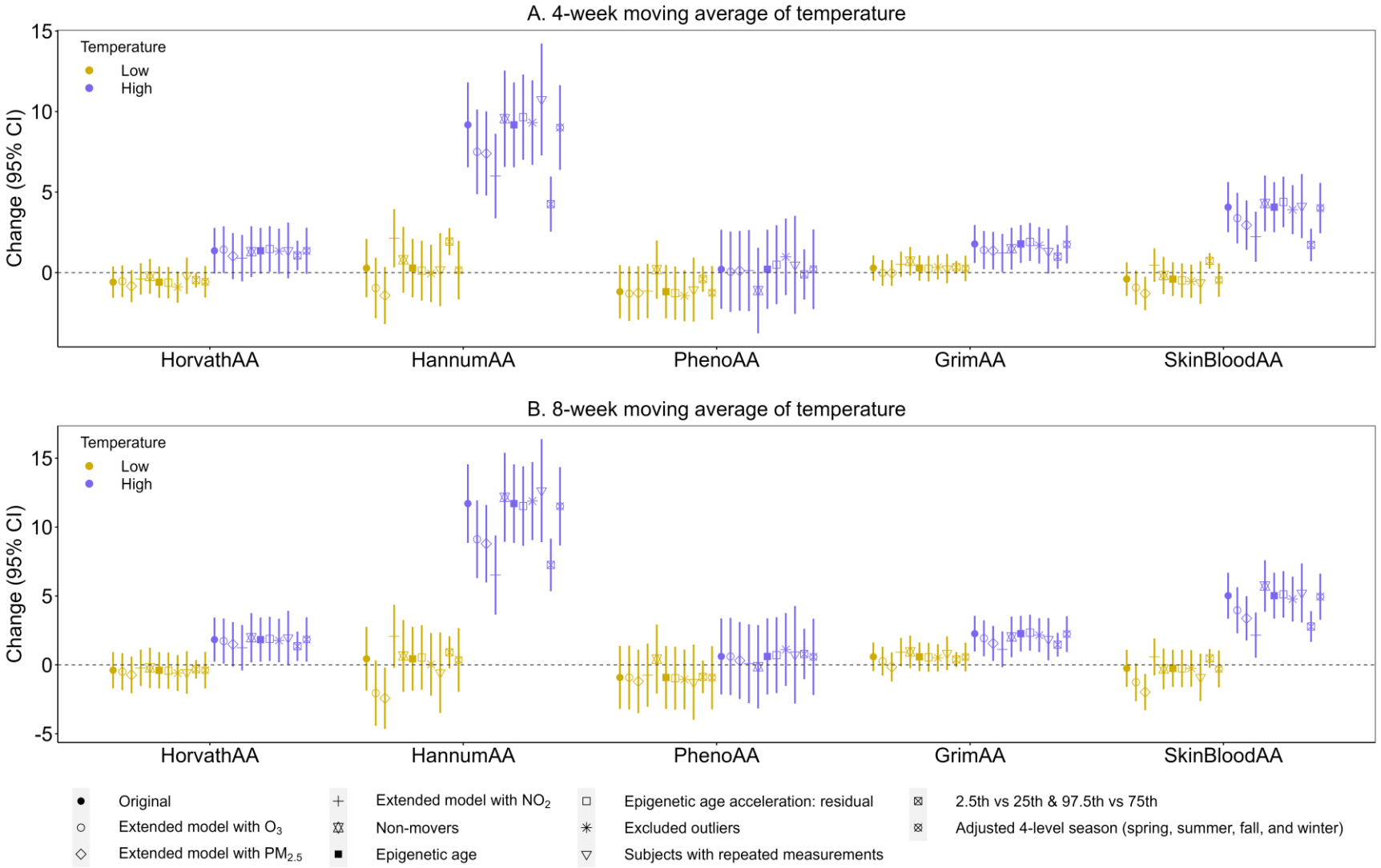


Figure S6. Sensitivity analysis: Medium-term effects of air temperature on epigenetic age acceleration.

Note: *Low*: effects of low temperature exposure, 2.5th percentile of temperature compared to median temperature. *High*: effects of high temperature exposure, 97.5th percentile of temperature compared to median temperature. The median temperature was 9.7℃; 2.5th percentile temperature was 1.5℃ and 1.4℃ for 4-week and 8-week moving averages of temperature, respectively; 97.5th percentile temperature was 18.5℃ and 18.3℃ for 4-week and 8-week moving averages of temperature, respectively. *HorvathAA*: Horvath's epigenetic age acceleration. *HannumAA*: Hannum's epigenetic age acceleration. *PhenoAA*: PhenoAge acceleration. *GrimAA*: GrimAge acceleration. *SkinBloodAA*: Epigenetic Skin and Blood Age acceleration. *Original*: main model. *Extended model with O_3*: additionally adjusted for O_3. *Extended model with $PM_{2.5}$*: additionally adjusted for $PM_{2.5}$. *Extended model with NO_2*: additionally adjusted for NO_2. *Non-movers*: restricted to the subpopulation that did not move throughout the study period. *Epigenetic age*: used epigenetic age metrics as outcomes. *Epigenetic age acceleration*: *residual*: residuals computed by linear regressing chronological age on epigenetic age. *Exclude outliers:* epigenetic age acceleration values outside 1.5 times the interquartile range were excluded. *Subject with repeated measurements*: included only participants with repeated measurements of epigenetic age acceleration. *2.5th vs 25th & 97.5th vs 75th:* extreme heat defined as the 97.5th percentile of temperature compared to the 75th percentile of temperature, and extreme cold as the 2.5th percentile of temperature compared to the 25th percentile of temperature. *Adjusted 4-level season (spring, summer, fall, and winter):* included season into the model as a four-factor category consisting of spring, summer, fall and winter.

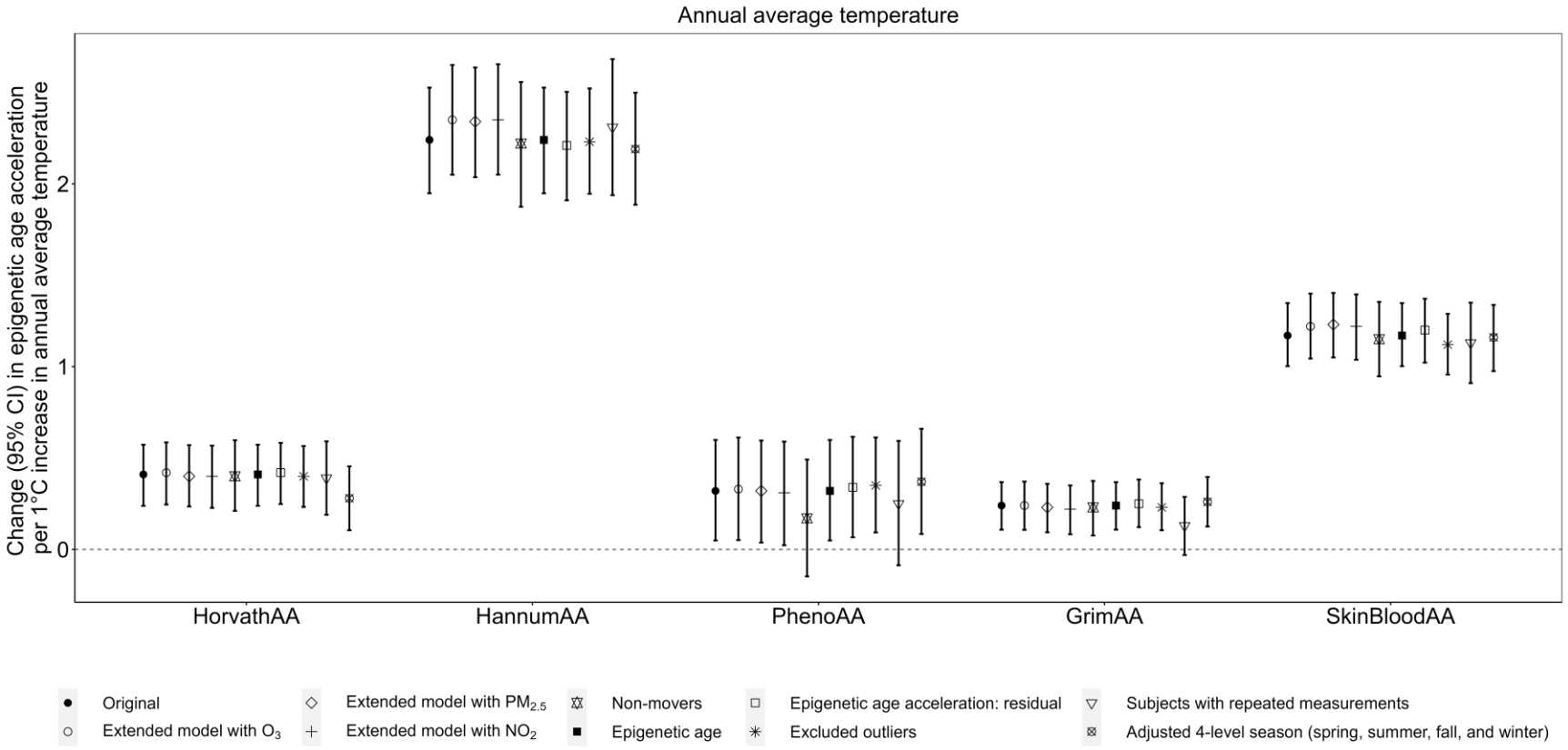


Figure S7. Sensitivity analysis: Long-term effects of annual average temperature on epigenetic age acceleration.

Note: *Original*: main model. *Extended model with O_3*: additionally adjusted for O_3. *Extended model with $PM_{2.5}$*: additionally adjusted for $PM_{2.5}$. *Extended model with NO_2*: additionally adjusted for NO_2. *Non-movers*: restricted to the subpopulation that did not move throughout the study period. *Epigenetic age*: used epigenetic age metrics as outcomes. *Epigenetic age acceleration*: *residual*: residuals computed by linearly regressing chronological age on epigenetic age. *Exclude outliers:* epigenetic age acceleration values outside 1.5

times the interquartile range were excluded. *Subject with repeated measurements*: included only participants with repeated measurements of epigenetic age acceleration. *Adjusted 4-level season (spring, summer, fall, and winter):* included season into the model as a four-factor category consisting of spring, summer, fall and winter.

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Publication III

Title: Effects of Short- And Medium-Term Exposures to Lower Air Temperature on 71 Novel Biomarkers of Subclinical Inflammation: Results from the KORA F4 Study

Authors: Wenli Ni, Susanne Breitner, Nikolaos Nikolaou, Kathrin Wolf, Siqi Zhang, Annette Peters, Christian Herder, Alexandra Schneider

Journal: Environmental Science & Technology

Status: Published

Volume: 57

Page: 12210-12221

Year: 2023

doi: 10.1021/acs.est.3c00302

Environmental Science & Technology

pubs.acs.org/est

Article

Effects of Short- And Medium-Term Exposures to Lower Air Temperature on 71 Novel Biomarkers of Subclinical Inflammation: Results from the KORA F4 Study

Published as part of the Environmental Science & Technology virtual special issue "The Exposome and Human Health".

Wenli Ni,* Susanne Breitner, Nikolaos Nikolaou, Kathrin Wolf, Siqi Zhang, Annette Peters, Christian Herder, and Alexandra Schneider

Cite This: *Environ. Sci. Technol.* 2023, 57, 12210−12221

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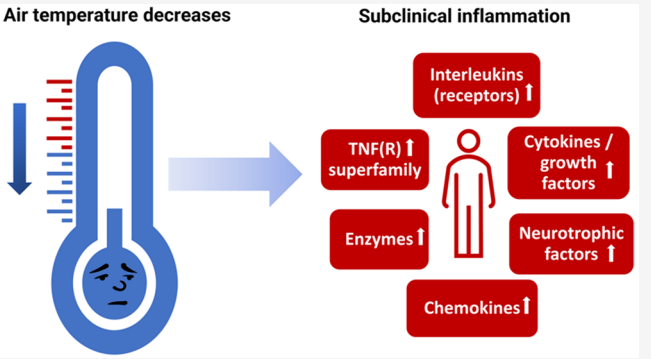
ABSTRACT: Increasing evidence has revealed that exposure to low temperatures is linked to a higher risk of chronic diseases and death; however, the mechanisms underlying the observed associations are still poorly understood. We performed a cross-sectional analysis with 1115 participants from the population-based KORA F4 study, which was conducted in Augsburg, Germany, from 2006 to 2008. Seventy-one inflammation-related protein biomarkers were analyzed in serum using proximity extension assay technology. We employed generalized additive models to explore short- and medium-term effects of air temperature on biomarkers of subclinical inflammation at cumulative lags of 0−1 days, 2−6 days, 0−13 days, 0−27 days, and 0−55 days. We found that short- and medium-term exposures to lower air temperature were associated with higher levels in 64 biomarkers of subclinical inflammation, such as Protein S100-A12 (EN-RAGE), Interleukin-6 (IL-6), Interleukin-10 (IL-10), C−C motif chemokine 28 (CCL28), and Neurotrophin-3 (NT-3). More pronounced associations between lower air temperature and higher biomarker of subclinical inflammation were observed among older participants, people with cardiovascular disease or prediabetes/diabetes, and people exposed to higher levels of air pollution ($PM_{2.5}$, NO_2, and O_3). Our findings provide intriguing insight into how low air temperature may cause adverse health effects by activating inflammatory pathways.



KEYWORDS: *short- and medium-term effects, air temperature, inflammation, cytokines*

1. INTRODUCTION

Climate change is an important public health issue that is characterized not only by an increasing frequency of extreme weather events but also by greater variability in temperature. Despite climate change, there will still be transient, unexpected temperature drops, and even if they are moderate and not extreme, they could still have an effect on health.[1] Increasing evidence from epidemiological studies revealed a U-shaped association between the air temperature and mortality. Of note, mortality increases both above and below a certain temperature optimum that appears to vary geographically.[2−4] Exposure to lower air temperature is also linked to a higher risk for chronic diseases.[5,6] Low temperatures sometimes contribute to more deaths than high temperatures.[3,4,7−9] For example, according to the Global Burden of Disease Study 2019, nonoptimal temperatures were a risk factor for global mortality, and low temperatures, compared to high temperatures, were associated with a greater mortality burden worldwide.[9] A study based on more than 1 million clinical visits for inflammation-related diseases in the Haiyuan and Yanchi counties in China found that low air temperature exposure was associated with an increased risk of inflammation-related diseases.[10]

However, the mechanisms underlying the association between the air temperature and both chronic diseases and mortality still need to be better understood. Biomarkers of inflammation have been associated with the development of

Received: January 13, 2023
Revised: July 26, 2023
Accepted: July 26, 2023
Published: August 8, 2023

many chronic diseases.[11−13] Several previous studies explored the association between air temperature and levels of biomarkers of inflammation.[14−21] However these studies were limited in focusing on only a small number of biomarkers (e.g., Interleukin-6 [IL-6]), and the findings remained inconsistent. Furthermore, most previous studies either investigated only subgroups of the general population (e.g., myocardial infarction survivors) or were based on experimental or panel studies with a small number of participants (though the panel study had greater internal validity), thus limiting the possibility of controlling for confounding or of generalizing the results.

Inflammatory processes are complex and have been shown to play a role in various chronic diseases.[11,22] Recent achievements in proteomic technologies have improved the detection of various inflammatory markers, but high-dimensional analyses between these markers and air temperature have not been conducted. To gain a more thorough understanding of how air temperature may cause adverse health effects through the systemic inflammatory pathway, we assessed short- and medium-term effects of air temperature (air temperature variability in space and time) on a multimarker panel of subclinical inflammation in a large population-based cohort in the Augsburg region, Germany. By analyzing a multimarker panel of biomarkers, we sought to capture a broad range of inflammatory effects and provide a more complete picture of the impact of the temperature on health. Furthermore, given that the different biomarkers of subclinical inflammation may have varying levels of sensitivity in detecting the effects of air temperature on health, we aimed to identify potential biomarkers that are more sensitive to temperature-related health responses, which may be recommended for use in future investigations.

2. MATERIALS AND METHODS

2.1. Study Population. We performed a cross-sectional analysis using data from the Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006−2008), which was a follow-up study of the population-based KORA S4 survey conducted in the city of Augsburg (Southern Germany) and its two surrounding districts during 1999−2001.[23] The latitude and longitude of Augsburg, Germany, are 48.366512 and 10.894446, respectively. The design of the study and data collection methods have been described in detail elsewhere.[23−26] The current study is based on 1115 subjects aged 62 to 81 years for whom a multimarker panel of biomarkers of inflammation (see section 2.3) was available.

The study was approved by the Ethics Board of the Bavarian Chamber of Physicians (Munich, Germany) in adherence to the Declaration of Helsinki. All participants gave written informed consent.

2.2. Exposure Assessment. We estimated German-wide and highly resolved (1 km × 1 km) daily mean, minimum, and maximum air temperature using a multistage regression-based modeling approach.[27] In order to achieve air temperature predictions with full spatial and temporal coverage across the country, we combined three stages. In the first stage, for days and grid cells where both monitor-based air temperature and satellite-derived land surface temperature were available, we trained a linear mixed effects model, including daily random intercepts and slopes for land surface temperature and several spatial predictors. In the second stage, we predicted air temperatures for grid cells without air temperature measurements but with available land surface temperature data using the model from the first stage. In the third stage, we regressed the second stage air temperature predictions against thin plate spline interpolated air temperature values for all remaining days and grid cells with neither air temperature measurements nor satellite land surface temperature available. In order to evaluate the performance of our models, we applied internal and external 10-fold cross-validation. All models showed excellent performance ($0.91 \leq R^2 \leq 0.98$) and low errors (1 °C < Root Mean Square Error (RMSE) < 2 °C). Especially in Augsburg, Germany, we extensively evaluated our model predictions by comparing them with measurements from an independent network of 82 HOBO-Logger devices[28] with similarly good results ($0.94 \leq R^2 \leq 0.99$, 0.99 °C ≤ RMSE ≤ 1.87 °C).

Details of the assessment of relative humidity (RH), particulate matter with an aerodynamic diameter <2.5 μm ($PM_{2.5}$), nitrogen dioxide (NO_2), and ozone (O_3) are given in Text S1 in the Supporting Information.

2.3. Measurement of Novel Systemic Biomarkers of Subclinical Inflammation. The OLINK Inflammation multiplex immunoassay (OLINK Proteomics, Uppsala, Sweden) used in this study includes 92 inflammation-related protein biomarkers from serum samples: pro- and anti-inflammatory cytokines, chemokines, growth factors, and factors involved in acute inflammatory and immune responses, angiogenesis, fibrosis, and endothelial activation. Serum samples were stored at −80 °C and thawed once before shipment to the OLINK Proteomics for biomarker measurement, which was performed in 2016−2017. The measurements were based on the proximity extension assay (PEA) technology that binds oligonucleotide-labeled antibody probe pairs to the respective target protein in the sample and uses a polymerase chain reaction for signal amplification.[29] The relative quantification of protein levels was expressed as normalized protein expression (NPX) arbitrary units on a Log2 scale. Details of the assessment of covariates are given in Text S2 in the Supporting Information.

An overview of all 92 analytes, including assay ID, abbreviated, full names, UniProt numbers, intra-assay coefficient of variation (CV), interassay CV, and limit of detection (LOD), is given in Table S1 (Supporting Information). As described before,[30] the intra- and interassay CVs were calculated based on the three control sera measured in duplicates on each plate ($n = 16$). Twenty biomarkers were excluded because of ≥25% of samples below the limit of detection (LOD), and one additional biomarker was excluded because of an interassay CV > 20%. For the remaining 71 analytes, sample values below the LOD were set to the LOD. In the final data set (71 biomarkers), the intra-assay CV was 3.6 ± 1.5% (mean ± SD), and the interassay CV was 8.4 ± 2.2% (mean ± SD).

2.5. Statistical Analysis. The characteristics of the study population were reported by frequency and percentage for categorical variables and mean and standard deviation (SD) for continuous variables. The levels of biomarkers of subclinical inflammation, meteorological variables, and air pollutants were summarized as mean, SD, 5%, 25%, median, 75%, and 95% percentiles. Spearman correlation analysis was used to evaluate the correlations.

We employed generalized additive models (GAMs) to explore short- and medium-term effects of the mean air temperature on biomarkers of subclinical inflammation.

https://doi.org/10.1021/acs.est.3c00302
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Biomarkers values outside of three times the interquartile range were excluded to avoid bias due to the presence of outliers. In order to explore the lagged and cumulative effects of air temperature, we investigated the effects of mean air temperature at 0−1, 2−6, and 0−13 days before blood draw for short-term effects and 0−27, and 0−55 days before blood draw for medium-term effects. Almost no appreciable deviations from linearity were found for exposure-response functions (spline with three degrees of freedom), so air temperature was included linearly in the GAMs (Figure S1, Supporting Information).

We controlled for potential confounders based on published literature and expert knowledge:[11,31] age, sex, education, smoking status, alcohol consumption, physical activity, height, waist circumference, systolic blood pressure, diastolic blood pressure, albumin, hematocrit, day of the week, season at blood draw (cold: April−September, warm: October−March), time trend (cubic spline with six degrees of freedom per year), and RH (cubic spline with three degrees of freedom) with the same lag period as the air temperature.

The results were expressed as percent changes of the outcome mean (with their 95% confidence intervals [CIs]) per 1-interquartile range (IQR) decrease in air temperature. We adjusted for multiple testing of different exposure windows and biomarkers of subclinical inflammation using the Benjamin-Hochberg false discovery rate (FDR). P (adjusted)-value <0.05 was considered statistically significant for all statistical tests.

For biomarkers of subclinical inflammation showing significant (adjusted p-value <0.05) associations with air temperature, further stratification analyses were conducted to examine effect modification by age (<70 years vs ≥70 years), sex (male vs female), cardiovascular disease (defined as a history of hypertension, angina pectoris, stroke, or myocardial infarction [yes vs no]), (pre)diabetes status (normal glucose tolerance vs prediabetes/diabetes), air pollutants with the same lag period as the air temperature ($PM_{2.5}$/NO_2/O_3: low [<median] vs high [≥ median]).

We performed several sensitivity analyses to assess the robustness of our results further. First, we additionally adjusted for medication intake (antihypertensive drugs or nonsteroidal anti-inflammatory drugs) in the main model. Second, we controlled for the presence of pre-existing conditions (cardiovascular disease, cancer, and chronic bronchitis or emphysema) by incorporating them as additional adjustment factors. Third, to control for potential confounding by air pollutants, the concentrations of three pollutants ($PM_{2.5}$, NO_2, and O_3 [continuous variables]) were additionally included in the main model, though separately, to avoid collinearity. Fourth, we accounted for wind speed and barometric pressure in the main model as additional adjustment factors. Fifth, participants with C-reactive protein (CRP) values greater than 10 mg/L were excluded (N = 47) because this might indicate acute infection. Sixth, we used the minimum and maximum air temperatures instead of the mean air temperature. Finally, to control for the confounding effect of season, we linearly regressed season on the biomarkers of subclinical inflammation and then calculated the respective residuals for further association analyses with air temperature.

All statistical analyses were performed by using R (version 4.1.2) with the "mgcv" package.

3. RESULTS

3.1. Study Population, Biomarkers of Inflammation, and Exposure Data. Table 1 describes the characteristics of the study population. The mean age in this study population was 70.4 years, 48.8% of the study population was female, 39.8% reported low physical activity, and only 7.4% were current smokers.

Table 1. Descriptive Statistics of Participant Characteristics[a]

	Mean ± SD/N (%) (n = 1115)
Age (years)	70.4 ± 5.5
Sex (female)	544 (48.8%)
Education (years)	11.0 ± 2.5
Smoking status	
Current smoker	82 (7.4%)
Former smoker	486 (43.6%)
Nonsmoker	544 (48.8%)
Physical activity	
Low	444 (39.8%)
Medium	429 (38.5%)
High	239 (21.4%)
Height (cm)	166 ± 9.0
Waist circumference (cm)	98.3 ± 12.2
Body mass index(kg/m^2)	28.7 ± 4.5
Systolic blood pressure (mmHg)	129 ± 19.8
Diastolic blood pressure (mmHg)	74.1 ± 10.0
Albumin(g/L)	43.7 ± 3.2
Haematocrit(L/L)	0.4 ± 0.03
Alcohol consumption (g/day)	13.8 ± 18.1
Cardiovascular disease (yes)	741 (66.5%)
Cancer (yes)	156 (14.0%)
Chronic bronchitis or emphysema (yes)	131 (11.7%)
Diabetes status	
Normal glucose tolerance	577 (51.7%)
Prediabetes	284 (25.5%)
Diabetes	231 (20.7%)
Medication intake	
Antihypertensive medication (yes)	657 (58.9%)
NSAIDs (yes)	48 (4.3%)
Season of examination	
Cold	744 (66.7%)
Warm	371 (33.3%)

[a]Note: SD: Standard deviation. Physical activity: low, almost no activity; medium, regularly or irregularly about 1 h per week; high, regularly more than 2 h per week. NSAIDs: Nonsteroidal anti-inflammatory drugs. Season: cold, April−September; warm, October−March.

Levels of biomarkers of subclinical inflammation are presented in Figure S2 and Table S2 (Supporting Information). Almost all correlations among the biomarkers of subclinical inflammation were positive and, in most cases, with low to moderate Spearman coefficients (Figure S3, Supporting Information).

Table 2 summarizes the meteorological variables and air pollutant levels to which our participants were exposed during the study period. The mean level of mean air temperature was 7.8 ± 6.1 °C (mean ± SD). Figure S4 shows the time series of mean air temperatures for participants in this study. High correlations were observed between air temperature variables (mean, minimum, and maximum air temperature) as well as

https://doi.org/10.1021/acs.est.3c00302
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Table 2. Descriptive Statistics of Meteorological Variables and Air Pollutants[b]

	Mean ± SD	Min	25%	Median	75%	Max
Mean air temperature (°C)	7.8 ± 6.1	−7.8	2.8	7.1	11.8	24.7
Minimum air temperature (°C)	3.8 ± 5.4	−13.2	0.0	3.0	7.0	17.1
Maximum air temperature (°C)	12.4 ± 7.5	−4.6	7.1	11.5	17.4	35.0
RH (%)	77.0 ± 9.9	46.5	70.9	78.2	84.0	94.5
$PM_{2.5}$ ($\mu g/m^3$)	14.8 ± 11.2	1.4	6.1	12.6	19.7	65.8
O_3 ($\mu g/m^3$)	38.6 ± 22.8	3.0	18.7	36.0	54.8	97.6
NO_2 ($\mu g/m^3$)	33.3 ± 11.9	10.4	23.3	32.4	41.2	77.9
Wind speed (m/s)	3.5 ± 2.1	0.9	1.9	3.0	4.4	15.3
Barometric pressure (hPa)	1018.1 ± 8.2	996.5	1013.0	1018.4	1023.9	1037.6

[b]Note: RH, relative humidity; $PM_{2.5}$, particulate matter with an aerodynamic diameter of ≤2.5 μm; O_3, ozone; NO_2, nitrogen dioxide.

