From
The Institute of Epidemiology II,
Helmholtz Zentrum München - German Research Center for Environmental Health (GmbH), Neuherberg, Germany
Director: PD Dr. A. Peters

Air Pollution, Air Temperature and the Influence on Cause-specific Mortality and Morbidity

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By
Stefanie Lanzinger
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Supervisor/Examiner: PD Dr. Annette Peters

Co-Examiners: PD Dr. Rudolf A. Jörres, Prof. Dr. Katja Radon, Prof. Dr. Alexander Baethmann

Co-Supervisor: Dr. Alexandra Schneider

Dean: Prof. Dr. med. dent. Reinhard Hickel

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Lanzinger, Stefanie

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List of Abbreviations

CI	Confidence Interval
DEPS	Diabetes and the Environmental Panel Study in Chapel Hill, North Carolina, USA
EPA	Environmental Protection Agency
E-Selectin	Soluble endothelial-leukocyte adhesion molecule
FMD	Flow-mediated dilatation
ICD	International Statistical Classification of Diseases and Related Health Problems
IQR	Interquartile range
NO	Nitric oxide
NO_2	Nitrogen dioxide
NTGMD	Nitroglycerin-mediated dilatation
PM	Particulate matter
PM_{10}	Particulate matter with an aerodynamic diameter < 10 µm
$PM_{2.5}$	Particulate matter with an aerodynamic diameter < 2.5 µm
$PM_{2.5\text{-}10}$	Coarse particles with an aerodynamic diameter between 2.5 and 10 µm
PNC	Particle number concentration
ppm	Parts per million
sICAM-1	Soluble intercellular adhesion molecule-1
sVCAM-1	Soluble vascular cell adhesion molecule-1
T2D	Type-2 diabetes
UFP	Ultrafine particles
UFIREG	Ultrafine particles – an evidence based contribution to the development of regional and European environmental and health policy
vWF	Von Willebrand factor
WHO	World Health Organization

Specification of my contribution to the manuscripts

As an investigator of the study "Ultrafine particles – an evidence based contribution to the development of regional and European environmental and health policy", which provided data for two of the manuscripts in this thesis, I played a significant role in the data collection, data management and statistical analysis. Furthermore, I wrote first drafts of several project reports and developed dissemination material (for more information see chapter 7).

For the three manuscripts discussed in this thesis, I developed the research questions together with my supervisors. Moreover, I conducted the statistical analysis, interpreted the results and wrote the first complete draft for all manuscripts. The manuscript entitled "Associations between ultrafine and fine particles and mortality in five central European cities – Results from the UFIREG study" was accepted on 8th of December by the journal Environment International. I prepared a revised version of the manuscript with the title "Ultrafine and fine particles and hospital admissions in Central Europe, results from the UFIREG study" based on reviewers' suggestions and submitted the revision on 18th of December to the American Journal of Respiratory and Critical Care Medicine. The manuscript entitled "The impact of decreases in air temperature and increases in ozone on markers of endothelial function in individuals having type-2 diabetes" was published in Environmental Research volume 134 in 2014.

Although I am the first author of the three manuscripts, I will refer to "we" throughout this thesis.

1 Summary

Epidemiological studies have shown that short-term changes in air pollution and air temperature have an impact on cardiovascular and respiratory health. However, only a few studies have investigated the association between ultrafine particles (UFP) with a diameter <100 nm (0.1 μm) and cardio-respiratory health so far and especially results from Eastern Europe are rare. Moreover, finding the underlying biological mechanisms, which explain the relationships between environmental changes and cardiovascular health, remains a major challenge. Investigating specific characteristics such as endothelial function in association with environmental stimuli might help to clarify potential biological pathways.

The main objectives of this thesis were to investigate short-term associations between air pollution and daily cause-specific mortality as well as hospital admissions in five Central and Eastern European cities (Augsburg, Chernivtsi, Dresden, Ljubljana and Prague) within the EU-project UFIREG. To investigate potential biological mechanisms explaining the associations between air pollution or air temperature and cardiovascular diseases we also examined the influence of short-term changes in air temperature and ozone on markers of endothelial function in individuals with type-2 diabetes (T2D) within the DEPS panel study in Chapel Hill in North Carolina, USA.