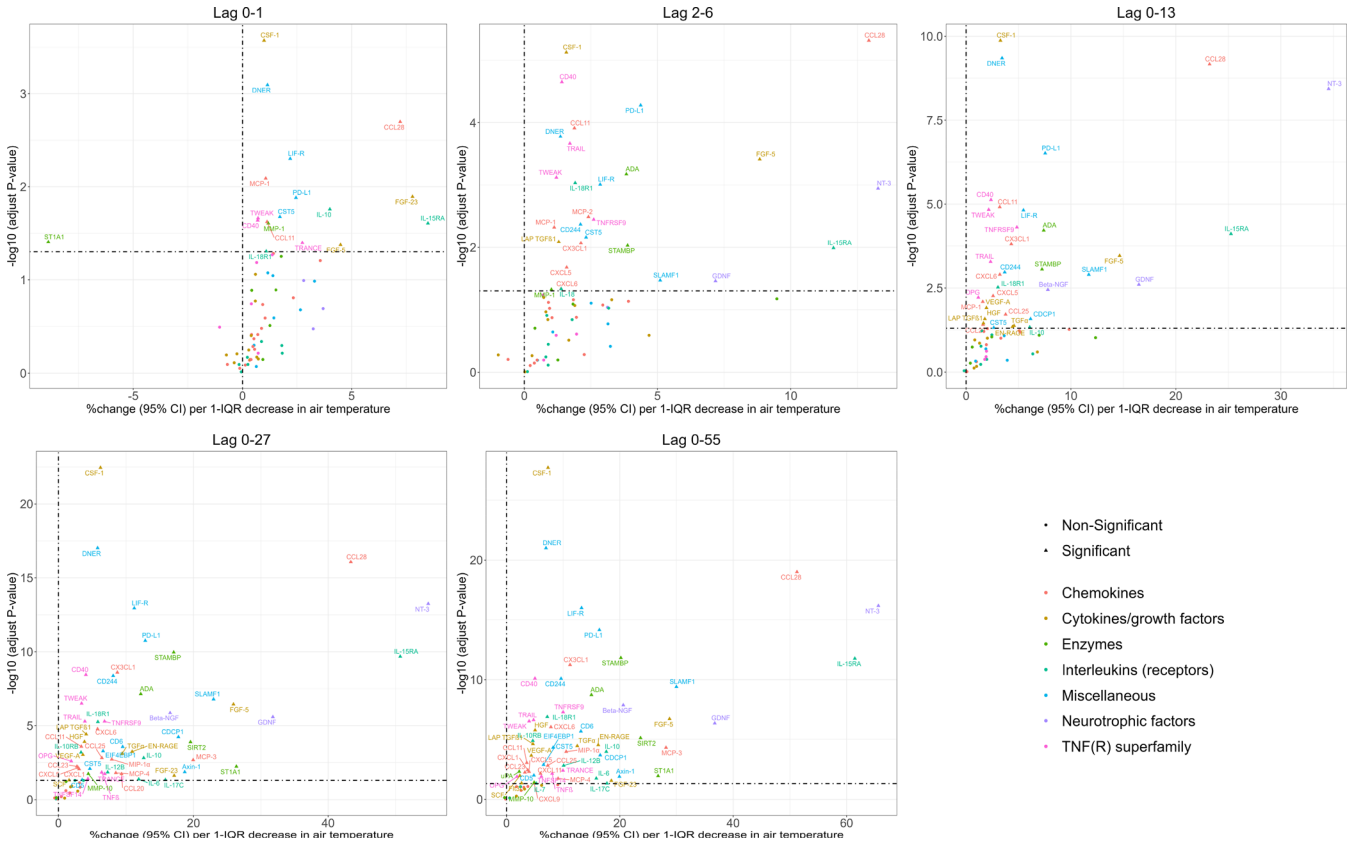

Figure 1. Volcano plots presenting the associations between short- and medium-term exposures to air temperature per 1-IQR decrease with 71 biomarkers of subclinical inflammation. Note: the 1-IQR decrease was 9.2 °C for lags 0−1 days, 8.9 °C for lags 2−6 days, 8.4 °C for lags 0−13 days, 9.0 °C for lags 0−27 days, and 9.4 °C for lags 0−55 days.

between $PM_{2.5}$, NO_2, and wind speed, while weak to moderate correlations were observed between other meteorological variables and other air pollutants (Figure S5, Supporting Information).

3.2. Short- and Medium-Term Effects of Air Temperature on 71 biomarkers of Subclinical Inflammation. The short- and medium-term effects of air temperature on 71 biomarkers of subclinical inflammation are shown in Figure 1 and 2, Figures S6 and S7 (Supporting Information). For a brief overview, a 1-IQR decrease in air temperature was significantly associated with increases in 64 biomarkers of subclinical inflammation (40 significant associations for short-term effects and 60 significant associations for medium-term effects), such as Protein S100-A12 (EN-RAGE), IL-6, Interleukin-10 (IL-10), C−C motif chemokine 28 (CCL28), Neurotrophin-3 (NT-3), and Interleukin-15 receptor subunit alpha (IL-15RA). Of these significant associations, there were associations for 17 biomarkers of subclinical inflammation at lag 0−1 days, 28 biomarkers of subclinical inflammation at lag 2−6 days, 35 biomarkers of subclinical inflammation at lag 0−13 days, 55 biomarkers of subclinical inflammation at lag 0−27 days, and 59 biomarkers of subclinical inflammation at lag 0−55 days. The number of significant associations with biomarkers of subclinical inflammation and their effect estimates increased with an increasing number of lag days. Figure S8 summarizes the associations between short- and medium-term exposures to air temperature per 1 °C decrease with 71 biomarkers of subclinical inflammation.

Venn diagrams (Figure S9, Supporting Information) show 13 overlapping biomarker associations at different exposure windows for short-term effects, 54 overlapping biomarker associations at different exposure windows for medium-term

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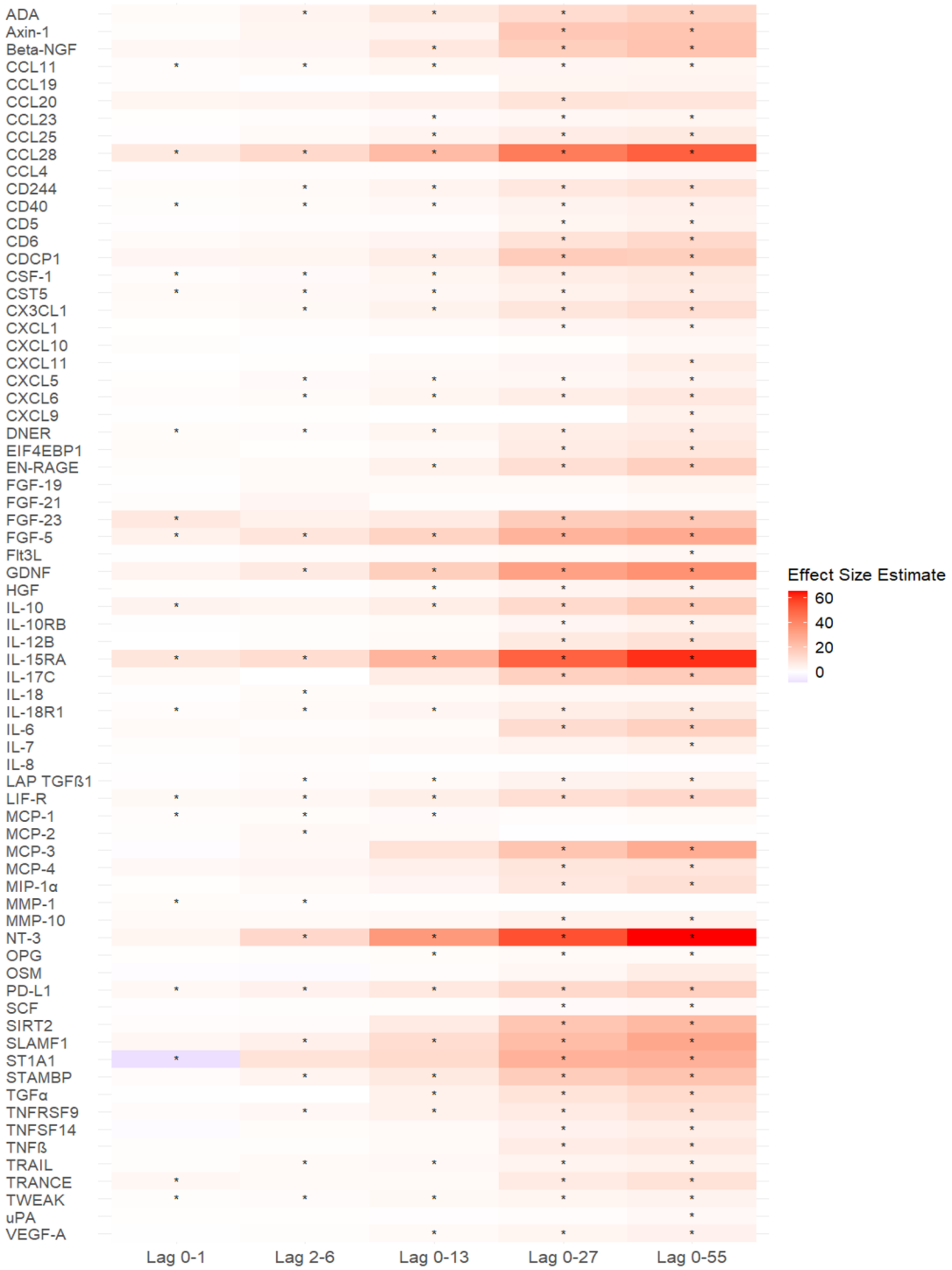

Figure 2. Heatmap of associations between short- and medium-term exposure to air temperature and 71 biomarkers of subclinical inflammation. Note: * *P* (adjusted)-value <0.05, Effect size estimate: percent changes of the outcome mean per 1-IQR decrease in air temperature.

effects, and 12 overlapping biomarker associations (Eotaxin [CCL11], CCL28, CD40L receptor [CD40], Macrophage colony-stimulating factor 1 [CSF-1], Cystatin D [CST5], Delta and Notch-like epidermal growth factor-related receptor [DNER], Fibroblast growth factor 5 [FGF-5], IL-15RA, Interleukin-18 receptor 1 [IL-18R1], Leukemia inhibitory factor receptor [LIF-R], Programmed cell death 1 ligand 1 [PD-L1], and Tumor necrosis factor [Ligand] superfamily, member 12 [TWEAK]) at different exposure windows for short- and medium-term effects. The significant biomarker associations found at lag 0−27 days were almost completely replicated at lag 0−55 days.

*3.3. **Effect Modification.*** We found stronger effects of air temperature (i) on 22 biomarkers (e.g., Beta-nerve growth factor [Beta-NGF], C-X-C motif chemokine 6 [CXCL6], Glial cell line-derived neurotrophic factor [GDNF], IL-15RA, TNF-related apoptosis-inducing ligand [TRAIL], and TNF-related activation-induced cytokine [TRANCE]) in participants older than 70 years of age, (ii) on 22 biomarkers (e.g., C−C motif chemokine 23 [CCL23], CCL28, CST5, IL-10, TRAIL, and

https://doi.org/10.1021/acs.est.3c00302
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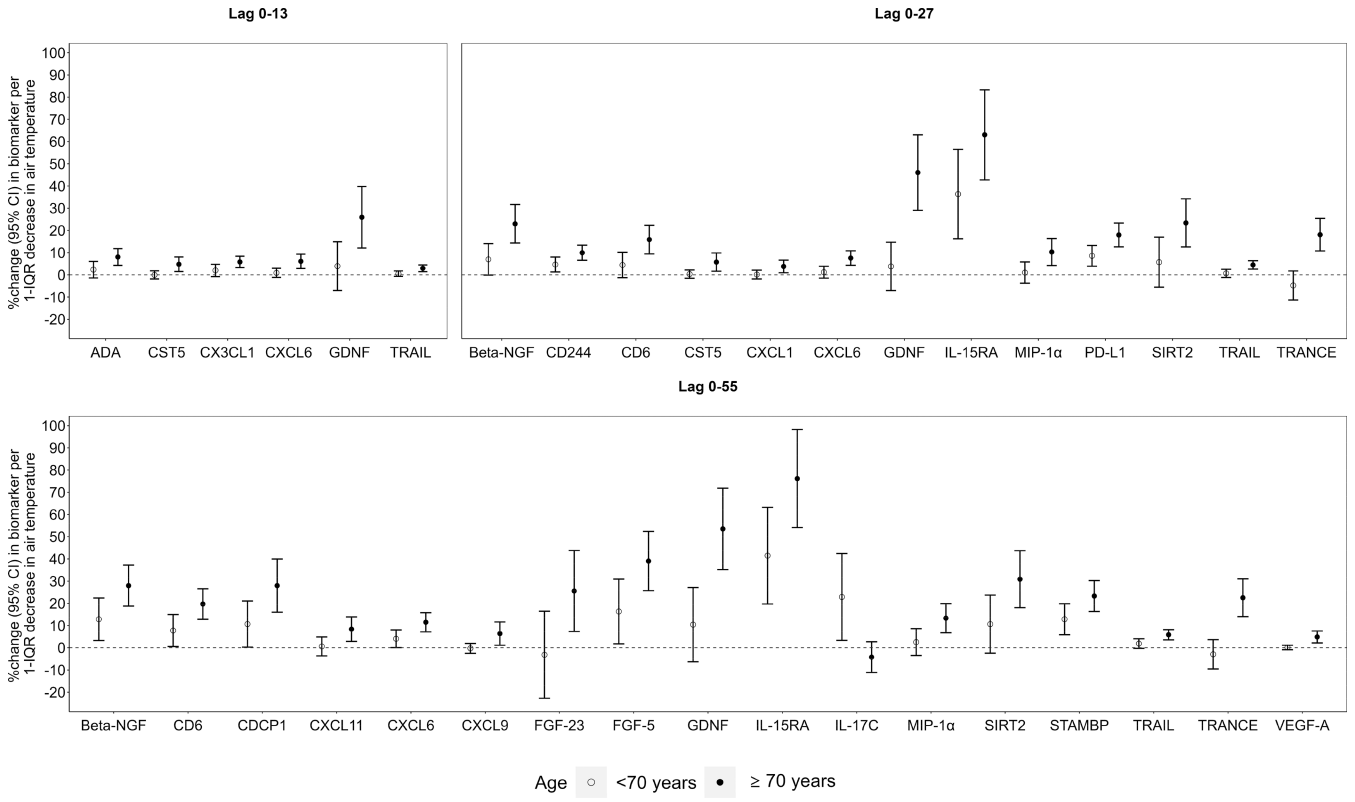

Figure 3. Short- and medium-term effects of air temperature on biomarkers of subclinical inflammation per 1-IQR decrease significantly modified by age. Note: the 1-IQR decrease was 8.4 °C for lags of 0−13 days, 9.0 °C for lags of 0−27 days, and 9.4 °C for lags of 0−55 days.

TRANCE) in participants with cardiovascular disease, and (iii) on 11 biomarkers (e.g., CCL28, CST5, and Interleukin-10 receptor subunit beta [IL-10RB]) in participants with prediabetes/diabetes compared to their respective counterparts (Figure 3, Figure 4, and Figure S10 [Supporting Information]). We also found stronger effects of air temperature on eight biomarkers (e.g., FGF-5, Neurotrophin-3 [NT-3]) in men than in women and on five biomarkers (e.g., IL-6) in women than in men (Figure S10, Supporting Information). Finally, we found stronger effects of air temperature on 21, 44, and 20 biomarkers in participants' exposure to higher levels of $PM_{2.5}$, NO_2, and O_3, respectively, than in those exposed to lower levels (Figures S11 and S12, Supporting Information).

3.4. ***Sensitivity Analysis.*** Overall, the results of the sensitivity analyses were consistent with those of the main analysis (data not shown). First, similar effect estimates were seen when additionally adjusting for medications, pre-existing conditions, air pollutants, or wind speed and barometric pressure in the model. Moreover, excluding study participants with CRP values greater than 10 mg/L did not affect the results (Figure S13, Supporting Information). Third, using minimum or maximum temperatures instead of the mean provided similar results. Finally, the findings were consistent when we used the residuals of the linear regression of season on biomarkers of subclinical inflammation.

4. Discussion. *4.1.* ***Summary of Key Results.*** To the best of our knowledge, this is the first study to investigate the effects of short- and medium-term exposures to air temperature on a multimarker panel of biomarkers of subclinical inflammation. Among the 71 biomarkers of subclinical inflammation, a lower air temperature showed statistically significant associations with higher levels in 64 biomarkers, after controlling for extensive potential confounding factors and correction for multiple tests.

4.2. ***Comparison with Current Evidence.*** Of the 71 biomarkers of subclinical inflammation reported in this study, only 5 of them (IL-6, Interleukin-8 [IL-8], IL-10, Monocyte chemotactic protein 1 [MCP-1], and Fibroblast growth factor 21 [FGF-21]) have previously been reported in association with air temperature in epidemiology studies, and most of these studies only explored short-term effects.[14−21] We found that a 1-IQR decrease in air temperature was significantly associated with higher levels of IL-6, IL-10, and MCP-1 for different lag windows up to 0−55 days. In contrast, there were no significant associations between the air temperature and IL-8 or FGF-21. Previous studies on older people (aged 60−82 years) or myocardial infarction survivors found that decreased air temperature was associated with increased IL-6 level at lag 1 day or 5-day moving average.[17,18] A panel study with a specific genetic background from KORA F4 study showed also decreased air temperature was associated with increased IL-6 level at lag 1 day, lag 4 day, and 5-day moving average; at the same time, no significant associations were seen in 187 people with type 2 diabetes and impaired glucose tolerance.[14] In addition, lower air temperature was associated with higher IL-6, IL-8, IL-10, or MCP-1 at lag 0 to lag 2 days in 35 people with type 2 diabetes in Shanghai, China.[16] Another study based on 77 healthy volunteers in North Carolina, United States, found that IL-8, but not MCP-1, was inversely associated with the air temperature of the previous day.[19] A crossover intervention study of 12 volunteers found that FGF-21 was higher at 19 °C than at 24 °C after 3−9 h.[20] In contrast, another experimental study of 19 healthy participants found that FGF-21 significantly decreased after 2 h of cold exposure

https://doi.org/10.1021/acs.est.3c00302
Environ. Sci. Technol. 2023, 57, 12210−12221

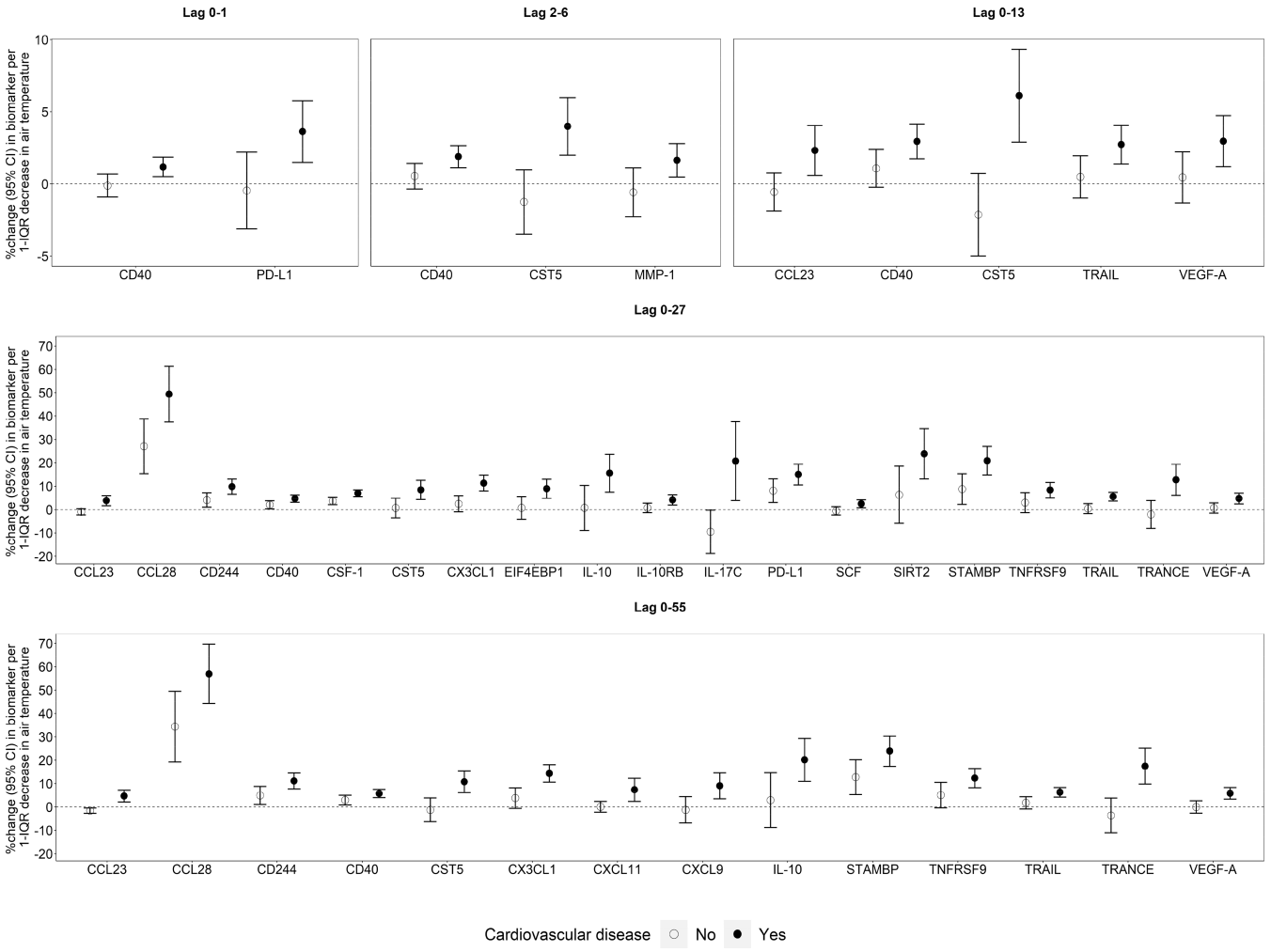

Figure 4. Short- and medium-term effects of air temperature on biomarkers of subclinical inflammation per 1-IQR decrease significantly when modified by cardiovascular disease. Note: the 1-IQR decrease was 9.2 °C for lags 0−1 days, 8.9 °C for lags 2−6 days, 8.4 °C for lags 0−13 days, 9.0 °C for lags 0−27 days, and 9.4 °C for lags 0−55 days.

in brown adipose tissue (BAT)-positive subjects, but not BAT-negative subjects.[21] Moreover, a study only focusing on men in the Greater Boston area, United States, found no associations between air temperature and IL-6 or IL-8 at lags 0 to 7, and 1-, 2-, 3-, and 4-week moving averages.[15] Therefore, our results of inverse associations between the air temperature and the biomarkers in our population-based setting mostly align with the current evidence from smaller studies based on selected populations and less extensive analyses of different lag times.

*4.3. **Duration of Effects.*** We found that medium-term effects of air temperature were stronger than short-term effects, with more significant biomarker associations and larger effect estimates. These findings suggest delayed effects of the lower air temperature on these biomarkers of subclinical inflammation. The larger effect sizes of the medium-term exposures could also be due, in part, to the cumulative impact of the low-temperature exposures. Of note, most previous studies on air temperature and inflammation have only investigated short-term associations, and the results of our study suggest these may be underestimated. Given that many of the temperature-sensitive biomarkers of inflammation have been related to the risk of various diseases and mortality (see section 4.6), our study indicates that adverse health effects of lower air temperature may not only be acute but relevant over at least two months.

*4.4. **Highlighted Biomarkers.*** Of the biomarkers we analyzed, (i) NT-3, IL-15RA, CCL28, FGF-5, and GDNF were about the top five biomarkers with the largest effects; (ii) CCL11, CCL28, CD40L CD40, CSF-1, CST5, DNER, FGF-5, IL-15RA, IL-18R1, LIF-R, PD-L1, and TWEAK were significantly associated with short- and medium-term effects across all exposure windows. These findings indicate that these potential biomarkers may be more sensitive to temperature-related health responses and may be better recommended in future studies for the detection of temperature-related adverse health responses.

*4.5. **Novel Associations.*** To the best of our knowledge, no epidemiological study investigated the effects of air temperature on the other 66 biomarkers of subclinical inflammation reported in our study. Hence, we substantially extended the current literature in this field. The present study identified significant associations for 64 biomarkers of subclinical inflammation, 61 of which were reported for the first time to be associated with lower air temperature exposures. In our analyses, we adjusted for a range of potential confounders and also for multiple testing (different exposure windows and biomarkers of subclinical inflammation). Covariates included

https://doi.org/10.1021/acs.est.3c00302
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not only standard demographic, anthropometric, and metabolic variables but also albumin and hematocrit to adjust for potential confounding effects between air temperature and changes in blood volume due to vasoconstriction and vasodilation. Furthermore, consistent results were obtained from multiple sensitivity analyses. We especially excluded participants with markedly high CRP values (>10 mg/L) who might have had an acute infection. The results remained stable, suggesting that these significant associations were not due to the confounding effects of acute infection. We used two exposure windows for medium-term effects and found nearly identical effects, again illustrating the stability of our results.

Our study may have several clinical implications. These are related to the (i) lag times, and thus the duration of temperature effects on subclinical inflammation, (ii) biomarkers that are regulated and have been previously found to be associated with morbidity and mortality, (iii) identification of subgroups within the population that show more pronounced responses to temperature changes than others, and (iiii) identification of interactive effects between lower air temperature and higher air pollution exposures on increased biomarkers of subclinical inflammation.

*4.6. **Mechanisms Linking Lower Air Temperature to Morbidity and Mortality.*** Exposure to low temperatures is associated with not only increased risks of various chronic diseases but also increased mortality.[6,8,32,33] Many previous studies reported that increased pro- and anti-inflammatory biomarkers (IL-6, EN-RAGE, and IL-10) were associated with increased risks of diabetes, cardiovascular disease, and mortality.[34−42] IL-6 is a pleiotropic cytokine with pro-inflammatory effects, which can induce atherosclerosis in cardiovascular disease.[43] EN-RAGE binds to RAGE, activating the pro-inflammatory NF-κB signaling, the typical innate immune system pathway involved in coronary heart disease pathogenesis.[36,44] IL-10 is a pleiotropic cytokine that is most widely recognized as an anti-inflammatory cytokine. However, previous studies found that upregulation of IL-10 was positively associated with the risk of cardiovascular events, although the association was not consistent.[38−40] Many of the other novel biomarkers of subclinical inflammation in this study are exploratory. However, they point toward cell−cell communication (e.g., chemokines involved in the cross-talk between innate and adaptive immunity), a role in immune responses, and neurological processes. Of note, the same assay allowed the identification of multiple biomarkers of inflammation associated with incident distal sensorimotor polyneuropathy[30] and impaired kidney function,[45,46] many of which were temperature-responsive in this study. In summary, our findings raise the possibility that lower air temperature exposure could affect the risk of multiple age-related and chronic diseases in addition to mortality partly through the effect on subclinical inflammation.

*4.7. **Susceptible Subgroups.*** We found that the effects of air temperature on biomarkers of subclinical inflammation were stronger in participants ≥70 years compared to participants <70 years. This may be related to the decline of body function and the thermoregulatory capacity in the elderly with age.[47,48] Also, people with underlying health conditions, such as cardiovascular disease and diabetes, were more vulnerable to temperature decreases. These observations are consistent with previous reports that low air temperature exposures increase the risks for both conditions.[6,49,50] Our findings lead to the hypothesis that subclinical inflammation may be one of the underlying mechanisms behind the associations of low temperature with cardiometabolic disease, which merits further studies in the future. Interestingly, we did not find evidence for consistent effect modification by sex (stronger effects of the air temperature on eight biomarkers in men and five biomarkers in women). Several previous studies found that the mortality among men exposed to low temperatures was higher than that among women,[51−53] while other studies showed opposite findings.[48,54] Overall, the role of sex in modifying temperature−mortality associations is not clear, which could at least partly be due to differential effects of air temperature on different biomarkers of subclinical inflammation that vary according to sex.

*4.8. **Interactive Effects between Lower Air Temperature and Higher Air Pollution.*** Strikingly, we found interactive effects between a lower air temperature and higher air pollution exposures on increased biomarkers of subclinical inflammation. Our findings suggest that given the adverse health effects of low temperature, the synergy of low temperature and air pollution aggravates the impact on health. Previous studies have showed that the potential interactive effect of low temperature and air pollution exposure on cardiovascular diseases have been found.[55−57] More importantly, lower air temperatures and higher air pollution exposures often coexist due to extensive use of coal, wood, diesel, or oil burn for heating during the colder temperature in many parts of the world, e.g., China. These highlights that integrated climate and air quality policies should be formulated to strengthen the response to the combined health threats of temperature and air pollution.

*4.9. **Limitations.*** Our study also has limitations. First, we conducted this study in one study area, limiting its generalizability beyond the study area but, most importantly, to areas in colder or warmer climate zones. Further cohort studies with similar research designs are necessary to corroborate our findings. Second, we used area-level exposure estimates rather than individual measurements, which may have led to a possible bias due to the misclassification of exposure and a potential underestimation of true associations. Third, as a cross-sectional study, it lacks the ability to observe changes over time. Additionally, despite adjusting for a range of covariates, residual confounding or unmeasured confounders may still be present. Finally, protein degradation is possible with long-term storage, but is expected to be nondifferential, i.e., should not depend on the air temperature exposure, which was the main exposure of the analyses. Therefore, the effects on our results are extremely unlikely.

In conclusion, we found that short- and medium-term exposures to lower air temperature were associated with higher levels in 64 biomarkers of subclinical inflammation, such as EN-RAGE, IL-6, IL-10, CCL28, and NT-3, some of which have been related to a higher risk of chronic diseases or mortality before. Our findings provide more insight into the complexity of the relationship between low air temperatures and adverse health effects and indicate that subclinical inflammation could be a relevant mediator meriting further studies

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c00302.

https://doi.org/10.1021/acs.est.3c00302
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Text S1, assessment of meteorological variables and air pollutants; Text S2, assessment of covariates; Table S1, biomarkers of subclinical inflammation in the OLINK Inflammation panel and assay characteristics; Table S2, levels of 71 biomarkers of subclinical inflammation in serum; Figure S1, exposure-response functions of air temperature and biomarkers of subclinical inflammation at lags 0−13 days; Figure S2, the levels of 71 biomarkers of subclinical inflammation in serum samples; Figure S3, spearman correlation between biomarkers of subclinical inflammation; Figure S4, time series of daily mean air temperature for participants in this study; Figure S5, correlation between meteorological variables and air pollutants; Figure S6, significant associations between short-term exposure to air temperature per 1-IQR decrease with biomarkers of subclinical inflammation (*P*-adjust <0.05); Figure S7, significant associations between medium-term exposure to air temperature per 1-IQR decrease with biomarkers of subclinical inflammation (*P*-adjust <0.05); Figure S8, volcano plots presenting the associations between short- and medium-term exposures to air temperature per 1 °C decrease with 71 biomarkers of subclinical inflammation; Figure S9, Venn diagrams of significant associations between short- and medium-term exposures to air temperature per 1-IQR decrease with biomarkers of subclinical inflammation; Figure S10, short- and medium-term effects of air temperature on biomarkers of subclinical inflammation per 1-IQR decrease significantly modified by sex or diabetes; Figure S11, short- and medium-term effects of air temperature on biomarkers of subclinical inflammation per 1-IQR decrease significantly modified by $PM_{2.5}$ or O_3; Figure S12, short- and medium-term effects of air temperature on biomarkers of subclinical inflammation per 1-IQR decrease significantly modified by NO_2; Figure S13, sensitivity analysis (participants with CRP values >10 mg/L were excluded): significant associations between short- and medium-term exposures to air temperature per 1-IQR decrease with biomarkers of subclinical inflammation (PDF)

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Author Contributions

▲C.H. and A.S. contributed equally and share last authorship.

Funding

This work was supported by a scholarship under the State Scholarship Fund by the China Scholarship Council (File No. 201906010316). The KORA study was initiated and financed by the Helmholtz Zentrum München − German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. Data collection in the KORA study is done in cooperation with the University Hospital of Augsburg. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC Health), Ludwig-

Maximilians-Universität, as part of LMUinnovativ. The German Diabetes Center is funded by the German Federal Ministry of Health (Berlin, Germany) and the Ministry of Culture and Science of the state North Rhine-Westphalia (Düsseldorf, Germany) and receives additional funding from the German Federal Ministry of Education and Research (BMBF) through the German Center for Diabetes Research (DZD e.V.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank all participants for their long-term commitment to the KORA study, the staff for data collection and research data management, and the members of the KORA Study Group (https://www.helmholtz-munich.de/en/epi/cohort/kora), who are responsible for the design and conduct of the study.

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https://doi.org/10.1021/acs.est.3c00302
Environ. Sci. Technol. 2023, 57, 12210−12221

At Supporting Information for

Effects of short- and medium-term exposures to lower air temperature on 71 novel biomarkers of subclinical inflammation: results from the KORA F4 study

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Text S1. Assessment of meteorological variables and air pollutants

Relative humidity (RH), particulate matter with an aerodynamic diameter <2.5 μm ($PM_{2.5}$), nitrogen dioxide (NO_2), ozone (O_3), wind speed, and barometric pressure were measured at fixed urban background monitoring sites operated by the Bavarian Environment Agency (LfU, Bayerisches Landesamt für Umwelt) in Augsburg, Germany.[1,2] Using Tapered Element Oscillating MicroBalance (TEOM model 1400A, ThermoFisherScientific) equipped with the Filter Dynamics Measurement System (FDMS, model 8500b; ThermoFisherScientific), $PM_{2.5}$ was measured at a single urban background site located 1 km south of the city center. The monitoring site for NO_2 was located about 2km north of the city center, and the monitoring sites for RH, O_3, wind speed, and barometric pressure were located about 5 km south of the city center. At least 75% of the hourly measurements had to be available to calculate the daily 24-hour average NO_2, maximum 8-hour average O_3, 24-hour average $PM_{2.5}$, and 24-hour average meteorological variables, respectively.

Text S2. Assessment of covariates

Information on participants' sociodemographic characteristics (age and sex), lifestyle (smoking status, alcohol intake, and physical activity), history of chronic diseases (hypertension, angina pectoris, stroke, myocardial infarction, and diabetes) and current use of medication (antihypertensive and nonsteroidal anti-inflammatory drugs), was collected via a computer-assisted personal interview and a self-administered questionnaire. Anthropometric measurements (height, body weight, and waist circumference) and blood pressure (systolic blood pressure, diastolic blood pressure) were measured during the physical examination.

Smoking was dichotomized as never smokers, former smoker, and current smokers (regular and occasional smokers) in this analysis. Alcohol intake was evaluated by participants self-reported

information on beverage-specific alcohol consumption (beer, wine, or spirits) on the last weekday and last weekend. Physical activity levels were based on self-reported time per week spent on physical activity during leisure time in summer and winter, and then were dichotomized as low physical activity (almost no activity), medium physical activity (regularly or irregularly about one hour per week), and high physical activity (regularly more than two hours per week) in present analysis.

Study participants without a diagnosis of diabetes underwent a standard oral glucose tolerance test (OGTT). Normal glucose tolerance was defined as a fasting glucose concentration of less than 110 mg/dL and a 2-hour glucose concentration of less than 140 mg/dL. Prediabetes was defined as a fasting glucose concentration of 110 -125 mg/dL, a 2-hour glucose concentration of 140-199 mg/dL, or a combination of both. Diabetes was defined as a fasting glucose concentration $\geq$ 126 mg/dL or a 2-hour glucose concentration $\geq$ 200 mg/dL during the OGTT, self-reported diagnosis of diabetes, or use of antidiabetic medication. Hypertension was defined using self-reported use of antihypertensive medication or blood pressure measurements (over 140/90 mm Hg). Angina pectoris was defined using self-reported history of physician diagnoses. Stroke or myocardial infarction was defined using self-reported history of physician diagnoses treated in hospital.

Medication data were collected using IDOM software (an instrument for database-supported online medication registration)[3], and included antihypertensive medication (antihypertensive medication, beta-blocker, diuretics, ACE inhibitors, calcium antagonists) and nonsteroidal anti-inflammatory drugs (NASID).

Table S1. Biomarkers of subclinical inflammation in the OLINK Inflammation panel and assay characteristics.

Assay ID	Biomarker	Full name	UniProt No	Gene symbol	Intra-assay CV (%)	Inter-assay CV (%)	LOD (NPX)	Percentage of samples below LOD
101_IL-8	IL-8	Interleukin-8	P10145	CXCL8	3	8.9	1.69	0
102_VEGF-A	VEGF-A	Vascular endothelial growth factor A	P15692	VEGFA	2.9	7.6	2.22	0
103_BDNF*	BDNF	Brain-derived neurotrophic factor	P23560	BDNF	2.8	8.1	0.09	26.9
105_MCP-3	MCP-3	Monocyte chemotactic protein 3 (CCL7)	P80098	CCL7	6.5	10.3	0.84	3
106_GDNF	GDNF	Glial cell line-derived neurotrophic factor	P39905	GDNF	8.5	8.4	0.7	9.5
107_CDCP1	CDCP1	CUB domain-containing protein 1	Q9H5V8	CDCP1	3.9	9.6	0.11	0
108_CD244	CD244	Natural killer cell receptor 2B4	Q9BZW8	CD244	2.2	8.9	0.66	0
109_IL-7	IL-7	Interleukin-7	P13232	IL7	2.7	6	1.04	0.1
110_OPG	OPG	Osteoprotegerin	O00300	TNFRSF11B	2.3	5	0.85	0
111_LAP TGF-beta-1	LAP TGFβ1	Latency-associated peptide transforming growth factor beta-1	P01137	TGFB1	10	16.6	0.61	0.1
112_uPA	uPA	Urokinase-type plasminogen activator	P00749	PLAU	2.5	4.6	0.81	0
113_IL-6	IL-6	Interleukin-6	P05231	IL6	4.4	10.7	1.15	2.1

114_IL-17C	IL-17C	Interleukin-17C	Q9P0M4	IL17C	6.2	6.9	0.95	22.3
115_MCP-1	MCP-1	Monocyte chemotactic protein 1 (CCL2)	P13500	CCL2	2.9	6	1.25	0
116_IL-17A*	IL-17A	Interleukin-17A	Q16552	IL17A	4.9	9.3	0.74	33
117_CXCL11	CXCL11	C-X-C motif chemokine 11	O14625	CXCL11	2.5	5.8	1.02	0
118_AXIN1	Axin-1	Axin-1	O15169	AXIN1	4.5	8.7	0.95	10.1
120_TRAIL	TRAIL	TNF-related apoptosis-inducing ligand (TNFSF10)	P50591	TNFSF10	2.7	7.2	0.59	0
121_IL-20RA*	IL-20RA	Interleukin-20 receptor subunit alpha	Q9UHF4	IL20RA	n/a	n/a	0.61	93
122_CXCL9	CXCL9	C-X-C motif chemokine 9	Q07325	CXCL9	3.2	5.4	0.7	0
123_CST5	CST5	Cystatin D	P28325	CST5	2.4	6.6	0.97	0
124_IL-2RB*	IL-2RB	Interleukin-2 receptor subunit beta	P14784	IL2RB	n/a	n/a	1.09	95.4
125_IL-1 alpha*	IL-1α	Interleukin-1 alpha	P01583	IL1A	n/a	n/a	1.77	96.9
126_OSM	OSM	Oncostatin-M	P13725	OSM	2.3	6.9	1.55	0.4
127_IL-2*	IL-2	Interleukin-2	P60568	IL2	n/a	n/a	0.68	99.9
128_CXCL1	CXCL1	C-X-C motif chemokine 1	P09341	CXCL1	2.5	5.8	1.73	0
129_TSLP*	TSLP	Thymic stromal lymphopoietin	Q969D9	TSLP	n/a	n/a	1.41	99.2
130_CCL4	CCL4	C-C motif chemokine 4	P13236	CCL4	2.8	5.8	1.38	0
131_CD6	CD6	T cell surface glycoprotein CD6 isoform	Q8WWJ7	CD6	3.8	12.9	1.55	0
132_SCF	SCF	Stem cell factor (c-Kit-ligand)	P21583	KITLG	2.1	5.8	1.08	0

133_IL-18	IL-18	Interleukin-18	Q14116	IL18	3	6.4	1.36	0
134_SLAMF1	SLAMF1	Signaling lymphocytic activation molecule (SLAM)	Q13291	SLAMF1	6.4	10.7	1.08	0
135_TGF-alpha	TGF-α	Transforming growth factor alpha	P01135	TGFA	3.1	9.5	-0.19	0
136_MCP-4	MCP-4	Monocyte chemotactic protein 4 (CCL13)	Q99616	CCL13	3	9.5	0.29	0
137_CCL11	CCL11	Eotaxin (CCL11)	P51671	CCL11	2.8	6.9	1.43	0
138_TNFSF14	TNFSF14	Tumor necrosis factor ligand superfamily member 14 (LIGHT)	O43557	TNFSF14	2.8	7.8	1.3	0
139_FGF-23	FGF-23	Fibroblast growth factor 23	Q9GZV9	FGF23	5	7.6	0.31	0.2
140_IL-10RA*	IL-10RA	Interleukin-10 receptor subunit alpha	Q13651	IL10RA	2.6	9.2	0.82	26.5
141_FGF-5	FGF-5	Fibroblast growth factor 5	Q8NF90	FGF5	4.3	8.7	0.61	2.5
142_MMP-1	MMP-1	Matrix metalloproteinase-1	P03956	MMP1	2.3	5.4	2.12	0
143_LIF-R	LIF-R	Leukemia inhibitory factor receptor	P42702	LIFR	3.4	10.1	1.2	0
144_FGF-21	FGF-21	Fibroblast growth factor 21	Q9NSA1	FGF21	3.1	7.3	0.84	0
145_CCL19	CCL19	C-C motif chemokine 19	Q99731	CCL19	2.9	7	0.9	0
148_IL-15RA	IL-15RA	Interleukin-15 receptor subunit alpha	Q13261	IL15RA	5.6	10.5	0.15	2.4
149_IL-10RB	IL-10RB	Interleukin-10 receptor subunit beta	Q08334	IL10RB	3.1	10.4	1.17	0

150_IL-22 RA1*	IL-22RA1	Interleukin-22 receptor subunit alpha-1	Q8N6P7	IL22RA1	n/a	n/a	1.55	99.8
151_IL-18R1	IL-18R1	Interleukin-18 receptor 1	Q13478	IL18R1	3	7.6	0.85	0
152_PD-L1	PD-L1	Programmed cell death 1 ligand 1	Q9NZQ7	CD274	4.8	9.6	1.87	0
153_Beta-NGF	Beta-NGF	Beta-nerve growth factor	P01138	NGF	3.6	7.6	1.02	0
154_CXCL5	CXCL5	C-X-C motif chemokine 5	P42830	CXCL5	2.7	5.8	1.57	0
155_TRANCE	TRANCE	TNF-related activation-induced cytokine (TRANCE, TNFSF11, RANKL, OPGL)	O14788	TNFSF11	4.6	8.8	1.32	0
156_HGF	HGF	Hepatocyte growth factor	P14210	HGF	2.5	7.5	1.11	0
157_IL-12B	IL-12B	Interleukin-12 subunit beta	P29460	IL12B	3.2	6.9	0.69	0
158_IL-24*	IL-24	Interleukin-24	Q13007	IL24	n/a	n/a	1.36	93.5
159_IL-13*	IL-13	Interleukin-13	P35225	IL13	n/a	n/a	1.14	94.9
160_ARTN*	Artemin	Artemin	Q5T4W7	ARTN	n/a	n/a	0.72	95.3
161_MMP-10	MMP-10	Matrix metalloproteinase-10 (SL-2)	P09238	MMP10	2.7	8.8	1.13	0
162_IL-10	IL-10	Interleukin-10	P22301	IL10	5.3	10.4	1.04	0
163_TNF*	TNFα	Tumor necrosis factor-alpha	P01375	TNF	n/a	n/a	1.08	95.9
164_CCL23	CCL23	C-C motif chemokine 23	P55773	CCL23	2.9	6.1	0.9	0
165_CD5	CD5	T-cell surface glycoprotein CD5	P06127	CD5	3.1	9.3	1.22	0

166_MIP-1 alpha	MIP-1α	Macrophage inflammatory protein-1alpha (C-C motif chemokine 3/CCL3)	P10147	CCL3	3.3	9	1.62	0
167_Flt3L	Flt3L	Fms-related tyrosine kinase 3 ligand	P49771	FLT3LG	2.7	8.1	1.28	0
168_CXCL6	CXCL6	C-X-C motif chemokine 6	P80162	CXCL6	2.4	8.9	1.26	0
169_CXCL10	CXCL10	C-X-C motif chemokine 10 (IP-10)	P02778	CXCL10	3.1	6	1.53	0
170_4E-BP1	EIF4EBP1	Eukaryotic translation initiation factor 4E-binding protein 1	Q13541	EIF4EBP1	3.8	8.6	0.66	0
171_IL-20*	IL-20	Interleukin-20	Q9NYY1	IL20	n/a	n/a	0.96	95.4
172_SIRT2	SIRT2	SIR2-like protein 2	Q8IXJ6	SIRT2	5.4	9.7	0.87	0
173_CCL28	CCL28	C-C motif chemokine 28	Q9NRJ3	CCL28	4.1	14.6	0.73	1
174_DNER	DNER	Delta and Notch-like epidermal growth factor-related receptor	Q8NFT8	DNER	2.3	8	0.71	0
175_EN-RAGE	EN-RAGE	Protein S100-A12 (EN-RAGE)	P80511	S100A12	4.9	11	0.78	0
176_CD40	CD40	CD40L receptor	P25942	CD40	2.5	7.8	0.81	0
177_IL-33*	IL-33	Interleukin-33	O95760	IL33	n/a	n/a	0.84	98
178_IFN-gamma*	IFNγ	Interferon-gamma	P01579	IFNG	n/a	n/a	0.94	98.5
179_FGF-19	FGF-19	Fibroblast growth factor 19	O95750	FGF19	2.9	8.4	0.68	0
180_IL-4*	IL-4	Interleukin-4	P05112	IL4	n/a	n/a	0.81	91.9

181_LIF*	LIF	Leukemia inhibitory factor	P15018	LIF	n/a	n/a	1.28	95
182_NRTN*	Neurturin	Neurturin	Q99748	NRTN	n/a	n/a	1.04	97.2
183_MCP-2	MCP-2	Monocyte chemotactic protein 2 (MCP-2, CCL8)	P80075	CCL8	2.7	8	1.27	0
184_CASP-8**	Caspase-8	Caspase-8	Q14790	CASP8	7.1	35.9	1.46	0.7
185_CCL25	CCL25	C-C motif chemokine 25	O15444	CCL25	3.2	7.6	0.7	0
186_CX3CL1	CX3CL1	Fractalkine	P78423	CX3CL1	3.7	10.8	1.31	0
187_TNFRSF9	TNFRSF9	Tumor necrosis factor receptor superfamily member 9	Q07011	TNFRSF9	3	9.7	1.31	0
188_NT-3	NT-3	Neurotrophin-3	P20783	NTF3	4.8	10.5	0.33	0.7
189_TWEAK	TWEAK	Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK)	O43508	TNFSF12	2.3	6.2	1.07	0
190_CCL20	CCL20	C-C motif chemokine 20	P78556	CCL20	3.2	7.1	0.89	0
191_ST1A1	ST1A1	Sulfotransferase 1A1	P50225	SULT1A1	6.4	14	0.69	4.5
192_STAMPB	STAMBP	STAM-binding protein	O95630	STAMBP	3.6	9.2	1.16	0
193_IL-5*	IL-5	Interleukin-5	P05113	IL5	n/a	n/a	1.21	72.6
194_ADA	ADA	Adenosine deaminase	P00813	ADA	3.7	8.8	0.99	0
195_TNFB	TNFβ	Tumor necrosis factor-beta (lymphotoxin-alpha/LT-alpha)	P01374	LTA	4.4	8.8	1.08	0
196_CSF-1	CSF-1	Macrophage colony-stimulating factor 1	P09603	CSF1	2.2	8.8	0.93	0

LOD, limit of detection; n/a, not applicable (NPX of control measurements below LOD); NPX, normalized protein expression values; *Data missing for more than 25% were excluded from analysis; ** Data for inter-assay CVs >20% were excluded from analysis.

Table S2. Levels of 71 biomarkers of subclinical inflammation in serum.

Biomarkers (NPX)	Groups	Mean ± SD	25%	Median	75%
ADA	Enzymes	3.8 ± 0.4	3.6	3.8	4.0
Axin-1	Miscellaneous	1.6 ± 0.5	1.2	1.5	1.9
Beta-NGF	Neurotrophic factors	1.9 ± 0.3	1.7	1.8	2.0
CCL11	Chemokines	8.7 ± 0.4	8.4	8.7	9.0
CCL19	Chemokines	10.0 ± 1.0	9.3	9.8	10.4
CCL20	Chemokines	4.9 ± 1.0	4.2	4.8	5.5
CCL23	Chemokines	10 ± 0.5	9.7	10.0	10.3
CCL25	Chemokines	6.7 ± 0.6	6.3	6.7	7.0
CCL28	Chemokines	1.6 ± 0.4	1.3	1.6	1.8
CCL4	Chemokines	8.2 ± 0.6	7.9	8.2	8.6
CD244	Miscellaneous	5.6 ± 0.3	5.3	5.6	5.8
CD40	TNF(R) superfamily	10.3 ± 0.3	10.1	10.3	10.5
CD5	Miscellaneous	5.4 ± 0.4	5.1	5.4	5.6
CD6	Miscellaneous	4.6 ± 0.5	4.3	4.6	4.9
CDCP1	Miscellaneous	3.4 ± 0.7	3.0	3.3	3.8
CSF-1	Cytokines/growth factors	7.8 ± 0.2	7.6	7.8	8.0
CST5	Miscellaneous	6.5 ± 0.5	6.1	6.4	6.8
CX3CL1	Chemokines	6.3 ± 0.4	6.0	6.3	6.5
CXCL1	Chemokines	9.4 ± 0.5	9.1	9.4	9.7
CXCL10	Chemokines	9.5 ± 0.8	9.0	9.4	9.9
CXCL11	Chemokines	7.9 ± 0.7	7.5	7.9	8.3

CXCL5	Chemokines	11.5 ± 0.7	11.1	11.6	12.1
CXCL6	Chemokines	8.7 ± 0.6	8.3	8.7	9.1
CXCL9	Chemokines	7.5 ± 0.8	6.9	7.4	8.0
DNER	Miscellaneous	8.3 ± 0.3	8.1	8.3	8.5
EIF4EBP1	Miscellaneous	7.0 ± 0.6	6.6	7.0	7.4
EN-RAGE	Cytokines/growth factors	5.1 ± 0.8	4.5	5.0	5.6
FGF-19	Cytokines/growth factors	7.8 ± 0.9	7.2	7.8	8.4
FGF-21	Cytokines/growth factors	6.0 ± 1.1	5.3	5.9	6.6
FGF-23	Cytokines/growth factors	1.4 ± 0.5	1.1	1.4	1.7
FGF-5	Cytokines/growth factors	1.3 ± 0.3	1.1	1.3	1.5
Flt3L	Cytokines/growth factors	9.0 ± 0.4	8.7	9.0	9.2
GDNF	Neurotrophic factors	1.1 ± 0.3	0.9	1.1	1.3
HGF	Cytokines/growth factors	8.6 ± 0.4	8.4	8.6	8.9
IL-10	Interleukins (receptors)	2.5 ± 0.4	2.2	2.5	2.7
IL-10RB	Interleukins (receptors)	7.3 ± 0.3	7.1	7.3	7.5
IL-12B	Interleukins (receptors)	5.5 ± 0.7	5.0	5.4	5.9
IL-15RA	Interleukins (receptors)	0.8 ± 0.3	0.6	0.8	1.0
IL-17C	Interleukins (receptors)	1.4 ± 0.5	1.0	1.3	1.7
IL-18	Interleukins (receptors)	8.7 ± 0.6	8.4	8.7	9.1
IL-18R1	Interleukins (receptors)	7.5 ± 0.4	7.2	7.5	7.8
IL-6	Interleukins (receptors)	2.4 ± 0.7	1.9	2.3	2.8
IL-7	Interleukins (receptors)	4.7 ± 0.5	4.4	4.7	5.0
IL-8	Interleukins (receptors)	6.7 ± 0.5	6.3	6.7	7.0

LAP TGFβ1	Cytokines/growth factors	8.0 ± 0.3	7.7	8.0	8.2
LIF-R	Miscellaneous	4.7 ± 0.3	4.4	4.7	4.9
MCP-1	Chemokines	11.1 ± 0.4	10.8	11.1	11.4
MCP-2	Chemokines	9.5 ± 0.7	9.1	9.6	10
MCP-3	Chemokines	2.0 ± 0.5	1.6	1.9	2.2
MCP-4	Chemokines	4.1 ± 0.6	3.7	4.1	4.4
MIP-1α	Chemokines	4.5 ± 0.5	4.2	4.5	4.8
MMP-1	Enzymes	14.5 ± 0.7	14	14.6	15.0
MMP-10	Enzymes	6.4 ± 0.6	6.1	6.4	6.8
NT-3	Neurotrophic factors	1.0 ± 0.3	0.8	1.0	1.2
OPG	TNF(R) superfamily	10.2 ± 0.3	10	10.2	10.5
OSM	Cytokines/growth factors	4.9 ± 0.6	4.5	4.9	5.3
PD-L1	Miscellaneous	4.3 ± 0.4	4.1	4.3	4.5
SCF	Cytokines/growth factors	9.9 ± 0.4	9.7	10	10.2
SIRT2	Enzymes	2.3 ± 0.5	2.0	2.3	2.6
SLAMF1	Miscellaneous	2.5 ± 0.5	2.2	2.5	2.9
ST1A1	Enzymes	2.0 ± 0.8	1.4	1.9	2.5
STAMBP	Enzymes	3.1 ± 0.4	2.8	3.0	3.3
TGFα	Cytokines/growth factors	4.6 ± 0.5	4.2	4.6	4.9
TNFRSF9	TNF(R) superfamily	6.3 ± 0.5	6.0	6.3	6.6
TNFSF14	TNF(R) superfamily	5.6 ± 0.5	5.3	5.6	6.0
TNFβ	TNF(R) superfamily	3.9 ± 0.5	3.6	3.9	4.1
TRAIL	TNF(R) superfamily	8.1 ± 0.3	7.9	8.1	8.3

TRANCE	TNF(R) superfamily	4.6 ± 0.6	4.2	4.6	5.0
TWEAK	TNF(R) superfamily	9.4 ± 0.3	9.2	9.4	9.6
uPA	Enzymes	9.9 ± 0.3	9.7	9.9	10.1
VEGF-A	Cytokines/growth factors	10.9 ± 0.5	10.6	10.9	11.3

Figure S1. Exposure-response functions of air temperature and biomarkers of subclinical inflammation at lags 0-13 days.