Our results of the UFIREG study indicated delayed and prolonged effects of UFP exposure on respiratory mortality and hospital admissions in Central and Eastern Europe. Although our results on UFP and respiratory health outcomes were not statistically significant, we consider these findings as potentially important. Further, we assume that the short study period might be one explanation for the non-significant associations. $PM_{2.5}$ exposure was associated with delayed and prolonged effects on cardiovascular and respiratory mortality and morbidity. We observed a stronger association between $PM_{2.5}$ and respiratory hospital admissions compared to results from other European regions and the U.S.

Our findings of the DEPS panel study suggested a linear association between temperature decreases as well as ozone increases and markers of endothelial function in individuals with T2D. We observed immediate and delayed decreases in flow-mediated dilatation of the brachial artery, a marker of endothelial function, in association with a decrease in temperature as well as with an increase in ozone.

This thesis adds to the existing knowledge in the field of short-term effects of air pollution since it reports results on the association between UFP and cause-specific mortality and morbidity from Central and Eastern European cities. Results of the UFIREG project indicated that it is important to integrate UFP into routine measurement networks to provide data for future short- and long-term epidemiological studies. Further studies are needed investigating the association between UFP and (cause-specific) mortality and morbidity at multiple locations using harmonized UFP measurements.

This thesis also discusses short-term changes in endothelial function in response to temperature decreases and ozone increases. We conclude that endothelial dysfunction might be one of the possible biological pathways explaining the association between environmental changes and cardiovascular events that have been observed worldwide.

2 Zusammenfassung

Epidemiologische Studien haben gezeigt, dass sich Tag-zu-Tag-Veränderungen in Luftschadstoffkonzentrationen sowie der Lufttemperatur auf die menschliche Gesundheit auswirken. Bisher haben jedoch nur wenige Studien den Zusammenhang zwischen ultrafeinen Partikeln (UFP) mit einem Durchmesser <100 nm (0.1 μm) und kardio-respiratorischen Gesundheitsparametern untersucht. Des Weiteren gibt es bisher wenige Ergebnisse aus osteuropäischen Ländern. Die Untersuchung spezifischer Merkmale wie zum Beispiel die Endothelfunktion, geben einen Einblick in mögliche biologische Mechanismen, die Herzkreislauferkrankungen, die im Zusammenhang mit Umweltveränderungen auftreten, erklären können.

Die Hauptziele dieser Dissertation waren, die Kurzzeitauswirkungen von ultrafeinen und feinen Partikeln auf die tägliche ursachenspezifische Mortalitäts- und Krankheitsrate in fünf zentral- und osteuropäischen Städten (Augsburg, Czernowitz, Dresden, Ljubljana und Prag) im Rahmen des EU-Projekts UFIREG zu untersuchen. Darüber hinaus untersuchten wir Veränderungen der Endothelfunktion bei Patienten mit Typ-2 Diabetes (T2D) in Zusammenhang mit einem kurzzeitigen Temperaturanstieg sowie einem Anstieg der Ozonkonzentration. Dieser Zusammenhang wurde innerhalb der DEPS Panel Studie in Chapel Hill, North Carolina, USA analysiert und könnte einen Aufschluss über mögliche biologische Mechanismen geben, wie Umweltveränderungen zu Herzkreislauferkrankungen führen können.

Ergebnisse des UFIREG Projekts zeigten einen Anstieg der Todesfälle und Krankenhauseinweisungen auf Grund von respiratorischen Erkrankungen in Zusammenhang mit einer kumulativen UFP Exposition. Die Einflüsse von UFP auf respiratorische Erkrankungen und Todesursachen waren zwar nicht statistisch signifikant, dennoch bewerten wir die Ergebnisse als wichtig. Die kurze Studienperiode könnte eine mögliche Erklärung für die nicht signifikanten Ergebnisse sein. Darüber hinaus zeigte ein Anstieg der $PM_{2.5}$-Konzentration einen Zusammenhang mit kardiovaskulärer sowie respiratorischer Mortalität und Morbidität. Unsere Ergebnisse des UFIREG Projekts zeigen einen stärkeren Zusammenhang zwischen $PM_{2.5}$ und respiratorischer Krankenhauseinweisungen als Studien, die in anderen europäischen Regionen und den USA durchgeführt wurden.