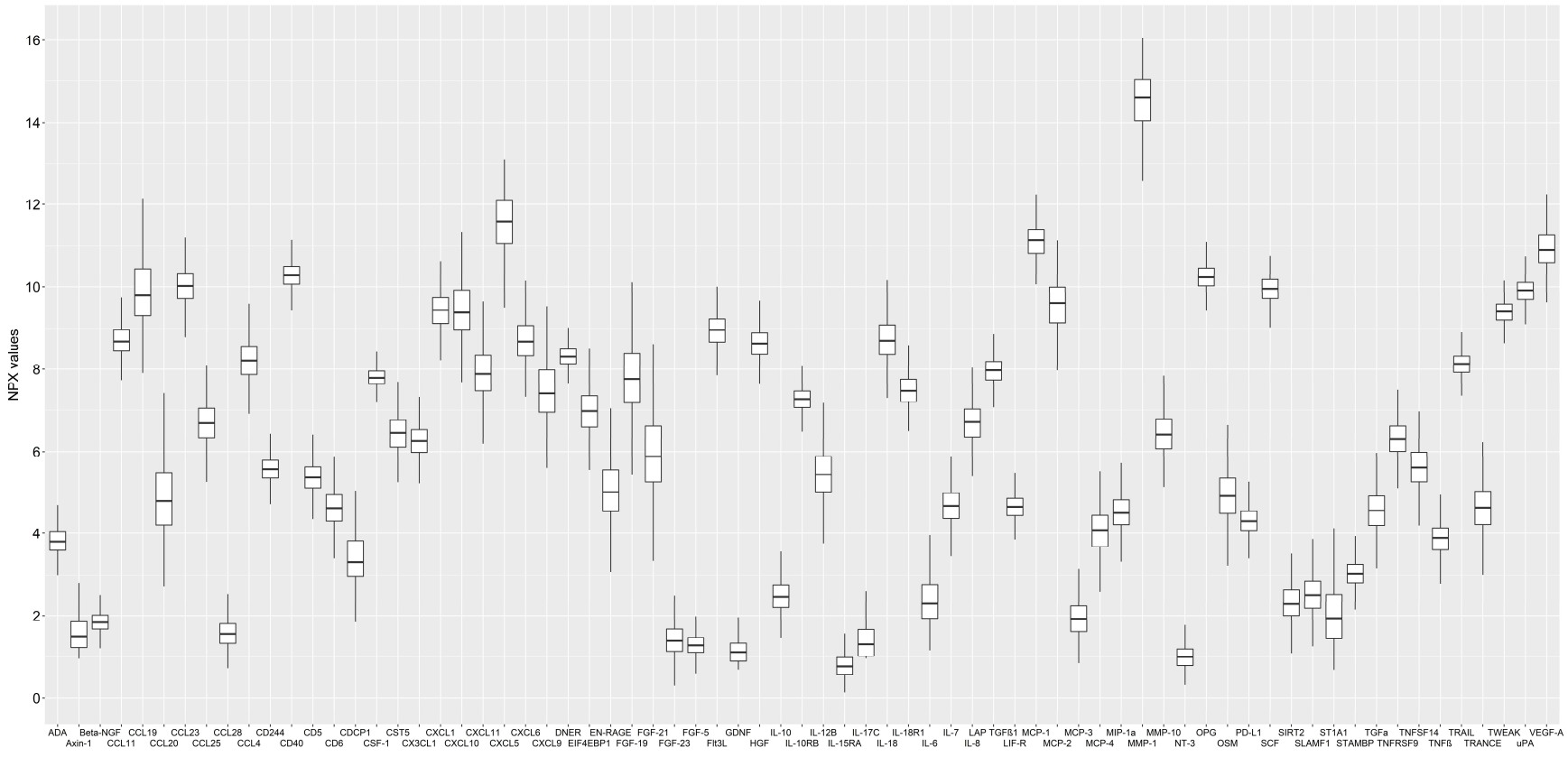

Figure S2. The levels of 71 biomarkers of subclinical inflammation in serum samples.

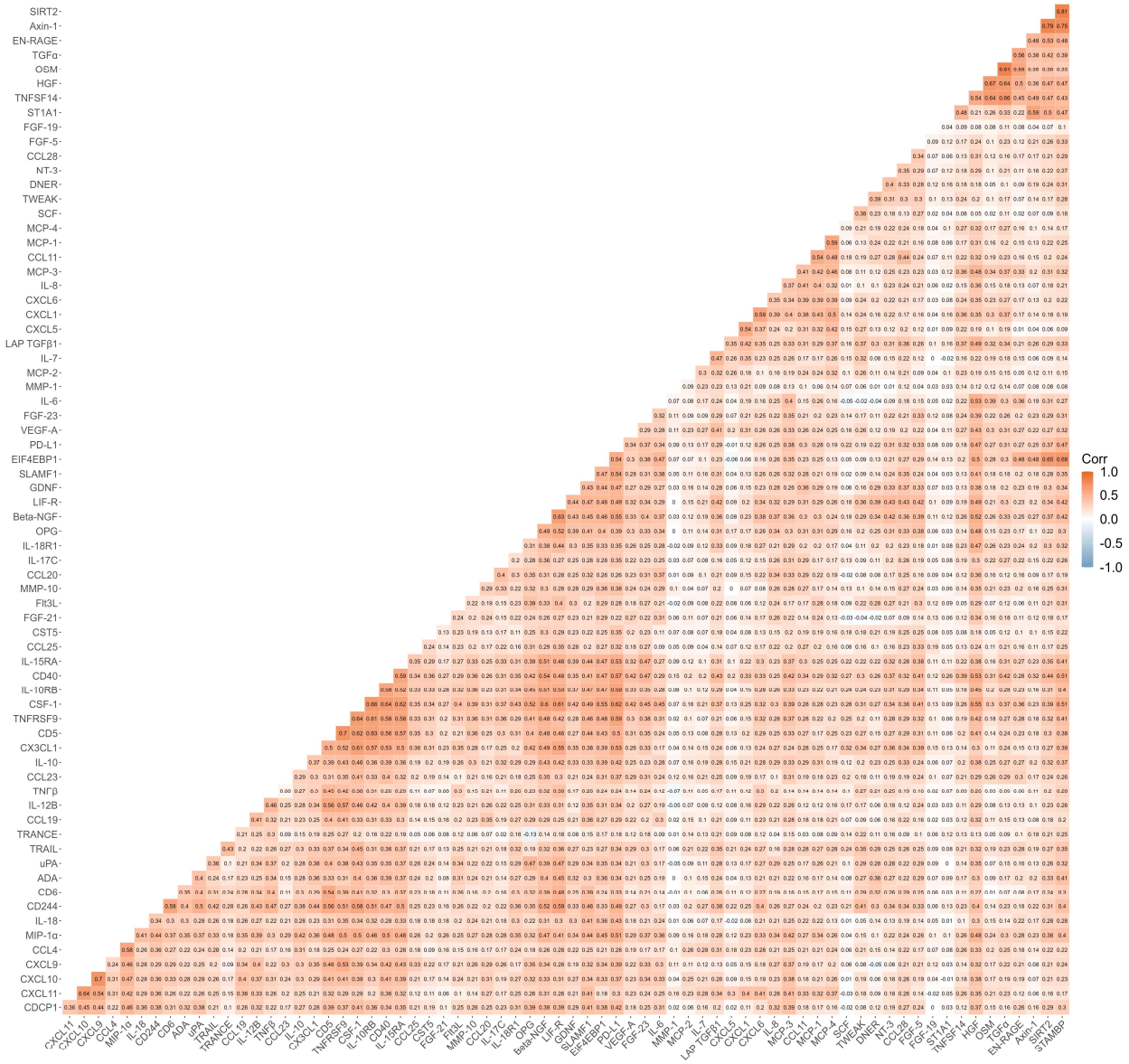

Figure S3. Spearman correlation between biomarkers of subclinical inflammation.

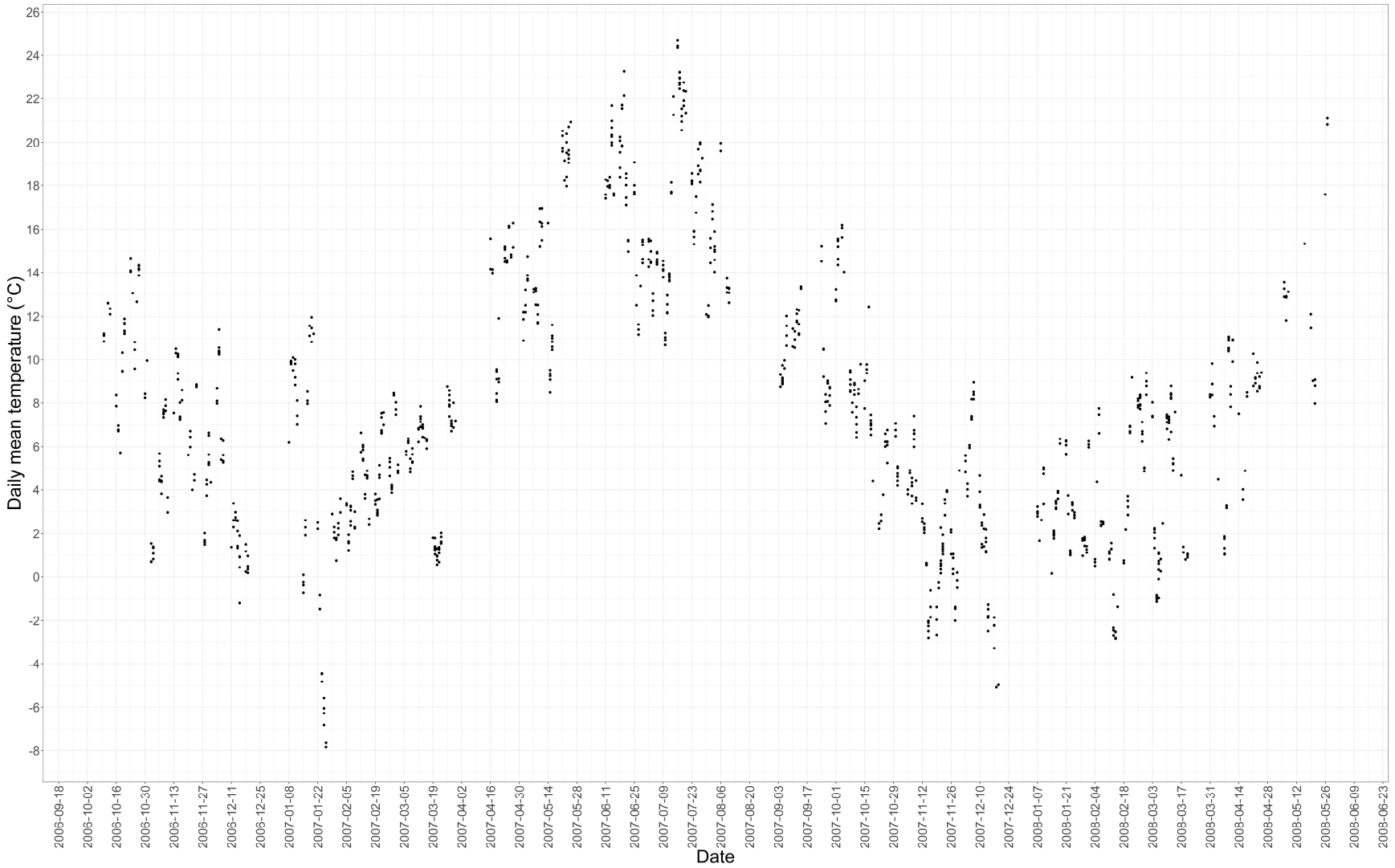

Figure S4. Time series of daily mean air temperature for participants in this study.

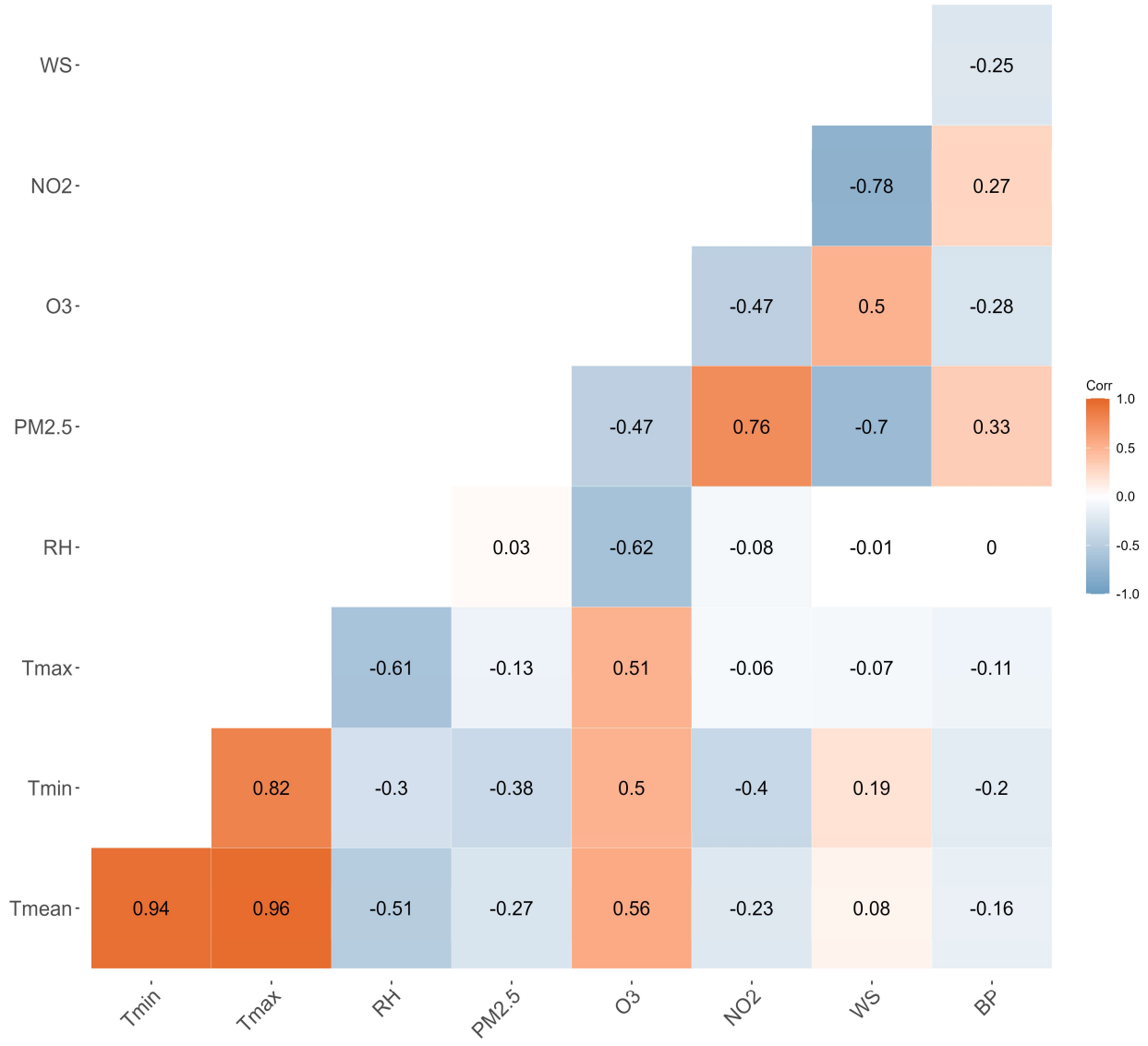

Figure S5. Correlation between meteorological variables and air pollutants.

Note: *Tmean***:** daily mean air temperature; *Tmin*: daily minimum air temperature; *Tmax*: daily maximum air temperature; *RH*: relative humidity; O_3*:* ozone; $PM_{2.5}$ particulate matter with an aerodynamic diameter of ≤2.5 μm; NO_2: nitrogen dioxide, *WS:* wind speed; *BP:* Barometric pressure.

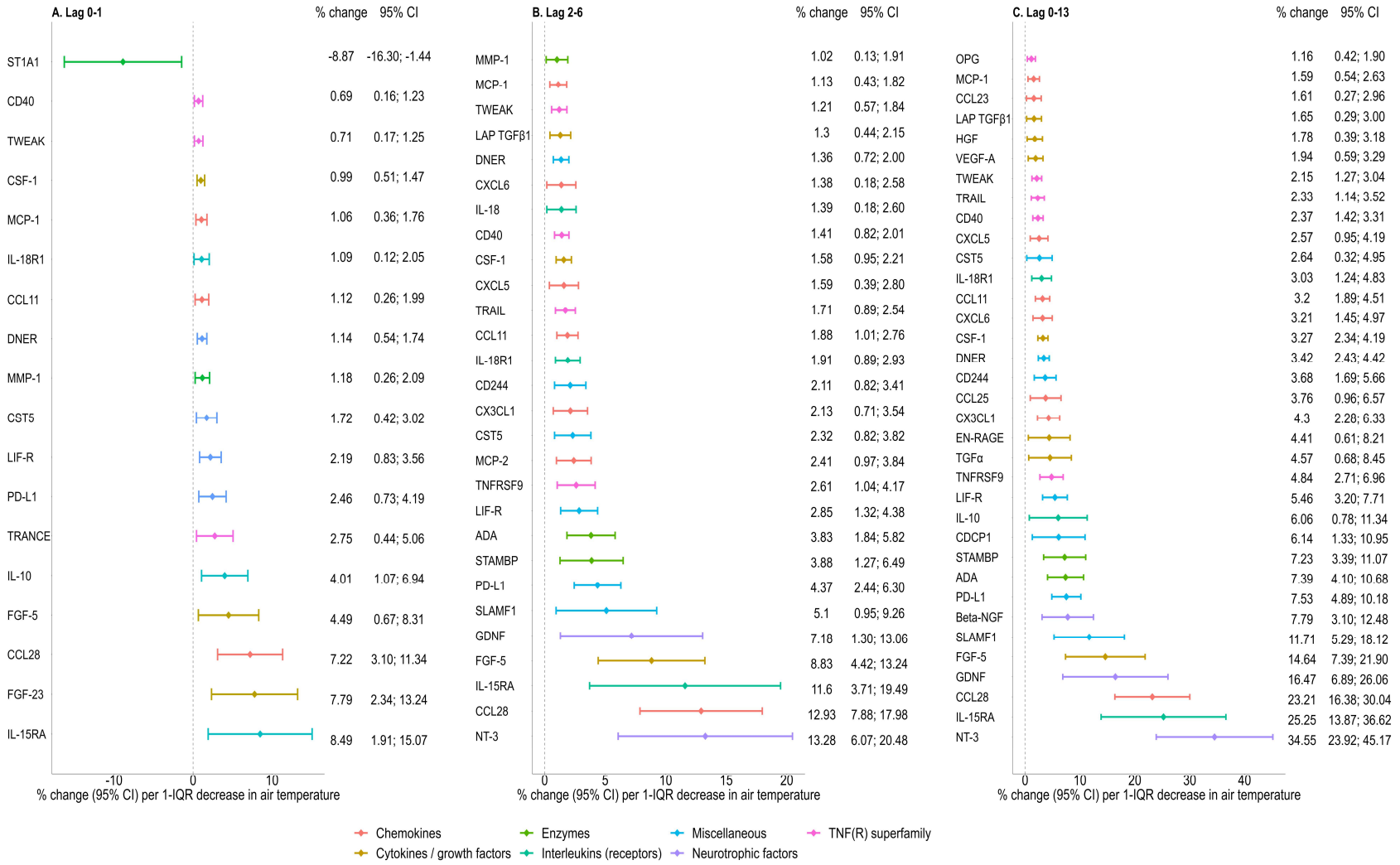
A. Lag 0-1
% change
95% CI
ST1A1 -8.87 -16.30; -1.44
CD40 0.69 0.16; 1.23
TWEAK 0.71 0.17; 1.25
CSF-1 0.99 0.51; 1.47
MCP-1 1.06 0.36; 1.76
IL-18R1 1.09 0.12; 2.05
CCL11 1.12 0.26; 1.99
DNER 1.14 0.54; 1.74
MMP-1 1.18 0.26; 2.09
CST5 1.72 0.42; 3.02
LIF-R 2.19 0.83; 3.56
PD-L1 2.46 0.73; 4.19
TRANCE 2.75 0.44; 5.06
IL-10 4.01 1.07; 6.94
FGF-5 4.49 0.67; 8.31
CCL28 7.22 3.10; 11.34
FGF-23 7.79 2.34; 13.24
IL-15RA 8.49 1.91; 15.07
-10 0 10
% change (95% CI) per 1-IQR decrease in air temperature
B. Lag 2-6
% change
95% CI
MMP-1 1.02 0.13; 1.91
MCP-1 1.13 0.43; 1.82
TWEAK 1.21 0.57; 1.84
LAP TGFβ1 1.3 0.44; 2.15
DNER 1.36 0.72; 2.00
CXCL6 1.38 0.18; 2.58
IL-18 1.39 0.18; 2.60
CD40 1.41 0.82; 2.01
CSF-1 1.58 0.95; 2.21
CXCL5 1.59 0.39; 2.80
TRAIL 1.71 0.89; 2.54
CCL11 1.88 1.01; 2.76
IL-18R1 1.91 0.89; 2.93
CD244 2.11 0.82; 3.41
CX3CL1 2.13 0.71; 3.54
CST5 2.32 0.82; 3.82
MCP-2 2.41 0.97; 3.84
TNFRSF9 2.61 1.04; 4.17
LIF-R 2.85 1.32; 4.38
ADA 3.83 1.84; 5.82
STAMBP 3.88 1.27; 6.49
PD-L1 4.37 2.44; 6.30
SLAMF1 5.1 0.95; 9.26
GDNF 7.18 1.30; 13.06
FGF-5 8.83 4.42; 13.24
IL-15RA 11.6 3.71; 19.49
CCL28 12.93 7.88; 17.98
NT-3 13.28 6.07; 20.48
0 5 10 15 20
% change (95% CI) per 1-IQR decrease in air temperature
C. Lag 0-13
% change
95% CI
OPG 1.16 0.42; 1.90
MCP-1 1.59 0.54; 2.63
CCL23 1.61 0.27; 2.96
LAP TGFβ1 1.65 0.29; 3.00
HGF 1.78 0.39; 3.18
VEGF-A 1.94 0.59; 3.29
TWEAK 2.15 1.27; 3.04
TRAIL 2.33 1.14; 3.52
CD40 2.37 1.42; 3.31
CXCL5 2.57 0.95; 4.19
CST5 2.64 0.32; 4.95
IL-18R1 3.03 1.24; 4.83
CCL11 3.2 1.89; 4.51
CXCL6 3.21 1.45; 4.97
CSF-1 3.27 2.34; 4.19
DNER 3.42 2.43; 4.42
CD244 3.68 1.69; 5.66
CCL25 3.76 0.96; 6.57
CX3CL1 4.3 2.28; 6.33
EN-RAGE 4.41 0.61; 8.21
TGFα 4.57 0.68; 8.45
TNFRSF9 4.84 2.71; 6.96
LIF-R 5.46 3.20; 7.71
IL-10 6.06 0.78; 11.34
CDCP1 6.14 1.33; 10.95
STAMBP 7.23 3.39; 11.07
ADA 7.39 4.10; 10.68
PD-L1 7.53 4.89; 10.18
Beta-NGF 7.79 3.10; 12.48
SLAMF1 11.71 5.29; 18.12
FGF-5 14.64 7.39; 21.90
GDNF 16.47 6.89; 26.06
CCL28 23.21 16.38; 30.04
IL-15RA 25.25 13.87; 36.62
NT-3 34.55 23.92; 45.17
0 10 20 30 40
% change (95% CI) per 1-IQR decrease in air temperature
Chemokines
Cytokines / growth factors
Enzymes
Interleukins (receptors)
Miscellaneous
Neurotrophic factors
TNF(R) superfamily

Figure S6. Significant associations between short-term exposure to air temperature per 1-IQR decrease with biomarkers of subclinical inflammation (P-adjust <0.05).

1-IQR decrease was 9.2°C for lags 0-1 days, 8.9°C for lags 2-6 days, 8.4°C for lags 0-13 days, 9.0°C for lags 0-27 days, and 9.4°C for lags 0-55 days. *ADA:* Adenosine deaminase, *Beta-NGF*: Beta-nerve growth factor, *CCL11*: Eotaxin, *CCL23*: C-C motif chemokine 23, *CCL25*: C-C motif chemokine 25, *CCL28*: C-C motif chemokine 28, *CD244*: Natural killer cell receptor 2B4, *CD40*: CD40L receptor, *CDCP1*: CUB domain-containing protein 1, *CSF-1*: Macrophage colony-stimulating factor 1, *CST5*: Cystatin D, *CX3CL1*: Fractalkine, *CXCL5*: C-X-C motif chemokine 5, *CXCL6*: C-X-C motif chemokine 6, *DNER*: Delta and Notch-like epidermal growth factor-related receptor, *EN-RAGE*: Protein S100-A12, *FGF-23*: Fibroblast growth factor 23, *FGF-5*: Fibroblast growth factor 5, *GDNF*: Glial cell line-derived neurotrophic factor, *HGF*: Hepatocyte growth factor, *IL-10*: Interleukin-10, *IL-15RA*: Interleukin-15 receptor subunit alpha, *IL-18*: Interleukin-18, *IL-18R1*: Interleukin-18 receptor 1, *LAP TGFβ1*: Latency-associated peptide transforming growth factor beta-1, *LIF-R*: Leukemia inhibitory factor receptor, *MCP-1*: Monocyte chemotactic protein 1, *MCP-2*: Monocyte chemotactic protein 2, *MMP-1*: Matrix metalloproteinase-1, *NT-3*: Neurotrophin-3, *OPG*: Osteoprotegerin, *PD-L1*: Programmed cell death 1 ligand 1, *SLAMF1*: Signaling lymphocytic activation molecule, *ST1A1*: Sulfotransferase 1A1, *STAMBP*: STAM-binding protein, *TGFα*: Transforming growth factor alpha, *TNFRSF9*: Tumor necrosis factor receptor superfamily member 9, *TRAIL*: TNF-related apoptosis-inducing ligand, *TRANCE*: TNF-related activation-induced cytokine, *TWEAK*: Tumor necrosis factor (Ligand) superfamily, member 1, *VEGF-A*: Vascular endothelial growth factor A.

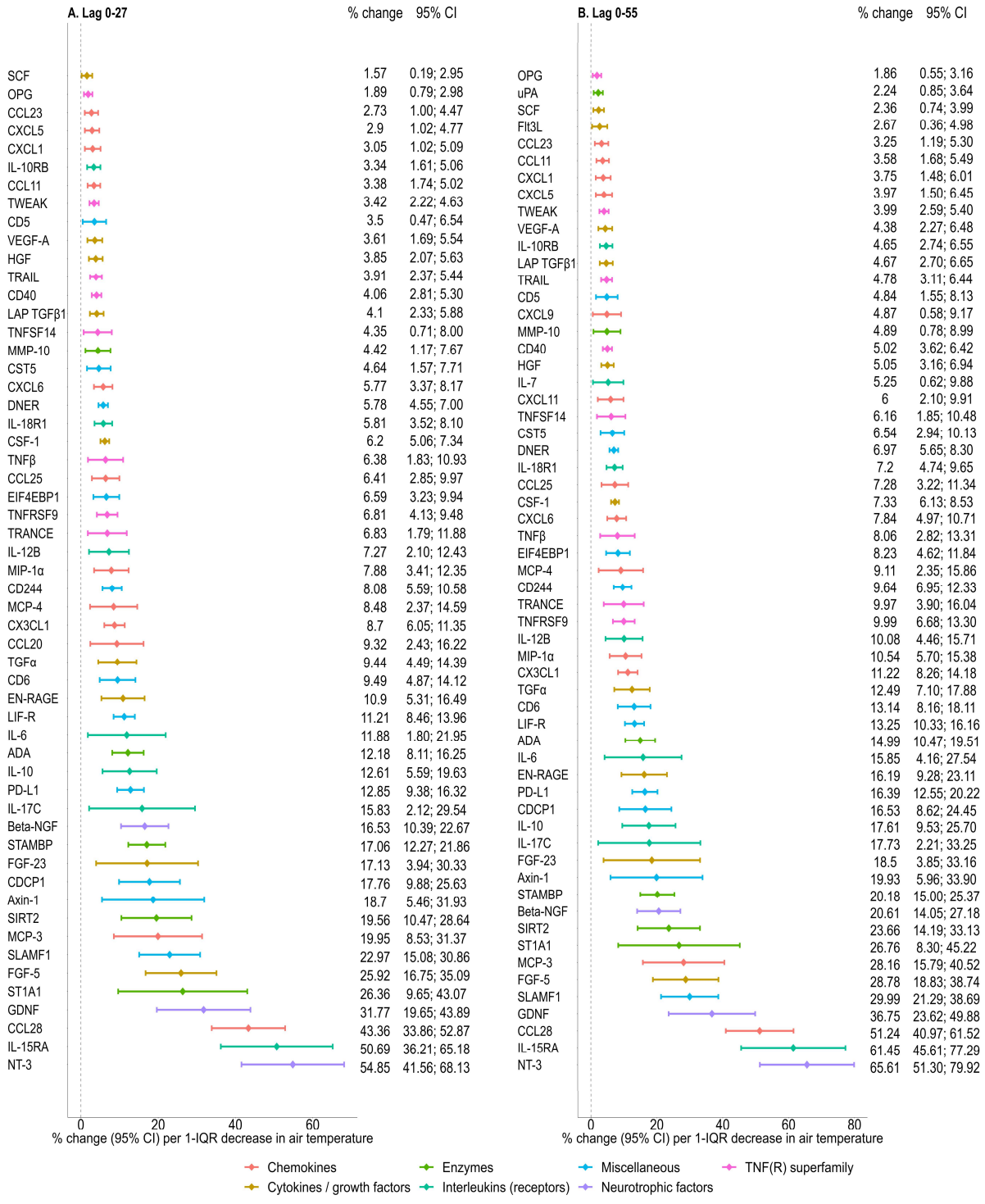

Figure S7. Significant associations between medium-term exposure to air temperature per 1-IQR decrease with biomarkers of subclinical inflammation (*P*-adjust <0.05).

1-IQR decrease was 9.2°C for lags 0-1 days, 8.9°C for lags 2-6 days, 8.4°C for lags 0-13 days, 9.0°C for lags 0-27 days, and 9.4°C for lags 0-55 days. *ADA:* Adenosine deaminase, *Beta-NGF*:

Beta-nerve growth factor, *CCL11*: Eotaxin, *CCL20*: C-C motif chemokine 20, *CCL23*: C-C motif chemokine 23, *CCL25*: C-C motif chemokine 25, *CCL28*: C-C motif chemokine 28, *CD244*: Natural killer cell receptor 2B4, *CD40*: CD40L receptor, *CD5*: T-cell surface glycoprotein CD5, *CD6*: T cell surface glycoprotein CD6 isoform, *CDCP1*: CUB domain-containing protein 1, *CSF-1*: Macrophage colony-stimulating factor 1, *CST5*: Cystatin D, *CX3CL1*: Fractalkine, *CXCL1*: C-X-C motif chemokine 1, *CXCL11*: C-X-C motif chemokine 11, *CXCL5*: C-X-C motif chemokine 5, *CXCL6*: C-X-C motif chemokine 6, *CXCL9*: C-X-C motif chemokine 9, *DNER*: Delta and Notch-like epidermal growth factor-related receptor, *EIF4EBP1*: Eukaryotic translation initiation factor 4E-binding protein 1, *EN-RAGE*: Protein S100-A12, *FGF-23*: Fibroblast growth factor 23, *FGF-5*: Fibroblast growth factor 5, *Flt3L*: Fms-related tyrosine kinase 3 ligand, *GDNF*: Glial cell line-derived neurotrophic factor, *HGF*: Hepatocyte growth factor, *IL-10*: Interleukin-10, *IL-10RB*: Interleukin-10 receptor subunit beta, *IL-12B*: Interleukin-12 subunit beta, *IL-15RA*: Interleukin-15 receptor subunit alpha, *IL-17C*: Interleukin-17C, *IL-18R1*: Interleukin-18 receptor 1, *IL-6*: Interleukin-6, *IL-7*: Interleukin-7, *LAP TGFβ1*: Latency-associated peptide transforming growth factor beta-1, *LIF-R*: Leukemia inhibitory factor receptor, *MCP-3*: Monocyte chemotactic protein 3, *MCP-4*: Monocyte chemotactic protein 4, *MIP-1α*: Macrophage inflammatory protein-1alpha, *MMP-10*: Matrix metalloproteinase-10, *NT-3*: Neurotrophin-3, *OPG*: Osteoprotegerin, *PD-L1*: Programmed cell death 1 ligand 1, *SCF*: Stem cell factor, *SIRT2*: SIR2-like protein 2, *SLAMF1*: Signaling lymphocytic activation molecule, *ST1A1*: Sulfotransferase 1A1, *STAMBP*: STAM-binding protein, *TGFα*: Transforming growth factor alpha, *TNFRSF9*: Tumor necrosis factor receptor superfamily member 9, *TNFβ*: Tumor necrosis factor-beta, *TNFSF14*: Tumor necrosis factor ligand superfamily member 14, *TRAIL*: TNF-related apoptosis-inducing ligand, *TRANCE*: TNF-related activation-induced cytokine, *TWEAK*: Tumor necrosis factor (Ligand) superfamily, member 1, *uPA*: Urokinase-type plasminogen activator, *VEGF-A*: Vascular endothelial growth factor A.

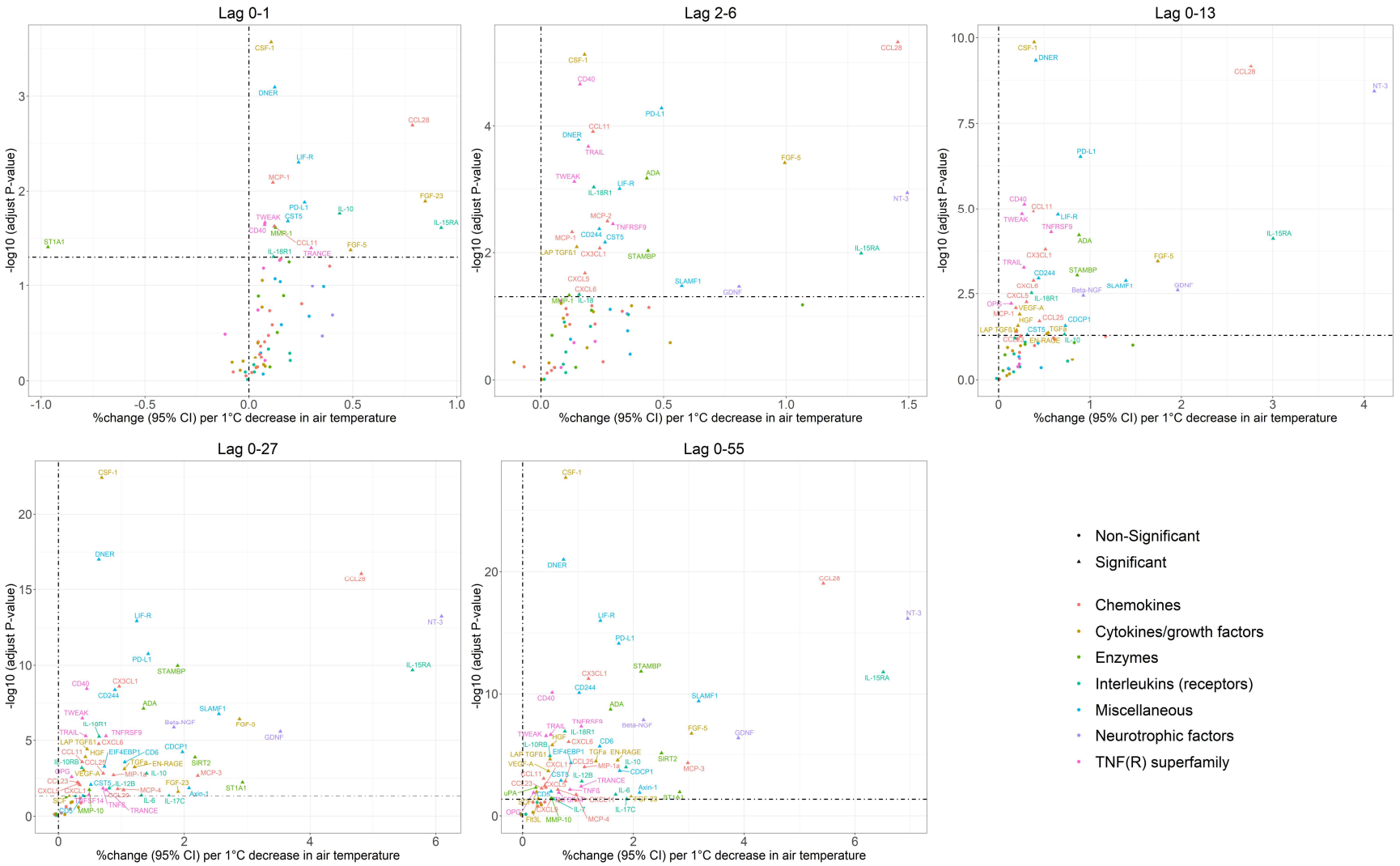
Lag 0-1
Lag 2-6
Lag 0-13
Lag 0-27
Lag 0-55
-log10 (adjust P-value)
%change (95% CI) per 1°C decrease in air temperature
Non-Significant
Significant
Chemokines
Cytokines/growth factors
Enzymes
Interleukins (receptors)
Miscellaneous
Neurotrophic factors
TNF(R) superfamily

Figure S8. Volcano Plots presenting the associations between short- and medium-term exposures to air temperature per 1°C decrease with 71 biomarkers of subclinical inflammation.

1-IQR decrease was 9.2°C for lags 0-1 days, 8.9°C for lags 2-6 days, 8.4°C for lags 0-13 days, 9.0°C for lags 0-27 days, and 9.4°C for lags 0-55 days. *ADA:* Adenosine deaminase, *Beta-NGF*: Beta-nerve growth factor, *CCL11*: Eotaxin, *CCL20*: C-C motif chemokine 20, *CCL23*: C-C motif chemokine 23, *CCL25*: C-C motif chemokine 25, *CCL28*: C-C motif chemokine 28, *CD244*: Natural killer cell receptor 2B4, *CD40*: CD40L receptor, *CD5*: T-cell surface glycoprotein CD5, *CD6*: T cell surface glycoprotein CD6 isoform, *CDCP1*: CUB domain-containing protein 1, *CSF-1*: Macrophage colony-stimulating factor 1, *CST5*: Cystatin D, *CX3CL1*: Fractalkine, *CXCL1*: C-X-C motif chemokine 1, *CXCL11*: C-X-C motif chemokine 11, *CXCL5*: C-X-C motif chemokine 5, *CXCL6*: C-X-C motif chemokine 6, *CXCL9*: C-X-C motif chemokine 9, *DNER*: Delta and Notch-like epidermal growth factor-related receptor, *EIF4EBP1*: Eukaryotic translation initiation factor 4E-binding protein 1, *EN-RAGE*: Protein S100-A12, *FGF-23*: Fibroblast growth factor 23, *FGF-5*: Fibroblast growth factor 5, *Flt3L*: Fms-related tyrosine kinase 3 ligand, *GDNF*: Glial cell line-derived neurotrophic factor, *HGF*: Hepatocyte growth factor, *IL-10*: Interleukin-10, *IL-10RB*: Interleukin-10 receptor subunit beta, *IL-12B*: Interleukin-12 subunit beta, *IL-15RA*: Interleukin-15 receptor subunit alpha, *IL-17C*: Interleukin-17C, *IL-18*: Interleukin-18, *IL-18R1*: Interleukin-18 receptor 1, *IL-6*: Interleukin-6, *IL-7*: Interleukin-7, *LAP TGFβ1*: Latency-associated peptide transforming growth factor beta-1, *LIF-R*: Leukemia inhibitory factor receptor, *MCP-1*: Monocyte chemotactic protein 1, *MCP-2*: Monocyte chemotactic protein 2, *MCP-3*: Monocyte chemotactic protein 3, *MCP-4*: Monocyte chemotactic protein 4, *MIP-1α*: Macrophage inflammatory protein-1alpha, *MMP-1*: Matrix metalloproteinase-1, *MMP-10*: Matrix metalloproteinase-10, *NT-3*: Neurotrophin-3, *OPG*: Osteoprotegerin, *PD-L1*: Programmed cell death 1 ligand 1, *SCF*: Stem cell factor, *SIRT2*: SIR2-like protein 2, *SLAMF1*: Signaling lymphocytic activation molecule, *ST1A1*: Sulfotransferase 1A1, *STAMBP*: STAM-binding protein, *TGFα*: Transforming growth factor alpha, *TNFβ*: Tumor necrosis factor-beta, *TNFRSF9*: Tumor necrosis factor receptor superfamily member 9, *TNFSF14*: Tumor necrosis factor ligand superfamily member 14, *TRAIL*: TNF-related apoptosis-inducing ligand, *TRANCE*: TNF-related activation-induced cytokine, *TWEAK*: Tumor

necrosis factor (Ligand) superfamily, member 1, *uPA*: Urokinase-type plasminogen activator, *VEGF-A*: Vascular endothelial growth factor A.

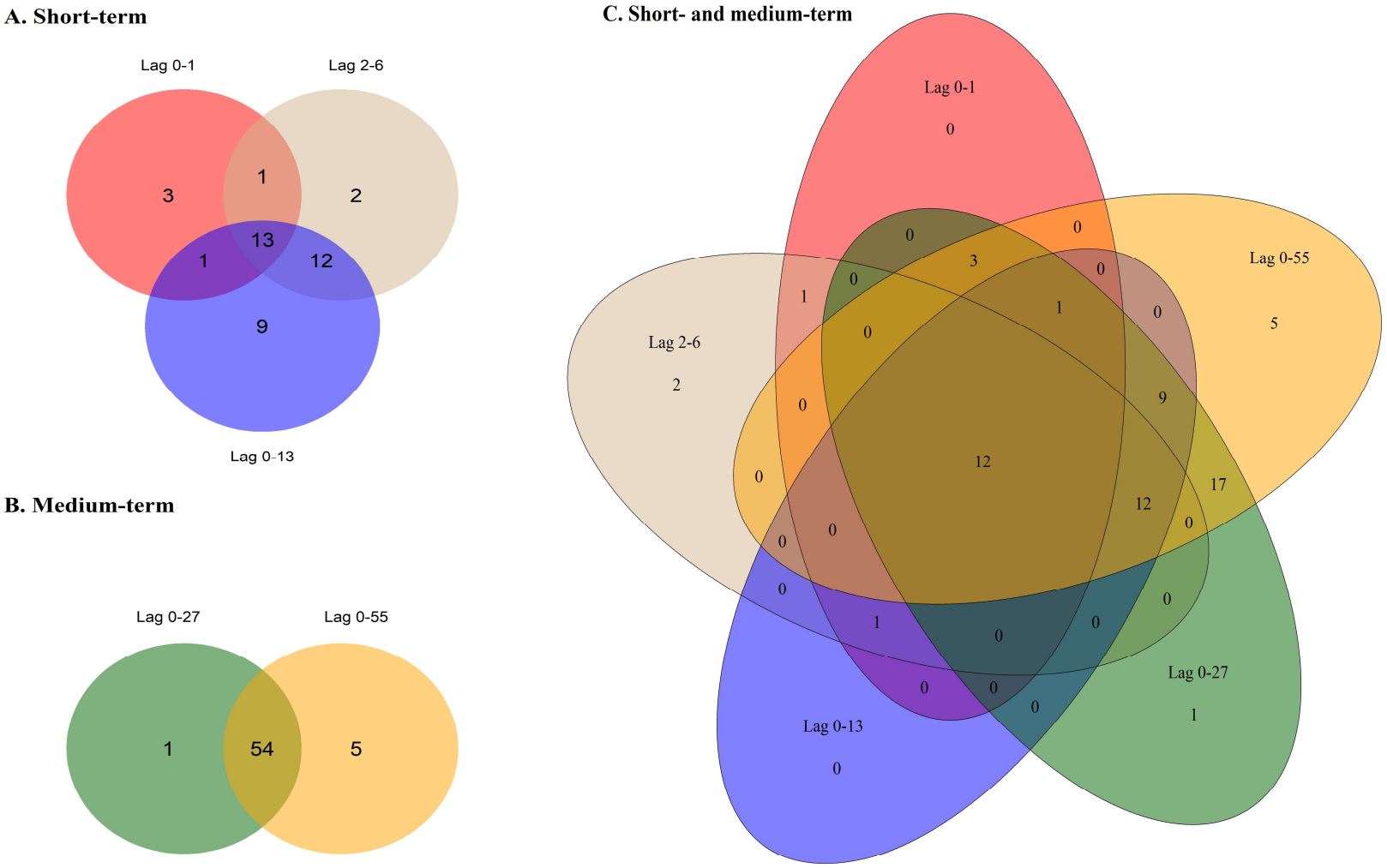

Figure S9. Venn diagrams of significant associations between short- and medium-term exposures to air temperature per 1-IQR decrease with biomarkers of subclinical inflammation.

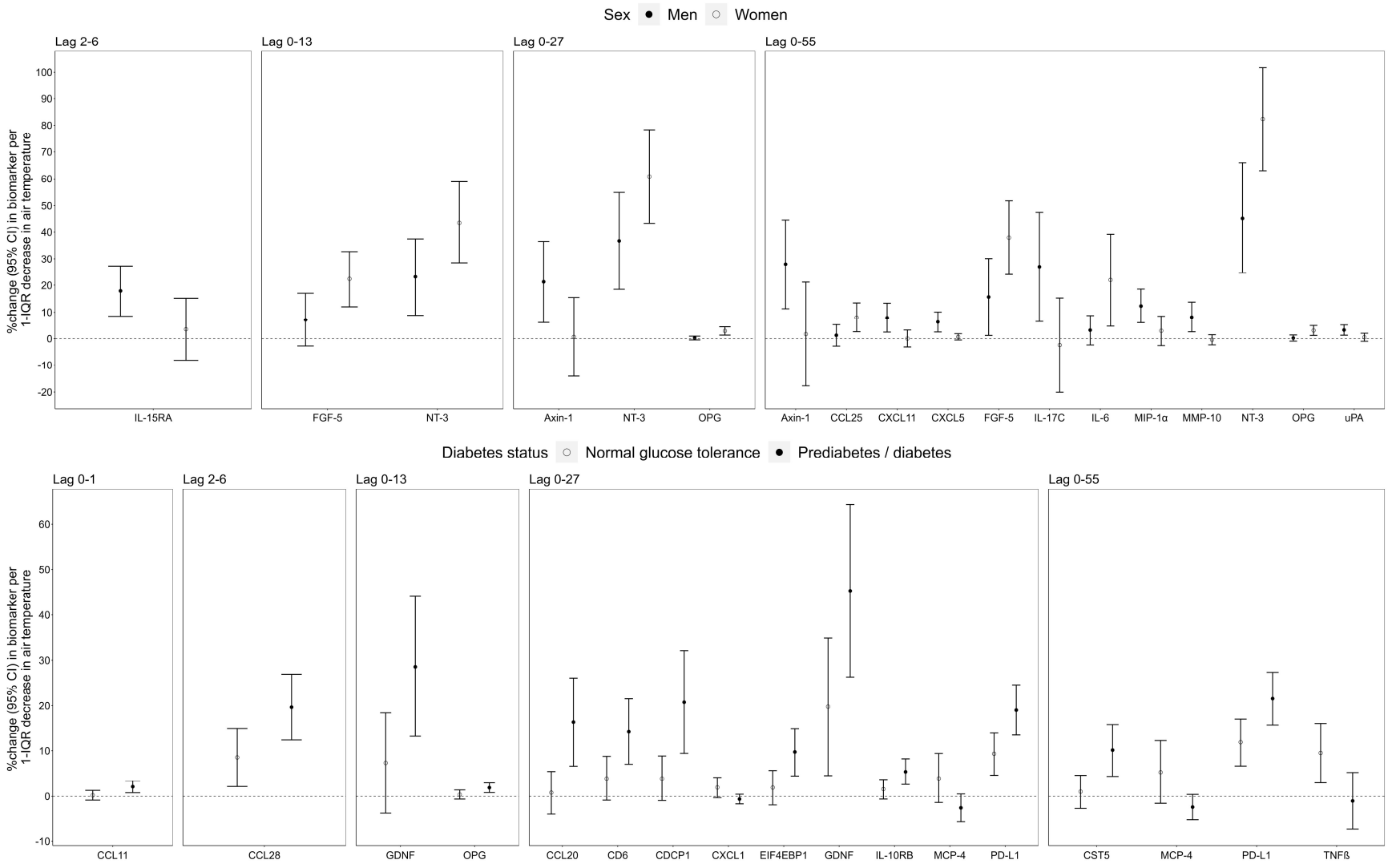
Sex Men Women
Lag 2-6
Lag 0-13
Lag 0-27
Lag 0-55
%change (95% CI) in biomarker per 1-IQR decrease in air temperature
IL-15RA
FGF-5
NT-3
Axin-1
NT-3
OPG
Axin-1
CCL25
CXCL11
CXCL5
FGF-5
IL-17C
IL-6
MIP-1α
MMP-10
NT-3
OPG
uPA
Diabetes status Normal glucose tolerance Prediabetes / diabetes
Lag 0-1
Lag 2-6
Lag 0-13
Lag 0-27
Lag 0-55
%change (95% CI) in biomarker per 1-IQR decrease in air temperature
CCL11
CCL28
GDNF
OPG
CCL20
CD6
CDCP1
CXCL1
EIF4EBP1
GDNF
IL-10RB
MCP-4
PD-L1
CST5
MCP-4
PD-L1
TNFß

Figure S10. Short- and medium-term effects of air temperature on biomarkers of subclinical inflammation per 1-IQR decrease significantly modified by sex or diabetes.

1-IQR decrease was 9.2°C for lags 0-1 days, 8.9°C for lags 2-6 days, 8.4°C for lags 0-13 days, 9.0°C for lags 0-27 days, and 9.4°C for lags 0-55 days. *CCL11*: Eotaxin, *CCL20*: C-C motif chemokine 20, *CCL25*: C-C motif chemokine 25, *CCL28*: C-C motif chemokine 28, *CD6*: T cell surface glycoprotein CD6 isoform, *CDCP1*: CUB domain-containing protein 1, *CST5*: Cystatin D, *CX3CL1*: Fractalkine, *CXCL1*: C-X-C motif chemokine 1, *CXCL11*: C-X-C motif chemokine 11, *CXCL5*: C-X-C motif chemokine 5, *EIF4EBP1*: Eukaryotic translation initiation factor 4E-binding protein 1, *FGF-5*: Fibroblast growth factor 5, *GDNF*: Glial cell line-derived neurotrophic factor, *IL-10RB*: Interleukin-10 receptor subunit beta, *IL-15RA*: Interleukin-15 receptor subunit alpha, *IL-17C*: Interleukin-17C, *IL-6*: Interleukin-6, *MCP-4*: Monocyte chemotactic protein 4, *MIP-1α*: Macrophage inflammatory protein-1alpha, *MMP-10*: Matrix metalloproteinase-10, *NT-3*: Neurotrophin-3, *OPG*: Osteoprotegerin, *PD-L1*: Programmed cell death 1 ligand 1, *TNFβ*: Tumor necrosis factor-beta, *uPA*: Urokinase-type plasminogen activator.

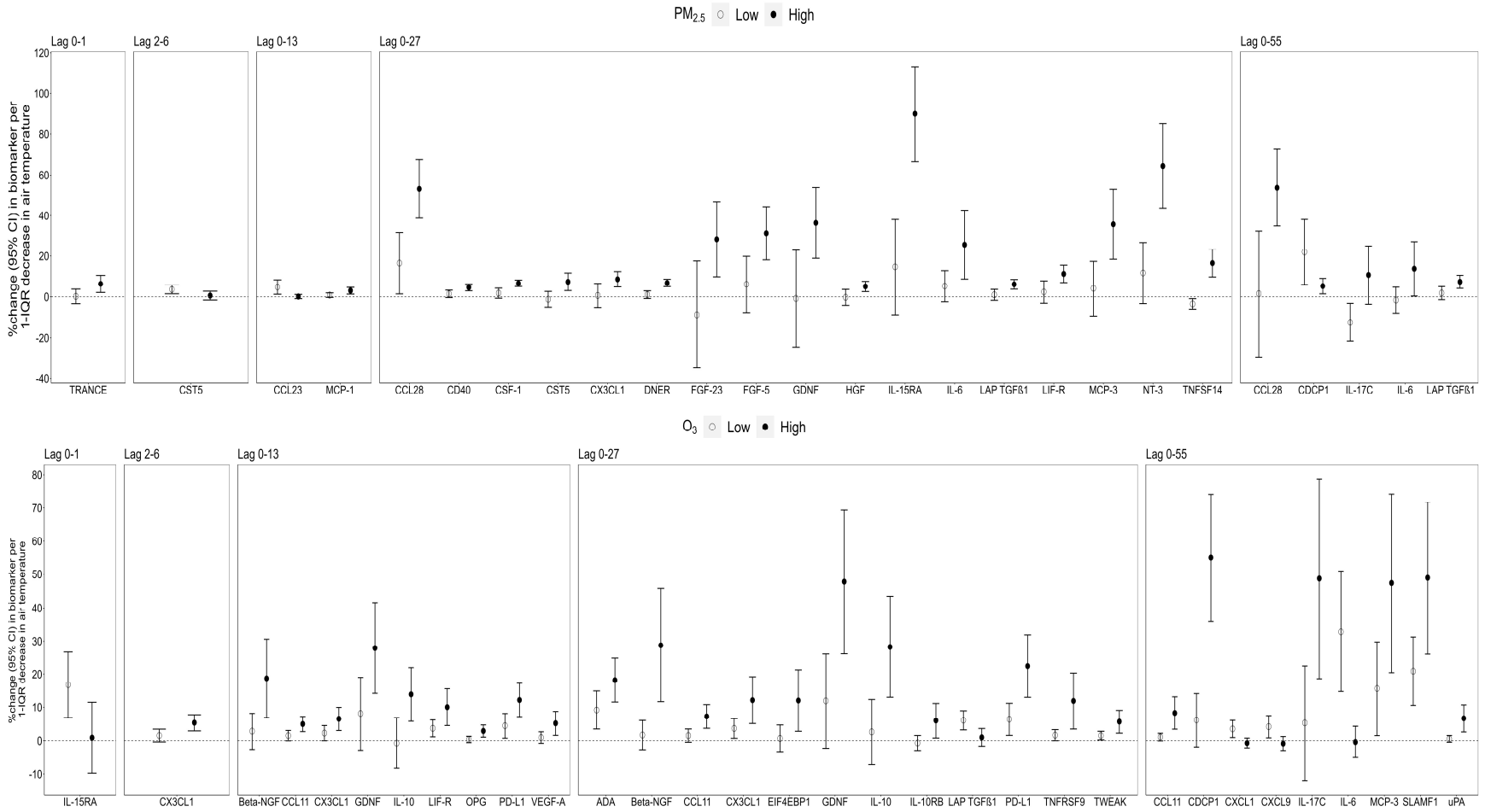

Figure S11. Short- and medium-term effects of air temperature on biomarkers of subclinical inflammation per 1-IQR decrease significantly modified by $PM_{2.5}$ or O_3.