Die Ergebnisse der DEPS Panel Studie weisen auf eine lineare Assoziation zwischen einem Temperaturabfall sowie Ozonanstieg und Veränderungen in der Endothelfunktion bei T2D hin. Die flussvermittelte Dilatation der Arteria brachialis, ein Marker für die Endothelfunktion, nahm unmittelbar und mit einer Verzögerung von einem Tag ab wenn die Lufttemperatur sank bzw. wenn die Ozonkonzentration anstieg.

Diese Dissertation zeigt Ergebnisse zu den Kurzzeitauswirkungen von UFP auf die tägliche ursachenspezifische Mortalitäts- und Morbiditätsrate in zentral- und osteuropäischen Städten und leistet daher einen wichtigen Beitrag zum aktuellen Wissensstand. Des Weiteren zeigen die Ergebnisse des UFIREG Projekts, dass es wichtig ist, UFP in Routinemessnetzwerke zu integrieren, um Daten für zukünftige Studien zu den Kurz- und

Langzeiteffekten von UFP zu liefern. Weitere multizentrische Studien mit harmonisierten UFP Messungen sind notwendig, um den Zusammenhang zwischen UFP und (ursachenspezifischen) Todesursachen und Krankenhauseinweisungen in verschiedenen Regionen zu untersuchen. Diese Arbeit diskutiert darüber hinaus Veränderungen in der Endothelfunktion bei Temperaturabfall und Ozonkonzentrationsanstieg. Die Ergebnisse deuten darauf hin, dass eine Endotheldysfunktion einen möglichen Mechanismus für die weltweit beobachteten Herzkreislauferkrankungen im Zusammenhang mit Umweltveränderungen darstellt.

3 Introduction

Air pollution has been shown to be a major environmental risk factor for health and was estimated to cause 3.7 million premature deaths worldwide (1). Particulate matter (PM) is of particular importance since it affects more people than any other air pollutant (1). Many long- and short-term epidemiological studies have shown that high levels of air pollution have a strong impact on cardiovascular and respiratory health (1). However, besides exposure to air pollution, short-term changes in air temperature have also been associated with adverse health effects. For example, both high and low temperatures were associated with increases in cardio-respiratory mortality and morbidity (2-5).

3.1 The influence of air pollution and air temperature on (cause-specific) mortality and morbidity

The impact of air pollution and air temperature on mortality and morbidity was studied in several single as well as multi-city studies (4, 6, 7). There is strong evidence for an association between PM with an aerodynamic diameter smaller than 10 µm (PM_{10}) or 2.5 µm ($PM_{2.5}$) and cardio-respiratory mortality and morbidity (7, 8). Only a few studies have investigated the association between ultrafine particles (UFP) with a diameter <100 nm (0.1 µm) and (cause-specific) mortality and morbidity worldwide. Moreover, European research activities on the health effects of UFP were primarily conducted in Western European countries and studies from Eastern Europe are needed to get an overall picture (9). The deposition and clearance in the respiratory tract differ between UFP and larger particles such as PM_{10} and $PM_{2.5}$. While larger particles deposit mainly in the upper and lower respiratory tract, UFP can penetrate deeply into the pulmonary alveoli and can be translocated with the blood stream to other organs (9, 10). Therefore, it is assumed that UFP might have at least partly independent health effects compared to larger particles (8, 9, 11, 12).

Extreme temperatures during heat waves and cold spells have been shown to increase mortality and morbidity (13-16). However, also short-term changes in moderate temperature were associated with adverse health effects. A study conducted in 15 European cities reported increases in natural and cause-specific mortality in association with a 1°C decrease in temperature between October and March (6). Breitner and colleagues (3) found increases in cardiovascular mortality with both increases and decreases in temperature in three cities in Bavaria, Germany. A meta-analysis conducted by Turner et al. (4) reported weak associations between a 1°C increase in air temperature and respiratory hospital admissions, but no association between temperature and cardiovascular hospital admissions. However, the authors pointed out that temperature decreases were shown to be associated with cardiovascular morbidity (4).