1-IQR decrease was 9.2°C for lags 0-1 days, 8.9°C for lags 2-6 days, 8.4°C for lags 0-13 days, 9.0°C for lags 0-27 days, and 9.4°C for lags 0-55 days. *ADA:* Adenosine deaminase, *Beta-NGF*: Beta-nerve growth factor, *CCL11*: Eotaxin, *CCL23*: C-C motif chemokine 23,

CCL25: C-C motif chemokine 25, *CCL28*: C-C motif chemokine 28, *CD40*: CD40L receptor, *CDCP1*: CUB domain-containing protein 1, *CSF-1*: Macrophage colony-stimulating factor 1, *CST5*: Cystatin D, *CX3CL1*: Fractalkine, *CXCL1*: C-X-C motif chemokine 1, *CXCL9*: C-X-C motif chemokine 9, *DNER*: Delta and Notch-like epidermal growth factor-related receptor, *EIF4EBP1*: Eukaryotic translation initiation factor 4E-binding protein 1, *FGF-23*: Fibroblast growth factor 23, *FGF-5*: Fibroblast growth factor 5, *GDNF*: Glial cell line-derived neurotrophic factor, *HGF*: Hepatocyte growth factor, *IL-10*: Interleukin-10, *IL-10RB*: Interleukin-10 receptor subunit beta, *IL-15RA*: Interleukin-15 receptor subunit alpha, *IL-17C*: Interleukin-17C, *IL-6*: Interleukin-6, *LAP TGFβ1*: Latency-associated peptide transforming growth factor beta-1, *LIF-R*: Leukemia inhibitory factor receptor, *MCP-1*: Monocyte chemotactic protein 1, *MCP-3*: Monocyte chemotactic protein 3, *NT-3*: Neurotrophin-3, *OPG*: Osteoprotegerin, *PD-L1*: Programmed cell death 1 ligand 1, *SLAMF1*: Signaling lymphocytic activation molecule, *TNFRSF9*: Tumor necrosis factor receptor superfamily member 9, *TNFSF14*: Tumor necrosis factor ligand superfamily member 14, *TRANCE*: TNF-related activation-induced cytokine, *TWEAK*: Tumor necrosis factor (Ligand) superfamily, member 1, *uPA*: Urokinase-type plasminogen activator, *VEGF-A*: Vascular endothelial growth factor A.

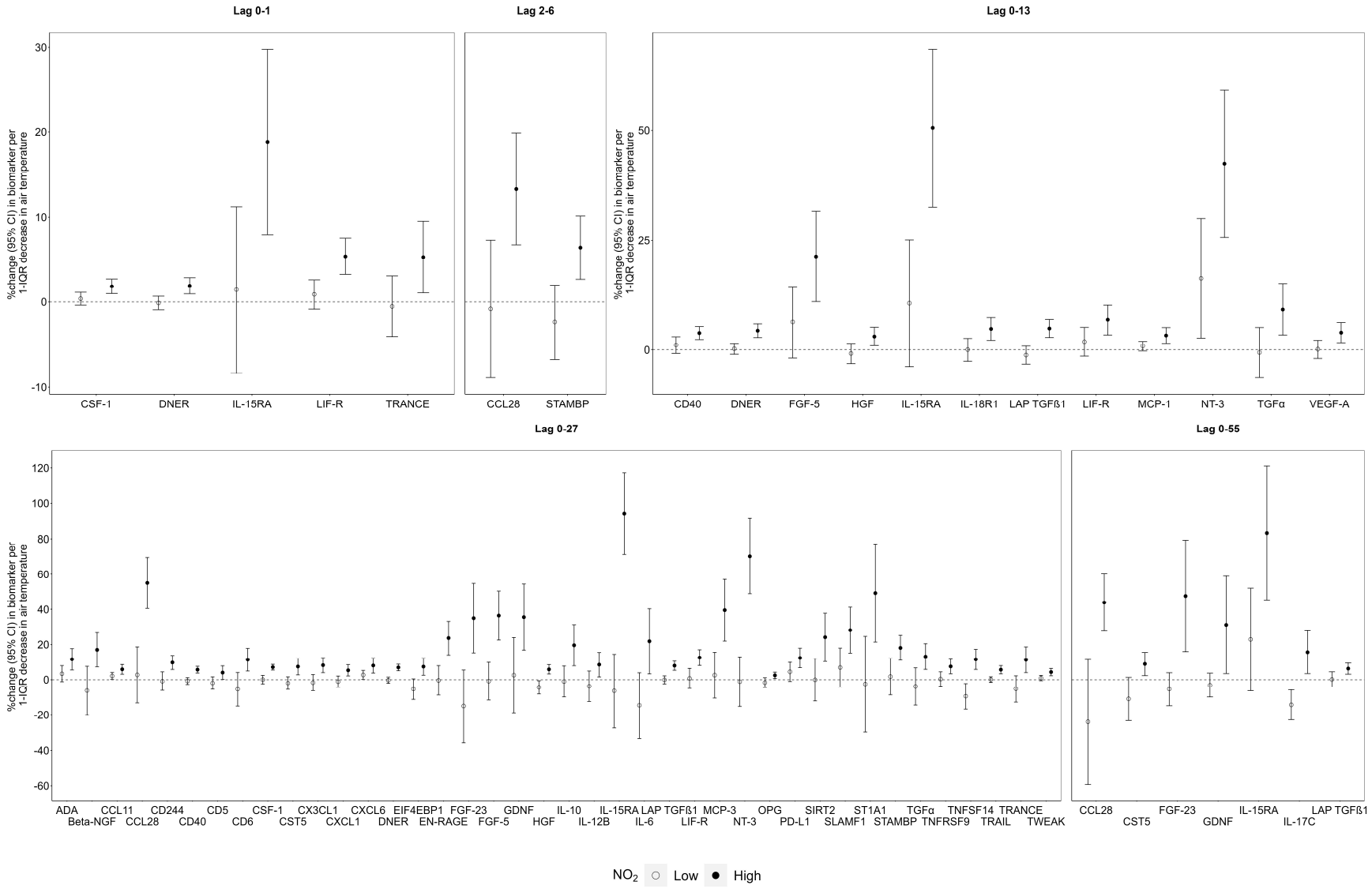
Lag 0-1
Lag 2-6
Lag 0-13
Lag 0-27
Lag 0-55
%change (95% CI) in biomarker per 1-IQR decrease in air temperature
CSF-1
DNER
IL-15RA
LIF-R
TRANCE
CCL28
STAMBP
CD40
FGF-5
HGF
IL-18R1
LAP TGFß1
MCP-1
NT-3
TGFα
VEGF-A
ADA
Beta-NGF
CCL11
CCL28
CD244
CD40
CD5
CD6
CSF-1
CST5
CX3CL1
CXCL1
CXCL6
DNER
EIF4EBP1
EN-RAGE
FGF-23
FGF-5
GDNF
HGF
IL-10
IL-12B
IL-15RA
IL-6
LAP TGFß1
LIF-R
MCP-3
NT-3
OPG
PD-L1
SIRT2
SLAMF1
ST1A1
STAMBP
TGFα
TNFRSF9
TNFSF14
TRAIL
TRANCE
TWEAK
CCL28
CST5
FGF-23
GDNF
IL-15RA
IL-17C
LAP TGFß1
NO2
Low
High

Figure S12. Short- and medium-term effects of air temperature on biomarkers of subclinical inflammation per 1-IQR decrease significantly modified by NO_2.

1-IQR decrease was 9.2°C for lags 0-1 days, 8.9°C for lags 2-6 days, 8.4°C for lags 0-13 days, 9.0°C for lags 0-27 days, and 9.4°C for lags 0-55 days. The Y-axis ranges are not comparable in terms of short- and medium-term effects, because visibility reasons. *ADA*: Adenosine deaminase, *Beta-NGF*: Beta-nerve growth factor, *CCL11*: Eotaxin, *CCL28*: C-C motif chemokine 28, *CD244*: Natural killer cell receptor 2B4, *CD40*: CD40L receptor, *CD5*: T-cell surface glycoprotein CD5, *CD6*: T cell surface glycoprotein CD6 isoform, *CSF-1*: Macrophage colony-stimulating factor 1, *CST5*: Cystatin D, *CX3CL1*: Fractalkine, *CXCL1*: C-X-C motif chemokine 1, *CXCL6*: C-X-C motif chemokine 6, *DNER*: Delta and Notch-like epidermal growth factor-related receptor, *EIF4EBP1*: Eukaryotic translation initiation factor 4E-binding protein 1, *EN-RAGE*: Protein S100-A12, *FGF-23*: Fibroblast growth factor 23, *FGF-5*: Fibroblast growth factor 5, *GDNF*: Glial cell line-derived neurotrophic factor, *HGF*: Hepatocyte growth factor, *IL-10*: Interleukin-10, *IL-12B*: Interleukin-12 subunit beta, *IL-15RA*: Interleukin-15 receptor subunit alpha, *IL-17C*: Interleukin-17C, *IL-18R1*: Interleukin-18 receptor 1, *IL-6*: Interleukin-6, *LAP TGFß1*: Latency-associated peptide transforming growth factor beta-1, *LIF-R*: Leukemia inhibitory factor receptor, *MCP-1*: Monocyte chemotactic protein 1, *MCP-3*: Monocyte chemotactic protein 3, *NT-3*: Neurotrophin-3, *OPG*: Osteoprotegerin, *PD-L1*: Programmed cell death 1 ligand 1, *SIRT2*: SIR2-like protein 2, *SLAMF1*: Signaling lymphocytic activation molecule, *ST1A1*: Sulfotransferase 1A1, *STAMBP*: STAM-binding protein, *TGFα*: Transforming growth factor alpha, *TNFRSF9*: Tumor necrosis factor receptor superfamily member 9, *TNFSF14*: Tumor necrosis factor ligand superfamily member 14, *TRAIL*: TNF-related apoptosis-inducing ligand, *TRANCE*: TNF-related activation-induced cytokine, *TWEAK*: Tumor necrosis factor (Ligand) superfamily, member 1, *VEGF-A*: Vascular endothelial growth factor A.

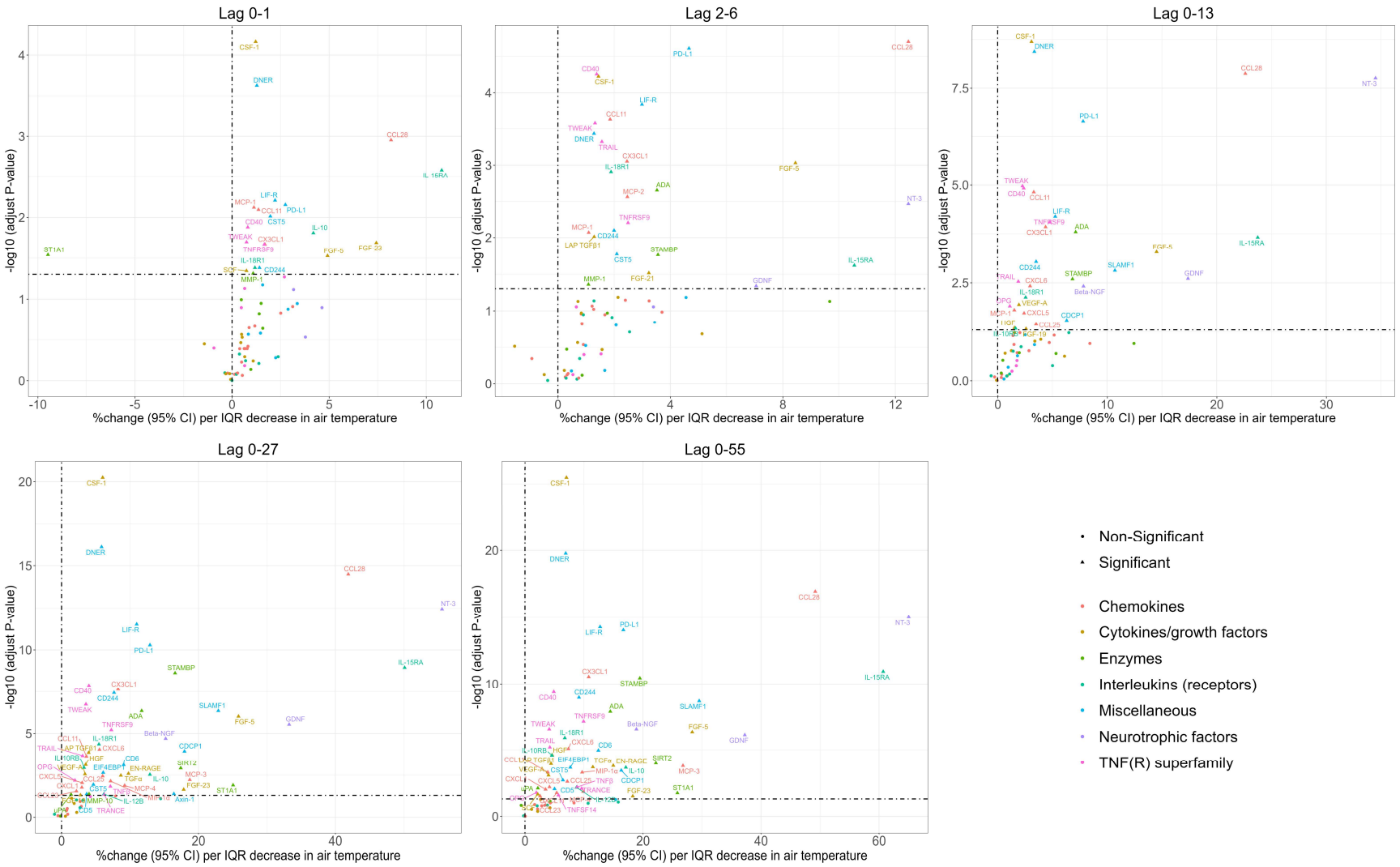
Lag 0-1
Lag 2-6
Lag 0-13
Lag 0-27
Lag 0-55
-log10 (adjust P-value)
%change (95% CI) per IQR decrease in air temperature
Non-Significant
Significant
Chemokines
Cytokines/growth factors
Enzymes
Interleukins (receptors)
Miscellaneous
Neurotrophic factors
TNF(R) superfamily

Figure S13. Sensitivity analysis (participants with CRP values > 10 mg/L were excluded): Significant associations between short- and medium-term exposures to air temperature per 1-IQR decrease with biomarkers of subclinical inflammation.

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Publication IV

Title:	Short-term effects of cold spells on plasma viscosity: Results from the KORA cohort study in Augsburg, Germany
Authors:	Wenli Ni, Alexandra Schneider, Kathrin Wolf, Siqi Zhang, Kai Chen, Wolfgang Koenig, Annette Peters, Susanne Breitner
Journal:	Environmental Pollution
Status:	Published
Volume:	302
Page:	119071
Year:	2022
doi:	10.1016/j.envpol.2022.119071

Environmental Pollution 302 (2022) 119071

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Short-term effects of cold spells on plasma viscosity: Results from the KORA cohort study in Augsburg, Germany☆

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ARTICLE INFO

Keywords:
Cold spell
Plasma viscosity

ABSTRACT

As the underlying mechanisms of the adverse effects of cold spells on cardiac events are not well understood, we explored the effects of cold spells on plasma viscosity, a blood parameter linked to cardiovascular disease. This cross-sectional study involved 3622 participants from the KORA S1 Study (1984–1985), performed in Augsburg, Germany. Exposure data was obtained from the Bavarian State Office for the Environment. Cold spells were defined as two or more consecutive days with daily mean temperatures below the 3rd, 5th, or 10th percentile of the distribution. The effects of cold spells on plasma viscosity were explored by generalized additive models with distributed lag nonlinear models (DLNM). We estimated cumulative effects at lags 0–1, 0–6, 0–13, 0–20, and 0–27 days separately. Cold spells (mean temperature <3rd, <5th or <10th percentile) were significantly associated with an increase in plasma viscosity with a lag of 0–1 days [%change of geometric mean (95% confidence interval): 1.35 (0.06–2.68), 1.35 (0.06–2.68), and 2.49 (0.34–4.69), respectively], and a lag of 0–27 days [18.81 (8.97–29.54), 17.85 (8.29–28.25), and 7.41 (3.35–11.0), respectively]. For the analysis with mean temperature <3rd or 10th percentile, we also observed significant associations at lag 0–20 days [8.34 (0.43–16.88), and 4.96 (1.68, 8.35), respectively]. We found that cold spells had significant immediate and longer lagged effects on plasma viscosity. This finding supports the complex interplay of multiple mechanisms of cold on adverse cardiac events and enriches the knowledge about how cold exposure acts on the human body.

1. Introduction

Numerous scientific reports have so far shown significant associations between low and high environmental temperatures and mortality and morbidity (Bunker et al., 2016; Ye et al., 2012). With climate change, populations will experience extreme events even more frequently – with often severe health consequences (Luber and McGeehin, 2008). Previous studies have shown that the amount of excess morbidity and mortality caused by cold spells may even exceed that caused by heat waves (Huynen et al., 2001; Lin et al., 2011; Ryti et al., 2016). Cold spells or low air temperatures appear to show several adverse health effects, particularly for cardiovascular diseases, such as ischemic stroke and myocardial infarction (Bhaskaran et al., 2010; Gao et al., 2019; Kysely et al., 2009; Wolf et al., 2009). Also, a systematic review and meta-analysis study found that cold spells were associated with increased mortality from cardiovascular diseases (Ryti et al., 2016).

However, the underlying biological mechanisms of the association between air temperature and health are not well understood. Several potential mechanisms have been proposed, including blood pressure variability and changes in lipid levels, blood markers of coagulation and

☆ This paper has been recommended for acceptance by Dr. Payam Dadvand.

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https://doi.org/10.1016/j.envpol.2022.119071
Received 12 October 2021; Received in revised form 11 January 2022; Accepted 25 February 2022
Available online 26 February 2022

inflammation, such as fibrinogen (Schauble et al., 2012; Schneider et al., 2008; Wang et al., 2017; Wu et al., 2017; Zhou et al., 2016). Previous studies about seasonal variations have shown that fibrinogen levels increased in winter (van der Bom et al., 1997; Woodhouse et al., 1994).

Plasma viscosity is an important determinant of blood flow rheology. It is influenced by various macromolecules, e.g. fibrinogen, lipoproteins, and certain immunoglobulins (Koenig et al., 1998). In addition, plasma viscosity has been shown to play a role in the progression or onset of ischemic heart disease, myocardial infarction, and atherosclerosis (Fuchs et al., 1987; Koenig et al., 1992; Koenig et al., 1998; Lowe et al., 2000; Lowe, 1987a, b; Peters et al., 2017). Increased plasma and blood viscosity were associated with an increased risk of mortality and morbidity of cardiovascular diseases (Koenig et al., 1992; Lowe et al., 1997; Peters et al., 2017; Skretteberg et al., 2010). Plasma viscosity is a factor contributing to platelet activation. The increased shear force of blood vessel walls caused by high levels of plasma viscosity can result in platelet aggregation (Ranucci et al., 2015). Increased blood viscosity may potentially promote the development of atherosclerosis though enhancing platelet adhesion to the subendothelium, increasing protein infiltration into the arterial wall, and changing the local shear forces at sites of atherogenesis (Lee et al., 1998; Ranucci et al., 2015). A previous study from our group found that plasma viscosity was high during a severe air pollution episode (Peters et al., 1997). However, the relationship between air temperature and plasma viscosity is incompletely understood until now. Though several previous studies reported that changes in air temperature or seasonal variations were associated with changes in plasma viscosity levels (Blumberg et al., 1999; Deveci et al., 2001; Frohlich et al., 1997; KA et al., 2020; Keatinge et al., 1984; Luo et al., 2012, 2014; Otto et al., 1996), these were either experimental with a small number of tested individuals or animal studies or focused on subgroups (e.g. men in the only epidemiological study). Thus overall, the findings remained inconsistent.

We therefore conducted this population-based epidemiological analysis to explore the effects of cold spells on plasma viscosity, using data collected between October 1984 and May 1985 in Augsburg, Germany (Peters et al., 1997).

2. Materials and methods

2.1. Study population

This cross-sectional study was based on the Cooperative Health Research in the Region of Augsburg (KORA) S1 study, which was performed in the city of Augsburg and the two adjacent counties Augsburg and Aichach-Friedberg, Germany, during October 1984 and May 1985. The latitude of Augsburg is 48.366512, and the longitude is 10.894446. The total number of KORA S1 participants was 4,022, with an age range of 25–64 years. Four hundred subjects without plasma viscosity measurements were excluded. Thus, in total, plasma viscosity measurements were available for 3622 participants. More details of the study design have been described elsewhere (Löwel et al., 2005). All study participants have given written informed consent. The Ethics Committees of the Bavarian Medical Association approved the study in adherence to the Declaration of Helsinki.

2.2. Laboratory procedures

The laboratory procedures have been described in detail previously (Koenig et al., 1991; Koenig et al., 1994; Peters et al., 1997). The measurement of plasma viscosity is quick, straightforward, reproducible, and it shows only minimal intra-individual variability (Koenig et al., 1998). In brief, blood samples were taken with short-term venous occlusion and minimal aspiration. EDTA-blood was then centrifuged at 3000 g for 15 min and finally stored at 4 °C for up to four days. Plasma viscosity was measured at 37 °C by a Coulter-Harkness capillary viscometer (Coulter Electronics, Luton, UK). The plasma viscosity test was repeated three times. In terms of quality control, a continuous comparison was made with water control. The coefficient of variation was 1.0%. There was no baseline shift during the data collection period. At irregular times, repetition was measured in a single-blind method. Its coefficient of variation was 2.0%, and 93% of the duplicates agreed within 5%. High-density lipoprotein (HDL) cholesterol and total cholesterol were measured by enzymatic methods (Keil et al., 1988). Lipid analyses were standardized on the World Health Organization lipid quality control reference laboratory in Prague.

2.3. Exposure assessment

Exposure data was obtained from the Bayerisches Landesamt für Umwelt (Bavarian State Office for the Environment). Daily 24-h averages of air temperature, relative humidity, and barometric pressure were measured at an urban background monitoring station located 7 km south of the city center (Chen et al., 2019b). Sulfur dioxide (SO_2), carbon monoxide (CO), and total suspended particulate (TSP) concentrations were measured at an urban background site located in the center of Augsburg (Peters et al., 1997). Air temperature, relative humidity, and barometric pressure measurements were available on 100% of the days. TSP, SO_2, and CO measurements were available on more than 85% of days during the study period (October 8, 1984, to May 24, 1985). A map of the study area and the monitoring sites was showed in Figure S1 (supplementary).

For short-term analysis (day-to-day variation), it is usually considered sufficient if the correlation between different sites over time is high, even if the absolute levels differ between stations. This means that the temperature data across various stations has to vary over time similarly, but it can vary on a different level. Our research group previously performed a correlation analysis on daily meteorological data from different sites from the German Weather Service (monitoring site located at the Augsburg airport), the Bavarian Environment Agency (LfU, site located in the southern part of the Augsburg urban area) and a fixed monitoring site located in the urban background in Augsburg, and found that daily air temperature was highly correlated between sites (Spearman correlation coefficients were >0.98). Therefore, we believe that using data from only one monitoring station for our analyses is sufficient for the exploration of temporal variation (short-term analysis).

2.4. Definition of cold spell

There is no standard definition of a cold spell. Multiple methods have been used to define a cold spell across different studies. Previous studies suggested that the definitions of cold spells with two or more consecutive days provided more precise statistical estimates than definitions based on single days (Chen et al., 2019a; Ma et al., 2021). In general, these vary according to the employed temperature threshold and duration of exposure. Therefore, we defined *cold spells* as daily mean temperature (Tmean) below the 3rd (cold spell <3%), 5th (cold spell <5%), and 10th (cold spell <10%) percentiles of the temperature distribution for two or more consecutive days during the study period, which means subnormal temperatures represented lowering of the temperature beyond the average level of the region.

2.5. Covariates

Participants completed a standardized interview including demographic characteristics (including age, education, sex, and occupational status), medical history, lifestyle characteristics (including smoking status, alcohol consumption, and physical activity), and medication intake (antihypertensive, lipid-lowering, antidiabetic medication, or platelet inhibitors). Besides, body height and weight were obtained via physical examinations, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

2.6. Statistical analysis

The individual subject-specific data have been linked to the time series of the daily exposures and respective lags by the date of examination. Our analysis explored the effects of cold spells on plasma viscosity using generalized additive models (GAM) with distributed lag nonlinear models (DLNM). Specifically, *cold spells* were defined as a binary variable with a value of 1 during the cold spell period and 0 for the non-cold spell days. To estimate the cumulative and lagged effects of the cold spells, we used a "cross-basis" function with a linear function for the relationship between cold spells and plasma viscosity and a natural cubic spline function for the lag-response dimension with two internal knots at equally spaced log values of lag days. The following GAM with DLNM was used: Yt = α+cb (CSt, lag, df = 2)+s (RH, 3, bs = 'cs')+s (BP, 3, bs = 'cs')+s (time trend, 3, bs = 'cs')+other covariances, where t is the date of observation, α is the intercept; CSt represents the cold spell on day t (0 = non-cold spell days, and 1 = cold spell days); Yt is the level of plasma viscosity on day t, cb means "cross-basis" function, in which a linear function and a natural cubic spline function with two dfs were used to estimate the linear and lagged effects of the cold spell, respectively; RH represents the relative humidity with 3 dfs; BP represents the barometric pressure with 3 dfs; bs = 'cs' is a cubic spline.

We estimated cumulative effects at lags 0–1, 0–6, 0–13, 0–20, and 0–27 days separately. A long lag structure of up to 27 days was intended to capture the cold spell exposure's cumulative effects completely (Chen et al., 2019a; Xie et al., 2013). Plasma viscosity levels (0.99 mPa s-1.76 mPa s) were natural log-transformed to increase the conformity to normal distributions of the residuals. We controlled for sex (male, female), age (years), BMI (kg/m^2), education (<10 years of education, ≥10 years of education but not a university degree, university degree), smoking status (never, former, current), alcohol consumption (g/day), physical activity (low: almost no exercise; medium: regularly or irregularly approx. 1 h per week; high: regularly more than 2 h per week), day of the week, season (warm: April–May; cold: October–March), SO_2 (μg/m^3), CO (mg/m^3), TSP (μg/m^3, as the three pollutants were not highly correlated) at lag 0, time trend (cubic spline with three degrees of freedom - df), relative humidity at lag 0 (cubic spline with three df), and barometric pressure at lag 0 (cubic spline with three df). We also explored the exposure response association across different lags for daily air temperature and plasma viscosity.

Also, we conducted stratification analyses for cold spell effects on plasma viscosity to examine effect modification by 1) sex (male vs. female), 2) age (<50 years vs. ≥ 50 years), 3) physical activity (low vs. medium or high), 4) overweight (<25 kg/m^2 vs. ≥ 25 kg/m^2), 5) hypertension (yes vs. no), 6) cardiovascular disease [defined as a history of hypertension, myocardial infarction, or stroke (yes vs. no)], and 7) area (urban vs. rural).

In order to assess the robustness of the results, we performed several sensitivity analyses. First, we alternatively defined *cold spells* as the daily mean temperature below the 3rd, 5th, and 10th percentiles of the temperature distribution with three or more consecutive days during the study period, and then explored the association between cold spells and plasma viscosity. Second, instead of Tmean, we used daily minimum temperature (Tmin), daily maximum temperature (Tmax), and mean apparent temperature (Tappmean) as exposure metrics to identify the effects of cold spells on plasma viscosity, respectively. Third, we applied three further adjustment sets to test the robustness of our results: the minimum model was only adjusted for age, sex, BMI, day of the week, time trend, season, SO_2, CO, TSP, relative humidity, and barometric pressure. In contrast, based on the main model, the maximum model additionally adjusted for medication intake (antihypertensive, lipid-lowering, antidiabetic medication, or platelet inhibitors), total cholesterol, and HDL. Further, we assessed a model without air pollution where we did not adjust for the three pollutants (TSP, SO_2, and CO) in the model. Fourth, we further adjusted for daily mean temperature in the model to examine the stability of our results. Fifth, we excluded plasma viscosity measurements below the 1st or above the 99th percentile to avoid overestimating effects induced by extreme blood marker values. Sixth, we excluded the participants who were examined in the warm season (April and May). Seventh, from 956 male participants, we had additional C-reactive protein (CRP) data. We excluded individuals with high CRP values (>10 mg/L), which may indicate ongoing infection. Eighth, the influenza virus was detected in human serum samples from people in Munich (50 km southeast of Augsburg) in February and March 1985 (Peters et al., 1997), indicating a potentially ongoing influenza wave at this time. We, therefore, included an indicator for February and March to control for this confounding factor. Ninth, we adjusted for relative humidity, barometric pressure, and the three pollutants (SO_2, CO, and TSP) with the same lag period as the cold spells for lag 0–1 and lag 0–6 days in the model (relative humidity and barometric pressure with "cross-basis" of distributed lag nonlinear model structure, and three pollutants with "cross-basis" of distributed lag linear model structure). We could not perform this sensitivity analysis for longer cumulative averages due to a large number of missing values in the three pollutants. Finally, we conducted three additional sensitivity analyses: model without time trend, model without season, and model without time trend and season.