Finding the underlying biological mechanisms explaining the relationships between environmental changes and cardiovascular health remains a major challenge. Investigating specific characteristics such as endothelial function in association with environmental stimuli might help in understanding potential biological mechanisms of the reported adverse health effects (7, 17-19).

3.2 Endothelial dysfunction as a potential biological mechanism

The endothelium is the inner layer of the blood vessels and maintains vascular homeostasis (20). Important vasoconstrictors and vasodilators are released by endothelial cells of the endothelium (20). Endothelial dysfunction refers to the inability of the arteries and arterioles to dilate fully and leads to an imbalance between vasodilatation and vasoconstriction (21). Flow-mediated dilatation (FMD) of the brachial artery is a marker of endothelial function and measures the endothelium-dependent increase in blood vessel diameter in response to reactive hyperemia (22). FMD occurs through the release of nitric oxide (NO) by the endothelial cells and reflects the bioavailability of NO. A decrease in FMD indicates a dysfunction of the vascular endothelium. Nitroglycerin-mediated dilatation (NTGMD) is an endothelial-independent mechanism and is an index of the vascular reactivity of the smooth muscle cells to nitroglycerin, which serves as an exogenous NO donor (22). A decreased NTGMD reflects an impaired function of the smooth muscle cells. To distinguish between endothelial-dependent and endothelial-independent mechanisms, it is important to investigate both FMD and NTGMD.

Studies reported a decrease in FMD in association with an increase in air pollution as well as with changes in air temperature (17, 18, 23, 24). Schneider and colleagues (18) reported an immediate decrease in FMD in association with increases in $PM_{2.5}$ in diabetic individuals. Moreover, increases in $PM_{2.5}$ were associated with a one day delayed decrease in NTGMD in the same study (18). This finding indicated that $PM_{2.5}$ immediately affected the bioavailability of NO, but had a delayed effect on the function of the smooth muscle cells. A further U.S. study conducted in Boston reported a decrease in brachial artery diameter in association with an increment in the 5-day $PM_{2.5}$-average in diabetics. Moreover, brachial artery diameter increased with a temperature increase, but no associations were found when investigating FMD and NTGMD (25). Inverse associations between UFP and endothelial function were also reported and discussed in a review of the physiological effects of UFP (26). For example, inhalation of high UFP levels during exercise was associated with decreases in FMD in healthy men in the U.S. (27). A further U.S. study on controlled laboratory exposure to ultrafine carbon particles reported reduced bioavailability of NO in healthy adults (28). However, no associations were found between UFP and endothelial function in diabetics in a study conducted in Boston (29).

Endothelial dysfunction has been shown to be a risk factor for cardiovascular diseases (30, 31). Impaired endothelial function leads to increased oxidative stress, promotes inflammation, allows thrombus formation and may trigger disruption of existing coronary plaques through vasoconstriction (30). NO plays an important role in inflammatory processes since it reduces the expression of inflammatory mediators and adhesion molecules. Hence, a decrease in NO production by endothelial cells in response to environmental stimuli leads to a promotion of inflammatory processes (30).

Individuals with type-2 diabetes (T2D) are especially vulnerable to a dysfunction of the vascular endothelium since it has been shown that T2D adversely affects small and larger blood vessels leading to micro- and macroangiopathy (32).

3.3 Specific objectives

The specific objectives of this thesis were:

1) To investigate short-term associations between air pollution and daily (cause-specific: cardiovascular and respiratory) mortality in five Central and Eastern European Cities.
2) To assess short-term associations between air pollution and daily cause-specific hospital admissions in five Central and Eastern European Cities.
3) To examine the influence of short-term changes in air temperature and ozone on markers of endothelial function in individuals with T2D in order to investigate potential biological mechanisms explaining the associations between air pollution and air temperature and cardiovascular diseases.

4 Methods

The EU-project "Ultrafine particles – an evidence based contribution to the development of regional and European environmental and health policy" (33) started in July 2011 and ended in December 2014. One of the main objectives of the UFIREG project was to investigate the association between ultrafine and fine particles and (cause-specific) mortality and morbidity in five Central and Eastern European Cities (specific objectives 1 and 2). Exposure assessment and statistical analyses were conducted based on a priori fixed and harmonized protocols. Daily counts of (cause-specific) mortality and hospital admissions were obtained for Augsburg and Dresden (Germany) from January 2011 to December 2012, Chernivtsi (Ukraine) from January 2013 to March 2014, Ljubljana (Slovenia) and Prague (Czech Republic) from January 2012 to December 2013 focusing on cardiovascular and respiratory diseases. The causes of death and primary diagnosis of hospital admissions were based on the International Statistical Classification of Diseases and Related Health Problems (ICD-10). We investigated deaths due to natural causes (ICD-10: A00-R99), deaths and hospital admissions due to cardiovascular (ICD-10: I00-I99) and respiratory diseases (ICD-10: J00-J99) as primary outcomes. Moreover, we examined hospital admissions due to diabetes (ICD-10: E10-E14) as an outcome of secondary interest.

Air pollutants and meteorological factors were measured at fixed monitoring sites in all cities. In all the five cities, UFP was measured using uniform measurement devices (differential or scanning mobility particle size spectrometers). Poisson regression models allowing for overdispersion were used to analyze the association between air pollutants and health outcomes for each city separately. The same confounder model was used for all cities based on a review of the current literature. The confounder model included long-term time-trend, day of the week, public holidays, vacation periods (to control for a decrease of the populations during Christmas, Easter and summer vacations), influenza epidemics (where available), air temperature and relative humidity. We analyzed single lags from lag 0 (same day of the event) up to lag 5 (five days prior to the event) and cumulative lags to represent immediate (2-day average: lag 0-1), delayed (average of lag 2-5) and prolonged (6-day average: lag 0-5) associations between air pollutants and health outcomes. City-specific effect estimates were pooled using meta-analysis methods.

In order to study potential biological mechanisms explaining cardiovascular events in association with environmental stimuli, we examined the impact of changes in air temperature and ozone on markers of endothelial function in individuals with T2D (specific objective 3). Within the project "Diabetes and the Environmental Panel Study in Chapel Hill, North Carolina, USA" (DEPS), FMD, NTGMD of the brachial artery and several blood markers representing endothelial function were repeatedly measured in 22 individuals with T2D. Between November 2004 and December 2005 four measurements per person were taken under controlled conditions on consecutive days at the U.S Environmental Protection Agency's (EPA) National Health and Environmental Effects Research Laboratory, Environmental Public Health Division in Chapel Hill, North Carolina. FMD was quantified as the percent change in brachial artery diameter after reactive hyperemia. NTGMD was quantified as the percent change in blood vessel diameter after nitroglycerin spray application. Soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble endothelial-leukocyte adhesion molecule (E-Selectin) and von Willebrand factor (vWF) were measured in blood. sICAM-1, sVCAM-1 and E-Selectin are specific markers for endothelial cell activation and vWF is a largely endothelium derived blood glycoprotein involved in platelet adhesion (22). Daily measurements of meteorological parameters, ozone and $PM_{2.5}$ were obtained from fixed monitoring sites. Twenty-four-hour averages of the examination day (lag 0), averages of 24-47 hours (lag 1), 48-71 hours (lag 2), 72-95 hours (lag 3), 96-119 hours (lag 4) and a 5-day average before the visits were considered to study immediate, delayed and cumulative effects of air temperature or ozone exposure on the health outcomes. Additive mixed-models were used to analyze the associations between air temperature decreases and ozone increases and markers of endothelial function. We adjusted for time-trend, day of the week, relative humidity and barometric pressure. When investigating associations with ozone, temperature was also included into the model. Moreover, interactive effects of air temperature and air pollution were studied. $PM_{2.5}$ and ozone were divided into low (<25%-quartile), medium (25%-74%) and high (≥75% quartile) concentrations and an interaction term was included in the model.

5 Results

5.1 Associations between air pollution and (cause-specific) mortality

Results on the association between air pollutants and (cause-specific) mortality from the UFIREG study are discussed in the manuscript entitled "Associations between ultrafine and fine particles and mortality in five central European cities – Results from the UFIREG study" (*Environment International,* accepted on 8th of December, see chapter 9.1). Within the manuscript, we also investigated particle number concentrations up to 800 nm (PNC), PM_{10}, coarse particles with an aerodynamic diameter between 2.5 and 10 µm ($PM_{2.5-10}$) and nitrogen dioxide (NO_2) as pollutants of secondary interest.