Effect estimates are presented as cumulative percent changes of the geometric outcome mean during cold-spell days comparing to non-cold spell days together with 95% confidence intervals (CIs). $P < 0.05$ was considered to be statistically significant for all statistical tests. We used R software version 4.0.3 to conduct all the analyses.

3. Results

3.1. Cold spells day

Cold spell episodes (defined as two or more consecutive days during the study period) were identified, and led to four days (<3rd), five days (<5th), and ten days (<10th) of cold spell days. Table 1 presents detailed information on cold spell days during our study period. Figure S2 (supplementary materials) shows the time series of the daily mean temperature of the study period.

3.2. Study population and exposure data

Table 2 presents the individual characteristics of the study participants and the plasma viscosity levels. The overall geometric mean level of plasma viscosity was 1.25 mPa s. The mean age and BMI were 45.1 years and 26.4 kg/m^2, respectively. Over half of the participants were overweight, 51.0% were male, 30.1% were current smokers, and 43.5% had low physical activity. Of the participants, 33.6% had hypertension and 34.3% had cardiovascular disease. Plasma viscosity and total cholesterol levels were significantly higher in participants in the different cold spell definition groups (Tmean <3rd, <5th, or <10th percentile) compared to non-cold spells groups. Participants in the different cold spell definition groups were significantly less educated than individuals in non-cold spells groups (supplementary materials, Table S1). We also compared the age distribution of 3622 participants with plasma viscosity to 400 participants without plasma viscosity (supplementary materials, Table S2). The mean age of 3622 participants

Table 1
Cold spell days identified based on different intensities.

Threshold	Threshold temperature	Cold spell days	Non-cold spell days	Sample size of study participants in cold spells	Number of cold episodes
<3rd	−18.2 °C	4	147	143	1
<5th	−17.8 °C	5	146	168	2
<10th	−11.5 °C	10	141	343	3

Table 2
Individual characteristics of the study participants and levels of plasma viscosity (N = 3622).

	Mean ± SD (Geometric mean[a])/N (%)
Plasma viscosity (mPa s)	1.26 ± 0.07 (1.25)
Age (years)	45.1 ± 11.3
Sex (male)	1849 (51.0)
Body mass index (kg/m^2)	26.4 ± 4.03
Overweight (yes: BMI≥25 kg/m^2)	2165 (59.8)
Smoking status	
Current smoker	1089 (30.1)
Former smoker	935 (25.8)
Never smoker	1597 (44.1)
Physical activity	
Low (no or <1h per week)	1576 (43.5)
Medium (~1h per week)	1459 (40.3)
High (~2h per week or more)	580 (16.0)
Alcohol consumption (g/day)	23.5 ± 29.6
Education	
<10 years of education	617 (17.0)
≥10 years of education but not a university degree	2690 (74.3)
University degree	314 (8.7)
Total cholesterol (mmol/L)	6.02 ± 1.22
High-density-lipoprotein (HDL; mmol/L)	1.48 ± 0.46
Hypertension (yes)	1217 (33.6)
Cardiovascular disease (yes)	1242 (34.3)
Medication intake	
Antihypertensive medication (yes)	305 (8.4)
Lipid-lowering medication (yes)	41 (1.1)
Antidiabetic medication (yes)	53 (1.5)
Platelet inhibitors (yes)	19 (0.5)
Area (urban)	1647 (45.5)

[a] Geometric mean, additionally shown for plasma viscosity.

with plasma viscosity was 45.1 years, and for the 400 participants without plasma viscosity it was 44.5 years. Age did not differ between the groups ($P > 0.05$).

The distributions of meteorological variables and air pollutants are presented in Table 3. During the study period, the median of the daily mean temperature was 2.4 °C. Fig. 1 shows the Spearman correlation coefficients (r) between meteorological variables and air pollutants. The correlation between the different temperature variables was high (r > 0.8). The correlation between temperature variables and air pollutants as well as among the three air pollutants was weak to moderate.

Table 3
Descriptive statistics of meteorological variables and air pollutants.

	Mean ± SD	Min	25%	Median	75%	Max
Tmean (°C)	1.4 ± 8.2	−24.8	−1.6	2.4	6.4	14.7
Tmin (°C)	−2.4 ± 7.9	−28.7	−4.3	−0.8	2.0	10.0
Tmax (°C)	5.7 ± 9.2	−20.9	1.1	5.7	12.7	25.7
Tappmean (°C)	−1.3 ± 8.2	−27.7	−4.2	−0.3	3.8	12.0
Relative humidity (%)	82.2 ± 9	56.6	76.6	83.4	89.7	97.0
Barometric pressure (hPa)	1015.5 ± 8.4	997.4	1009.5	1015.6	1022.5	1031.1
CO (mg/m^3)	4.4 ± 1.7	0.9	3.1	4.3	5.5	10.7
SO_2 (μg/m^3)	60.1 ± 46.9	12.1	31.3	51.8	68.9	223.1
TSP (μg/m^3)	54.2 ± 31.8	7.0	31.0	44.0	72.0	176.0

Tmean: daily mean temperature; *Tmin*: daily minimum temperature; *Tmax*: daily maximum temperature; *Tappmean*: mean apparent temperature; *CO*: carbon monoxide; *SO_2*: sulfur dioxide; *TSP*: total suspended particulate.

3.3. Cold spells and plasma viscosity

Fig. 2 presents the cumulative effects [percent change (95% CI) of geometric mean] of cold spells (using different definitions) on plasma viscosity at lag 0–1, lag 0–6, lag 0–13, lag 0–20, and lag 0–27 days. Cumulative effects of cold spells (Tmean <3rd, <5th or <10th percentile) were significantly associated with plasma viscosity with a lag of 0–1 days [%change: 1.35 (0.06–2.68), 1.35 (0.06–2.68), and 2.49 (0.34–4.69), respectively], and a lag of 0–27 days [%change: 18.81 (8.97–29.54), 17.85 (8.29–28.25), and 7.41 (3.35–11.0), respectively]. For the analysis with Tmean <3rd or 10th percentile, we also observed significant associations with lags 0–20 days [%change: 8.34 (0.43–16.88), and 4.96 (1.68, 8.35), respectively], which was similar in size for Tmean <5th percentile, but only borderline significant.

Fig. 3 presents the single-lag effects of the different cold spell definitions (Tmean <3rd, <5th or <10th percentile) on plasma viscosity: cold spells were significantly associated with increased levels of plasma viscosity at lag 0 or lag 1, and longer lag days (>14 days/16 days). We expanded the lag days up to 41 days in order to check when the effects go down. We found that the effects decreased after lag 19/20 and became non-significant after lag 27 for Tmean <5th or <10th percentile, and became non-significant after lag 34 for Tmean <3rd (supplementary materials, Figure S3). The number of participants for each lag day (0–27) during cold spells and non-cold spells (Tmean <10th percentile) are shown in Table S3 (supplementary materials).

When exploring the exposure response association across different lags (supplementary materials, Figure S4), the maximum effect of daily air temperature on plasma viscosity occurred at −24.79 °C and lag 0 (% change: 2.36 [95% CI: 0.99, 3.75], with the minimum effect of temperature at 14.9 °C selected as reference value).

3.4. Effect modification

Fig. 4 shows the estimated cumulative effects of the cold spell defined as Tmean <10th percentile on plasma viscosity at lag 0–1, lag 0–6, lag 0–13, lag 0–20, and lag 0–27 days modified by sex, age, overweight, cardiovascular disease, hypertension, physical activity, and area. Overall, we did not find significant effect modification by these participant characteristics (all $P > 0.05$ for the interaction term). The analysis of cold spells defined as Tmean <3rd and <5th percentiles gave similar results (not shown).

3.5. Sensitivity analysis

In general, the associations between cold spells and plasma viscosity remained almost similar in the sensitivity analyses (supplementary materials, Figure S5). Defining *cold spells* as the daily mean temperature below the 3rd, 5th, and 10th percentile of the temperature distribution with three or more consecutive days during the study period, resulted in similar effects compared to the main results. Alternative exposure metrics Tmin, Tmax, and Tappmean showed similar effect estimates as Tmean, except for Tmin at lag 0–1 days which was slightly decreased. We also observed similar estimates when alternatively adjusting for the minimum or maximum set of covariates, but effects estimates were considerably decreased when not adjusting for air pollutants. When additionally adjusting for daily air temperature, we observed similar results for the effects of cold spells. Moreover, the exclusion of extreme plasma viscosity values (1% and 99%) showed robust results. Exclusion of participants who were examined in the warm season or with elevated CRP value (>10 mg/L, n = 47) mainly confirmed our main results. Additional adjustment for potential influenza effects also resulted in similar estimates of the cold spells on plasma viscosity. The adjustment for relative humidity, barometric pressure, and the three pollutants using the same lag period as the cold spells in the model resulted in similar effects for lag 0–6 days compared to our main results, but non-significant effects for lag 0–1 days. Finally, the results for the model

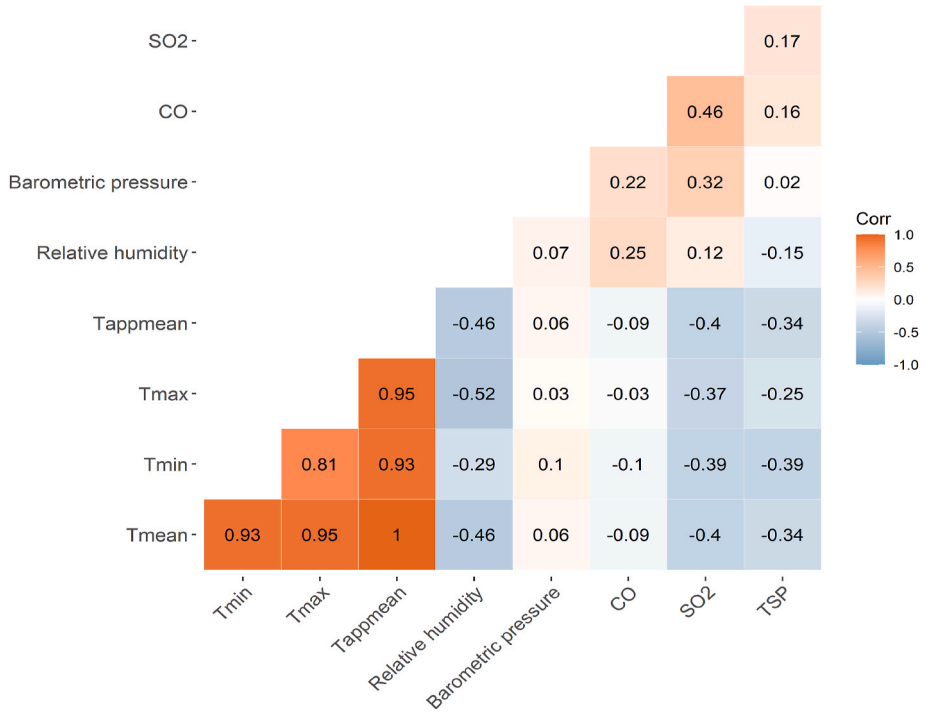

Fig. 1. Spearman correlation coefficients (r) between meteorological variables and air pollutants. *Tmean*: daily mean temperature; *Tmin*: daily minimum temperature; *Tmax*: daily maximum temperature; *Tappmean*: mean apparent temperature; *CO*: carbon monoxide; SO_2: sulfur dioxide; *TSP*: total suspended particulate.

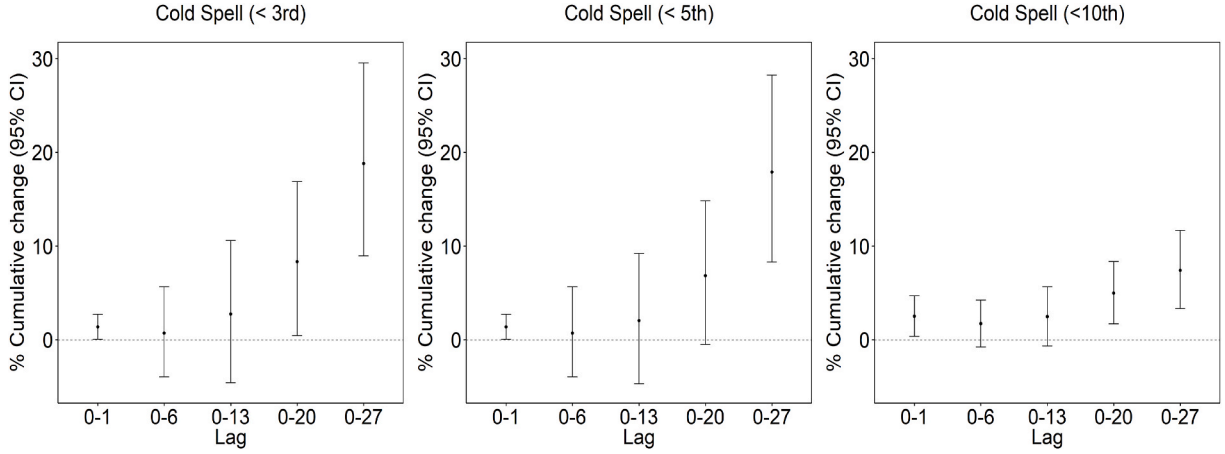

Fig. 2. Estimated cumulative effects [percent change (95% CI) of geometric mean] for the different cold spell definitions (Tmean<3^{rd}, <5^{th} or <10^{th} percentile) on plasma viscosity at lags 0–1, lags 0–6, lags 0–13, lags 0–20, and lags 0–27.Generalized additive models with distributed lag nonlinear models were adjusted for age, sex, BMI, education, smoking status, alcohol consumption, physical activity, day of the week, season, SO_2 at lag 0, CO at lag 0, TSP at lag 0, time trend (cubic spline with three degrees of freedom - df), relative humidity at lag 0 (cubic spline with three df), and barometric pressure at lag 0 (cubic spline with three df). *Cold spells* were defined as a binary variable with a value of 1 during the cold spell period and 0 for the non-cold spell days. Plasma viscosity levels were natural log-transformed in analyses, and the effect estimates are shown as the percent changes of geometric outcome mean.

without time trend, season, or time trend and season were consistent with the main results (not shown).

4. Discussion

To our knowledge, this is the most extensive population-based study to explore the effect of cold spells on plasma viscosity in the general population. In this epidemiological study, we found significant associations between cold spells and increased plasma viscosity.

An epidemiological study based on the MONICA project in Augsburg, Germany (Koenig et al., 1998), showed that plasma viscosity was associated with an increased risk of coronary heart disease. Viscosity was also a risk factor for subsequent cardiovascular events in stroke survivors in a further population-based study (Resch et al., 1992). Moreover, plasma viscosity, blood viscosity and fibrinogen were significantly associated with cardiovascular events in the Edinburgh Artery Study, based on a population aged 55–74 years (Lowe et al., 1997). Additional two studies also found that plasma viscosity and fibrinogen were significantly associated with ischemic heart disease (Sweetnam et al., 1996; Yarnell et al., 1991).

Our findings are generally consistent with reported observations from some of the previous experimental studies. Keatinge et al., for example, reported that healthy male (n = 4) and female (n = 4) subjects exposed to cold for 6 h showed significant increases in plasma and whole

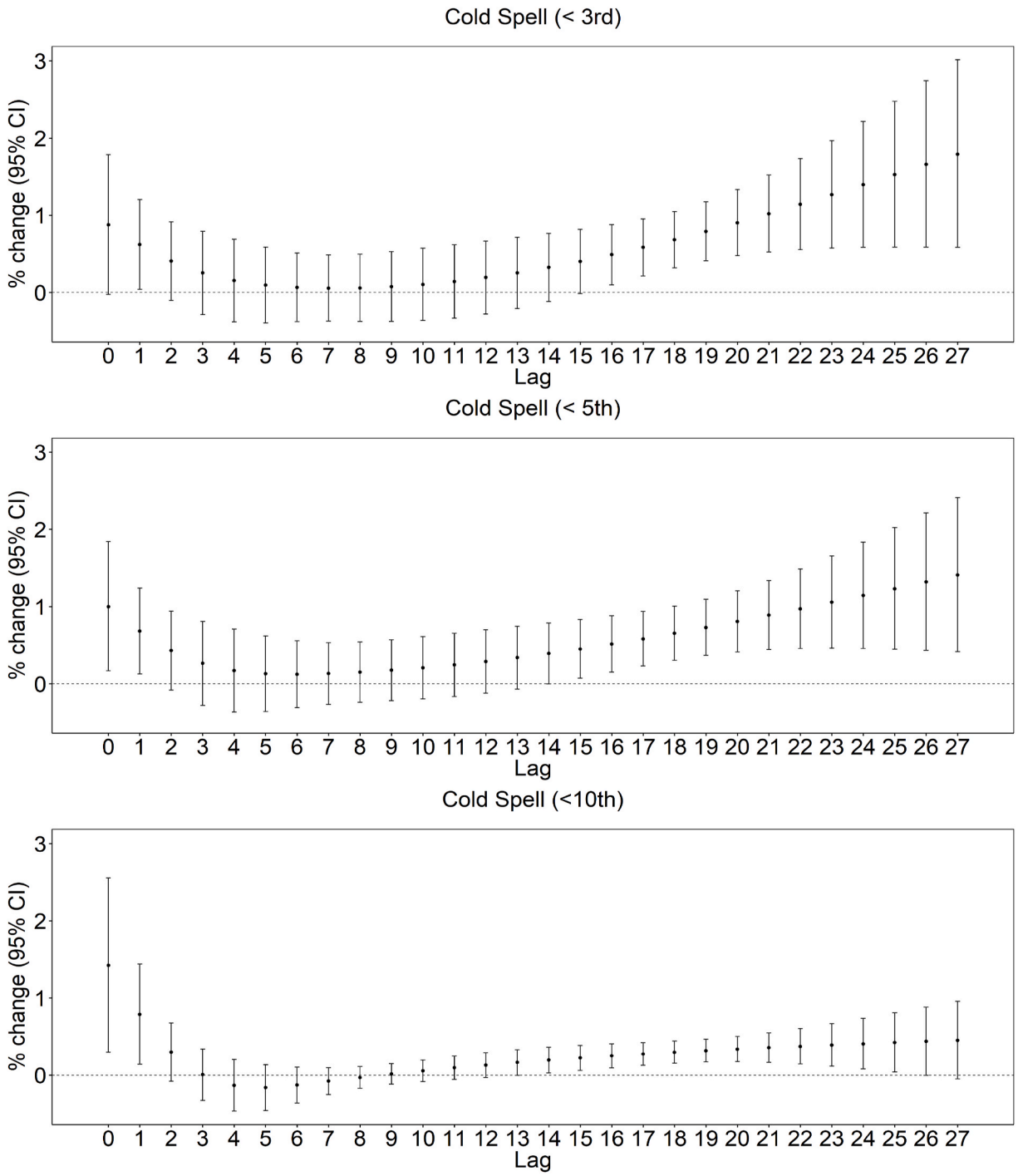

Fig. 3. Single-day lag structures (0–27 days) for effects of the three cold spell definitions on plasma viscosityGeneralized additive models with distributed lag nonlinear models were adjusted for age, sex, BMI, education, smoking status, alcohol consumption, physical activity, day of the week, season, SO_2 at lag 0, CO at lag 0, TSP at lag 0, time trend (cubic spline with three degrees of freedom - df), relative humidity at lag 0 (cubic spline with three df), and barometric pressure at lag 0 (cubic spline with three df). *Cold spells* were defined as a binary variable with a value of 1 during the cold spell period and 0 for the non-cold spell days. Plasma viscosity levels were natural log-transformed in analyses, and the effect estimates are showed as the percent changes of geometric outcome mean.

blood viscosity (Keatinge et al., 1984). Rostomily et al. found an increase in the mean blood viscosity by 19% for ten healthy volunteers immersed in mid-sternum in 10 °C water for 90 min (KA et al., 2020). Seasonal analyses also showed plasma viscosity variability with peak values during winter months in sixteen healthy volunteers (Frohlich et al., 1997). A further study in fourteen healthy middle-aged individuals reported blood viscosity levels of 5.73 mPa s in February, 5.60 mPa s in May, and 5.51 mPa s in August ($P < 0.05$), but there was no seasonal alteration in plasma viscosity level (Otto et al., 1996). However, results were not consistent across all studies. An epidemiological study focusing only on a male population (50–65 years) found that plasma viscosity levels did not differ between the coldest and the warmest month (Elwood et al., 1993). Three animal studies across rats also reported that blood viscosity significantly increased as temperature decreased (Blumberg et al., 1999; Luo et al., 2012, 2014). However, one further animal study showed plasma viscosity was significantly lower in hamsters exposed to relatively low temperatures for four weeks (Deveci et al., 2001).

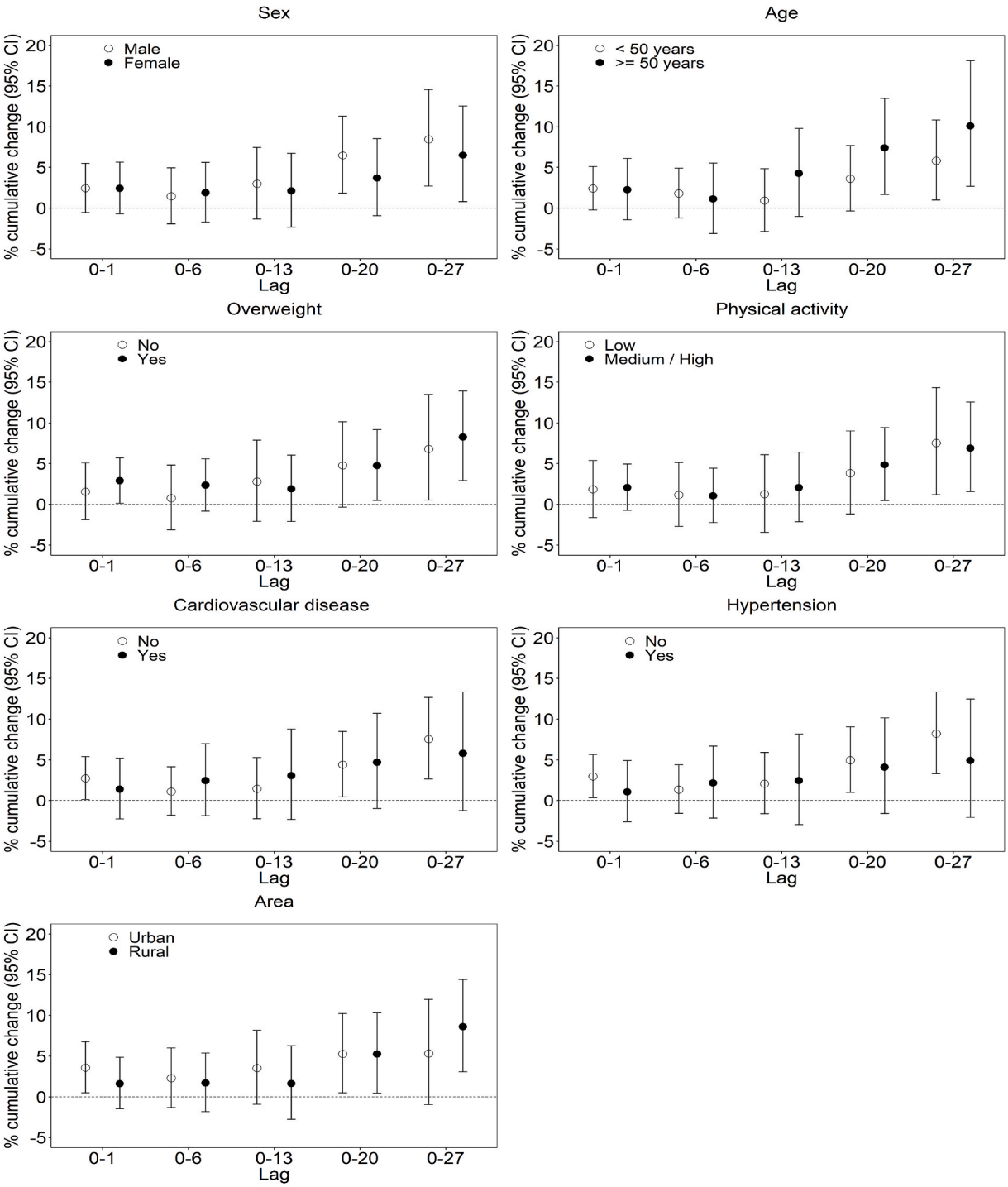

Fig. 4. Estimated cumulative effects [percent change (95% CI) of geometric mean] of the cold spell defined as Tmean <10th percentile on plasma viscosity at lags 0–1, lags 0–6, lags 0–13, lags 0–20, and lags 0–27 modified by sex, age, overweight, cardiovascular disease, hypertension, physical activity, and area.*Cold spells* were defined as a binary variable with a value of 1 during the cold spell period and 0 for the non-cold spell days. Plasma viscosity levels were natural log-transformed in analyses, and the effect estimates are showed as the percent changes of geometric outcome mean.

To our knowledge, this is the first research that explores the effects of different definitions of cold spells on plasma viscosity at different lag periods. Our results showed larger effect estimates for plasma viscosity during cold spells that were more intense (Tmean $<3^{rd}$: 18.81, Tmean $<5^{th}$: 17.85, Tmean $<10^{th}$: 7.41). We found that cold spells had both immediate (lag 0–1 days) but also longer lagged (up to 27 days) effects on plasma viscosity. This finding supports the complex interplay of multiple mechanisms of cold on adverse health. Previous studies have showed that cold temperatures had relatively longer lagged effects on health (Goodman et al., 2004; Guo et al., 2012; Wang et al., 2016). Our findings suggest that the delayed effects of cold spells on health are also significant and should be considered - the adhibition of shorter lag periods may undervalue the health effects of cold spells.

Previous work of our group studying associations between a severe air pollution episode and plasma viscosity showed an increased risk for extreme values of plasma viscosity after adjustment for meteorological variables and cardiovascular risk factors during the episode (characterized by elevated TSP, SO_2, and CO) (Peters et al., 1997). This study

adjusted for air pollution (TSP, SO_2, and CO) and found an increased plasma viscosity during cold spells. Moreover, we did not find significantly differing effects in any investigated subgroup (sex, age, overweight, physical activity, cardiovascular disease, and hypertension). This can be interpreted as robust effects of cold spells on plasma viscosity across the population, implying a high potential for generalizability of the findings of this study. Furthermore, we found similar results for different temperature metrics (Tmean, Tmin, Tmax, and Tappmean) in our study, again speaking for the stability of our results.

The mechanisms of how cold spells could lead to increases in plasma viscosity remain inconclusive. Plasma viscosity is determined basically by water content and macromolecular components of blood (Késmárky et al., 2008). One mechanism may be that exposure to cold causes vasoconstriction (which also elevates blood pressure), which induces a reversible plasma water shift from vascular to interstitial spaces, increasing the plasma viscosity levels (Freund et al., 1996; Vogelaere and Pereira, 2005; Young et al., 1987). And increasing respiratory water loss and diuresis may promote this process to a certain extent (Freund et al., 1996; Vogelaere and Pereira, 2005; Young et al., 1987). On the other hand, fibrinogen is one of the most important proteins in the process of blood coagulation, which is a major determinant of plasma viscosity (Jensen et al., 2004; Letcher et al., 1981; Matsuda and Murakami, 1976). Previous studies have found that fibrinogen levels were elevated in winter. Furthermore, both population and laboratory studies have shown that fibrinogen and other coagulation markers increase with acute or prolonged cold exposure (Nagelkirk et al., 2012; Parkkila et al., 2021). Though, the results from recent laboratory studies have shown inconclusive results. Increases in fibrinogen may therefore at least partially explain increased plasma viscosity during cold spells.

This study has several strengths. Firstly, to our knowledge, this is the most extensive population-based study to analyze the effect of cold spells on plasma viscosity. Secondly, the KORA cohort provided a wide range of information on patient characteristics allowing us to adjust comprehensively for confounders and investigate potential effect modifying factors. Thirdly, we carried out many sensitivity analyses to show that the results were reasonably robust.

A major limitation of this study is that the temperature data was derived from a fixed monitoring site rather than measuring individual exposure, which may induce exposure measurement error (Berkson type) and lead to underestimated and less precise cold spell effects. Secondly, this is only a single-center study in Augsburg, Germany, and the results may be limited concerning the generalizability to other regions and other ethnic groups. Thirdly, some potential confounding factors, such as dietary intake which might be associated with plasma viscosity (Naghedi-Baghdar et al., 2018), were not measured during the study and could thus not be considered in this analysis. Finally, as with other observational studies, we made the best efforts to control for the main confounders; however, there may remain some residual confounding because of unknown or unmeasured confounders in this study.

5. Conclusion

In conclusion, we found that cold spells had significant immediate and longer lagged effects on plasma viscosity. This finding supports the complex interplay of multiple mechanisms of cold on adverse cardiac events and enriches the knowledge about how cold exposure acts on the human body.

Credit author statement

Wenli Ni: Methodology, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Alexandra Schneider**: Methodology, Writing - review & editing, Supervision. **Kathrin Wolf**: Methodology, Writing - review & editing. **Siqi Zhang**: Methodology, Writing - review & editing. **Kai Chen**: Writing - review & editing. **Wolfgang Koenig**: Methodology, Writing - review & editing. **Annette Peters**: Writing review & editing, Supervision. **Susanne Breitner**: Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by a scholarship under the State Scholarship Fund by the China Scholarship Council (File No. 201906010316). The KORA Study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119071.

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Supplementary materials

Short-term effects of cold spells on plasma viscosity: results from the KORA cohort study in Augsburg, Germany

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Table S1 Characteristics of the participants and levels of plasma viscosity in cold spells and non-cold spells

	Mean ± SD / N (%)					
	Tmean <10th percentile		Tmean <5th percentile		Tmean <3rd percentile	
	Non-cold spells (n=3279)	Cold spells (n=343)	Non-cold spells (n=3454)	Cold spells (n=168)	Non-cold spells (n=3479)	Cold spells (n=143)
Plasma viscosity (mPa·s)*	1.25 ± 0.068	1.27 ± 0.074	1.26 ± 0.069	1.28 ± 0.075	1.26 ± 0.069	1.28 ± 0.076
Age (years)	45.3 ± 11.4	44.0 ± 11.1	45.1 ± 11.4	45.1 ± 11.0	45.1 ± 11.4	45.5 ± 10.9
Sex (male)	1671 (51.0)	178 (51.9)	1765 (51.1)	84 (50.0)	1778 (51.1)	71 (49.7)
Body mass index (kg/m²)	26.4 (4.1)	26.7 (4.1)	26.4 (4.1)	26.5 (3.9)	26.4 (4.1)	26.6 (3.8)
Overweight (yes: ≥25kg/m²)	1943 (59.3)	222 (64.7)	2056 (59.5)	109 (64.9)	2070 (59.5)	95 (66.4)
Smoking status						
Current smoker	991 (30.2)	98 (28.6)	1042 (30.2)	47 (28.0)	1046 (30.1)	43 (30.1)
Former smoker	852 (26.0)	83 (24.2)	897 (26.0)	38 (22.6)	909 (26.1)	26 (18.2)
Never smoker	1435 (43.8)	162 (47.2)	1514 (43.8)	83 (49.4)	1523 (43.8)	74 (51.7)
Physical activity						

Low (no or <1h per week)	1409 (43.0)	167 (48.7)	1500 (43.4)	76 (45.2)	1511 (43.4)	65 (45.5)
Medium (~1h per week)	1333 (40.7)	126 (36.7)	1391 (40.3)	68 (40.5)	1403 (40.3)	56 (39.2)
High (~2h per week or more)	530 (16.2)	50 (14.6)	556 (16.1)	24 (14.3)	558 (16.0)	22 (15.4)
Alcohol consumption (g/day)	23.7 ± 29.8	23.2 ± 26.8	23.7 ± 29.7	22.7 ± 24.8	23.7 ± 29.7	21.6 ± 24.0
Education*						
<10 years of education	538 (16.4)	79 (23.0)	576 (16.7)	41 (24.4)	581 (16.7)	36 (25.2)
≥10 years of education but not a university degree	2450 (74.7)	240 (70.0)	2577 (74.6)	113 (67.3)	2592 (74.5)	98 (68.5)
University degree	290 (8.8)	24 (7.0)	300 (8.7)	14 (8.3)	305 (8.8)	9 (6.3)
Total cholesterol (mmol/L)*	6.00 ± 1.21	6.20 ± 1.27	6.01 ± 1.21	6.33 ± 1.28	6.01 ± 1.21	6.37 ± 1.31
High-density-lipoprotein (HDL; mmol/L)	1.48 ± 0.46	1.46 ± 0.46	1.48 ± 0.46	1.45 ± 0.44	1.48 ± 0.46	1.45 ± 0.44

Hypertension (yes)	1098 (33.5)	119 (34.7)	1158 (33.5)	59 (35.1)	1168 (33.6)	49 (34.3)
Cardiovascular disease (yes)	1120 (34.2)	122 (35.6)	1182 (34.2)	60 (35.7)	1192 (34.3)	50 (35.0)
Medication intake						
Antihypertensive medication (yes)	275 (8.4)	30 (8.7)	287 (8.3)	18 (10.7)	291 (8.4)	14 (9.8)
Lipid-lowering medication (yes)	40 (1.2)	1 (0.3)	40 (1.2)	1 (0.6)	40 (1.1)	1 (0.7)
Antidiabetic medication (yes)	47 (1.4)	6 (1.7)	50 (1.4)	3 (1.8)	50 (1.4)	3 (2.1)
Platelet inhibitors (yes)	16 (0.5)	3 (0.9)	19 (0.6)	0 (0)	19 (0.5)	0 (0)
Area (urban) *	1524 (46.5)	123 (35.9)	1580 (45.7)	67 (39.9)	1594 (45.8)	53 (37.1)

*$P < 0.05$. *P*-Values for difference between non-cold spell and cold spell groups were test by Kruskal-Wallis rank sum tests for continuous variables and chi-square tests for categorical variables.

Table S2 Age distribution of the 3,622 participants with plasma viscosity compared to 400 participants without plasma viscosity

Groups	Mean	SD	25%	Median	75%	*P*-value
Participants with plasma viscosity	45.1	11.3	36.0	45.0	55.0	0.25
Participants without plasma viscosity	44.5	11.6	34.0	44.0	54.2	

Table S3 Number of participants for each lag day (0-27) during cold spells and non-cold spells (Tmean <10th percentile)

Lag	N's of participants in non-cold spells day	N's of participants in cold spells day
0	3,279	343
1	3,266	356
2	3,254	368
3	3,301	321
4	3,346	276
5	3,357	265
6	3,336	286
7	3,369	253
8	3,356	266
9	3,346	276
10	3,368	254
11	3,458	164
12	3,449	173
13	3,391	231
14	3,401	221
15	3,379	243
16	3,354	268
17	3,397	225
18	3,496	126
19	3,429	193

20	3,342	280
21	3,325	297
22	3,317	305
23	3,281	341
24	3,296	326
25	3,345	277
26	3,325	297
27	3,265	357

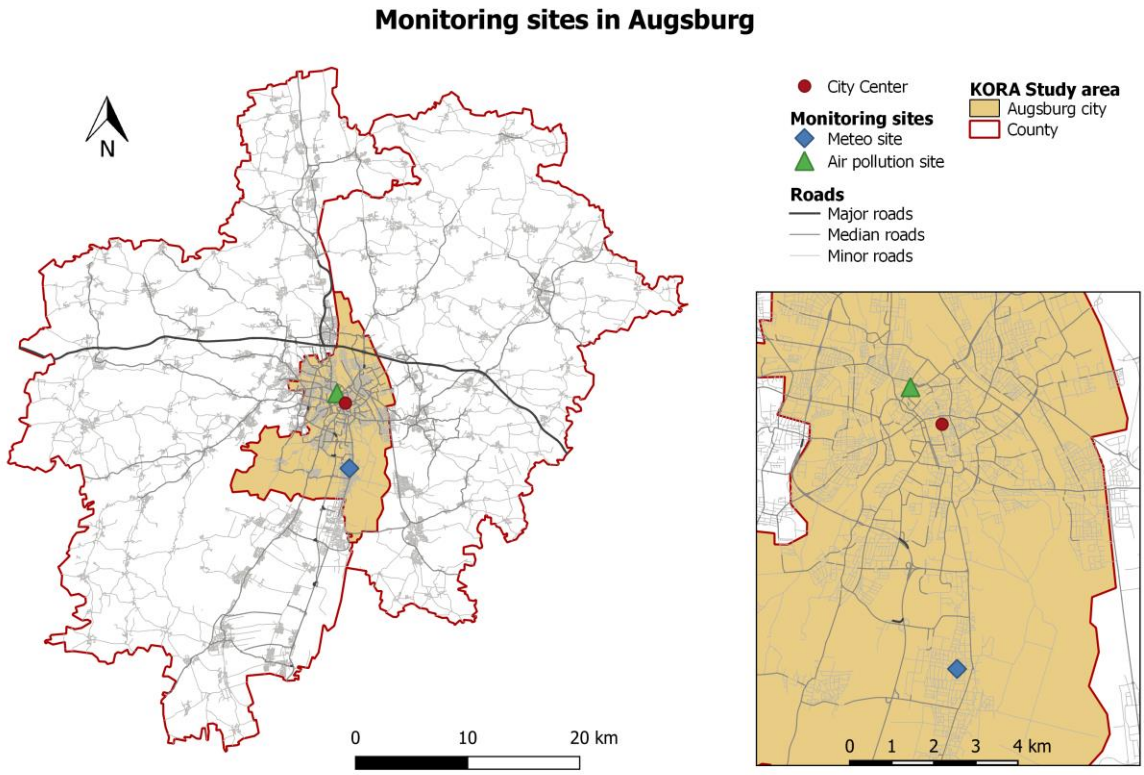

Figure S1 Map of the study area with meteorology (Meteo) and air pollution monitoring stations in the Augsburg area (light brown: city of Augsburg, on the right: county of Aichach-Friedberg, on the left: county of Augsburg)

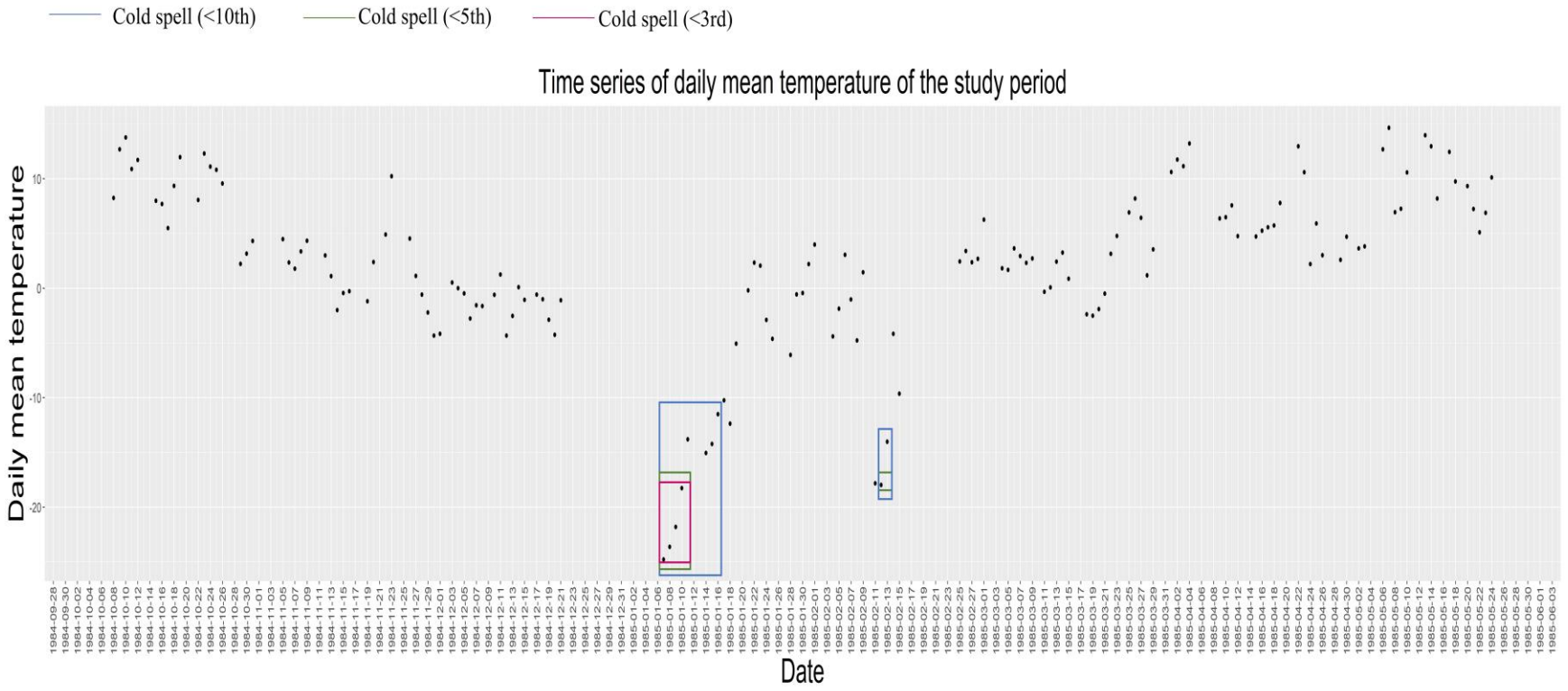

Figure S2. Time series of daily mean temperature during the study period

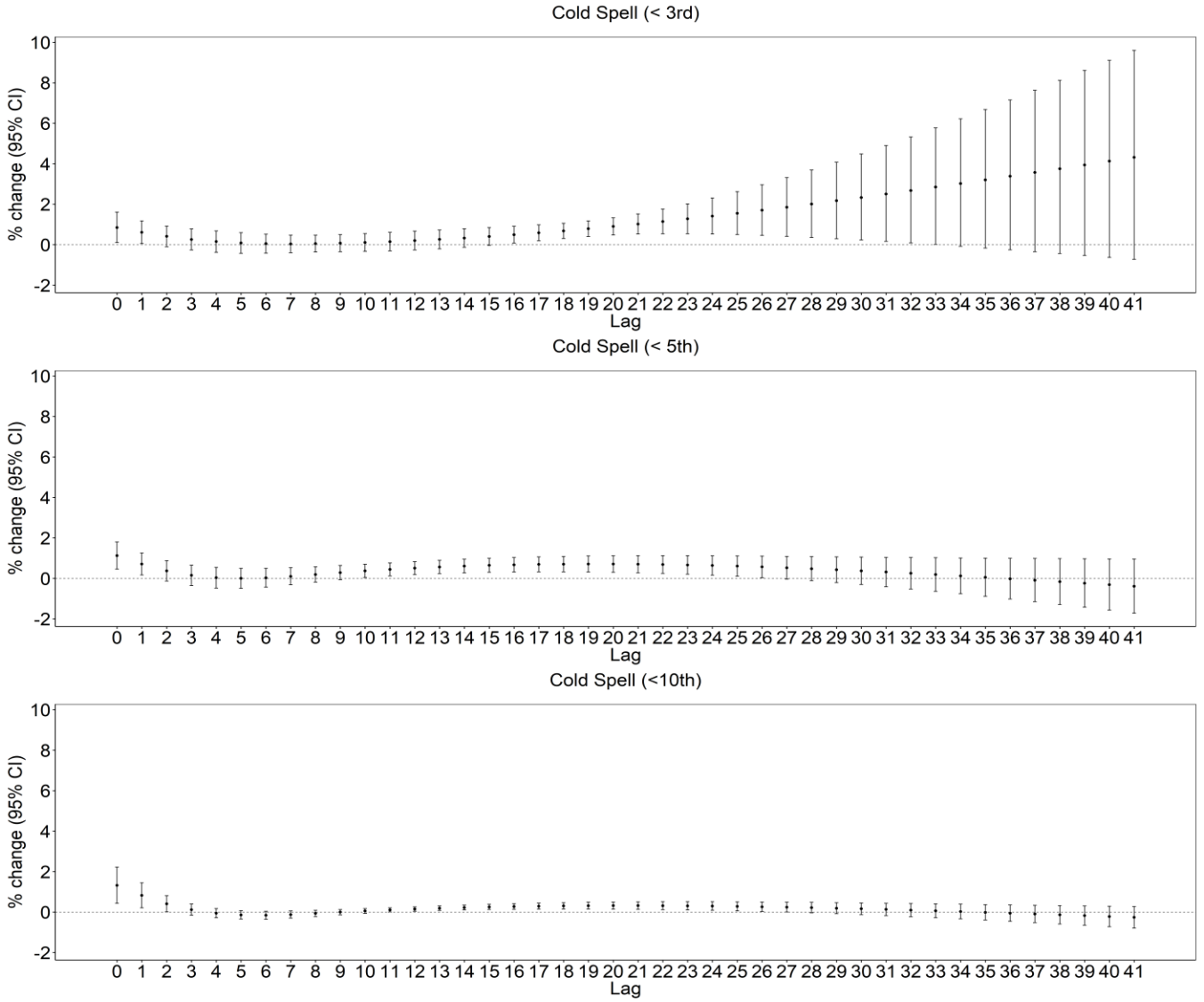

Figure S3. Single-day lag structures (0–41 days) for effects of the three cold spell definitions on plasma viscosity

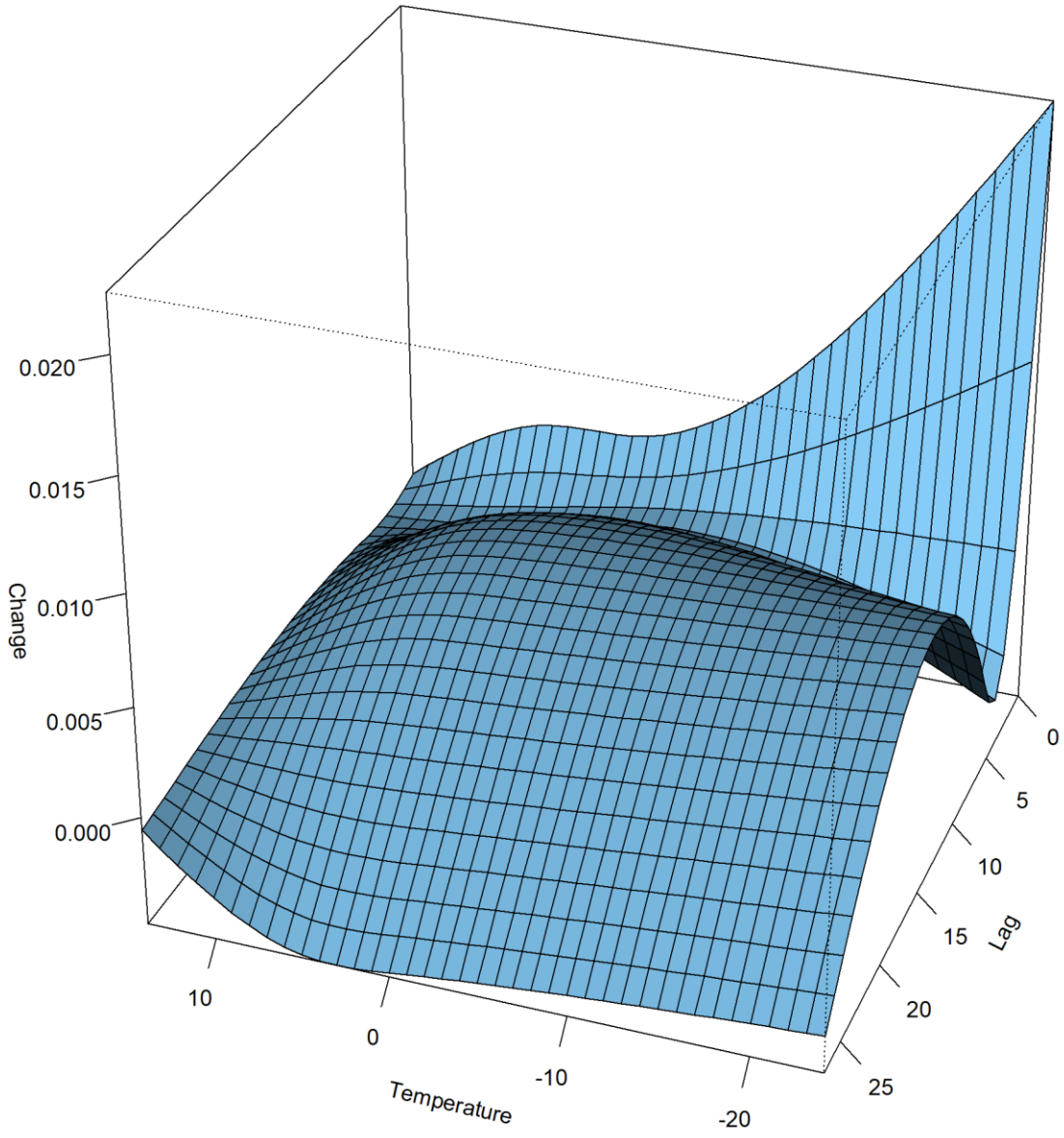

Figure S4. Three-dimensional plot of the exposure-lag-response for daily air temperature and plasma viscosity

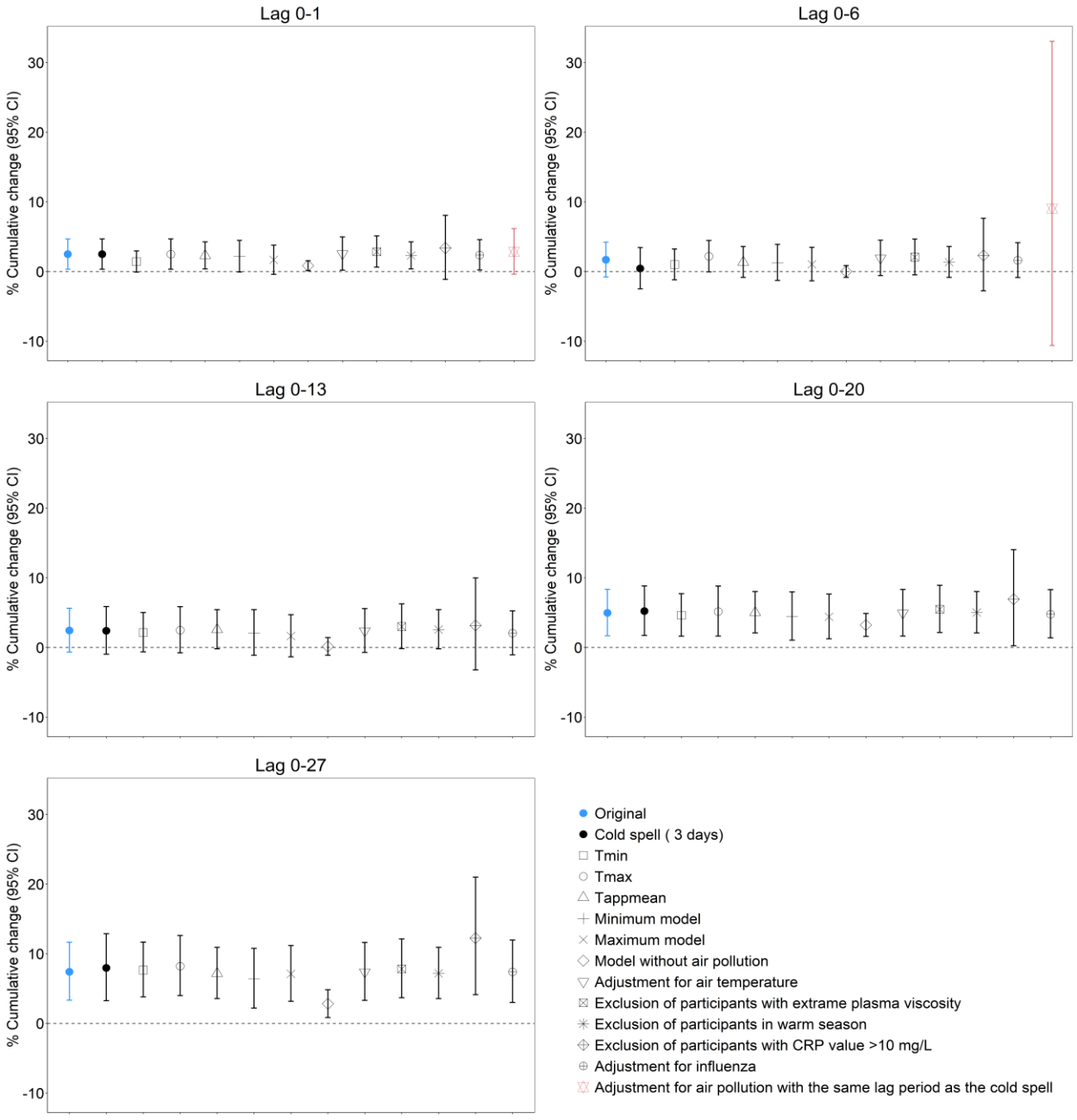

Figure S5. Sensitivity analysis: Estimated cumulative effects [percent change (95% CI) of geometric mean] of cold spells (defined by 10th percentile) on plasma viscosity at lags 0-1, lags 0-6, lags 0-13, lags 0-21, and lags 0–27.

Original: main model; *Tmin*: daily minimum temperature; *Tmax*: daily maximum temperature; *Tappmean*: mean apparent temperature.

Acknowledgements

First and foremost, I would like to express my deepest appreciation to my supervisors, Prof. Dr. Annette Peters, Director of the Institute of Epidemiology and Dr. Alexandra Schneider, Deputy Director of the Institute of Epidemiology, Head of Research Group "Environmental Risks" at Helmholtz München, for their invaluable guidance, mentorship, support and encouragement throughout my PhD journey. Their invaluable expertise, enthusiasm and patience were instrumental in the successful completion of these research projects. I am especially thankful for their insightful advice, engaging discussions, and invaluable feedback. I would also like to express my appreciation for their unwavering support in enabling me to attend international conferences and research stays.

I would like to thank my research team for their hard work and dedication. I am particularly grateful to Dr. Kathrin Wolf, Dr. Susanne Breitner, and Dr. Siqi Zhang for their thoughtful insights, productive discussions, support, and guidance during my research.

I would like to express my gratitude to Prof. Dr. Christian Herder, Dr. Cavin K Ward-Caviness, Dr. Melanie Waldenberger, and Dr. Rory Wilson, who provided invaluable feedback and support on my research.

I am deeply indebted to Chinese Scholarship Council for their financial support throughout my research.

To my parents and brother - words cannot express how grateful I am for your unconditional love and support throughout my educational journey.

Finally, I would like to thank all those who have contributed to my PhD projects in one way or another.