No associations between UFP or PNC and natural or cardiovascular mortality were found. However, our results indicated delayed and prolonged effects of UFP exposure on respiratory mortality. The association between UFP and respiratory mortality was not statistically significant, but we still consider these findings as potentially important as

results show a trend towards delayed and prolonged effects. A 2,750 particles/cm^3 increase (average interquartile range (IQR) across all cities) in the 6-day average of UFP was associated with a 9.9% [95%-confidence interval: -6.3%; 28.8%] increase in the pooled effect estimate of respiratory mortality. All cities except Ljubljana showed increases in the city-specific effect estimates of respiratory mortality; the strongest association was observed in Dresden. The associations between PNC and respiratory mortality were weaker but consistent.

In contrast to UFP and PNC, we found no associations between $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$ and respiratory mortality. However, a 12.4 µg/m^3 and 4.7 µg/m^3 (average IQR) increase in the $PM_{2.5}$- and $PM_{2.5-10}$-average of lag 2-5 was associated with increases in the pooled estimates of cardiovascular mortality by 3.0% [-2.7%; 9.1%] and 4.1% [0.4%; 8.0%], respectively. PM_{10} showed a similar association, but NO_2 was not associated with any mortality outcomes.

5.2 Associations between air pollution and cause-specific hospital admissions

The manuscript entitled "Ultrafine and fine particles and hospital admissions in Central Europe, results from the UFIREG study" (*American Journal of Respiratory and Critical Care Medicine*, revision submitted on 18th of December, see chapter 9.2) discusses the association between air pollutants and cause-specific hospital admissions in Augsburg, Chernivtsi, Dresden, Ljubljana and Prague. As a secondary objective of the manuscript, we also examined the short-term associations between PNC, PM_{10}, $PM_{2.5-10}$, NO_2 and cause-specific hospital admissions in these five cities.

We observed no association between UFP, PNC or NO_2 and cardiovascular hospital admissions. As for mortality, we found an association between an IQR increase in the 6-day average of UFP and respiratory hospital admissions. A 2,750 particles/cm^3 increase in the UFP-6-day average indicated an increase in the pooled effect estimate of respiratory hospital admissions by 3.4% [-1.7%; 8.8%]. Results of PNC were similar. Moreover, we found increases in cardiovascular (exposure average of lag 2-5: 1.8% [0.1%; 3.4%]) and respiratory (6-day average exposure: 7.5% [4.9%; 10.2%]) admissions per 12.4 µg/m^3 increase in $PM_{2.5}$. Associations between PM_{10} or $PM_{2.5-10}$ and cardiovascular and respiratory hospital admissions were similar to $PM_{2.5}$. A 15.4 µg/m^3 increase in the NO_2-average of lag 2-5 also led to an increased pooled estimate of hospital admissions due to respiratory diseases (5.1% [0.7%; 9.7%]). Moreover, our results indicated delayed and prolonged effects of UFP, PNC, $PM_{2.5}$ and PM_{10} exposure on hospital admissions due to diabetes.

5.3 The influence of short-term changes in air temperature and ozone on markers of endothelial function in individuals with type-2 diabetes

Our results on the association between air temperature or ozone and markers of endothelial function are reported in the manuscript with the title "The impact of decreases in air temperature and increases in ozone on markers of endothelial function in individuals having type-2 diabetes" (*Environmental Research,* 134: 331-338, 2014, see chapter 9.3).

Our findings of the DEPS study suggest a linear association between temperature decreases

as well as ozone increases and markers of endothelial function in individuals with T2D. We observed no changes in sVCAM-1, E-Selectin and vWF in association with a temperature decrease or an ozone increase. However, FMD decreased immediately (lag 0) in association with a 1°C decrease in air temperature (-2.2% [-4.7%; 0.3%]) and a 0.01 ppm (20 $\mu g/m^3$) increase in ozone (-14.6% [-26.3; -2.9%]). A 1°C temperature decrement was also associated with a one day delayed (-2.3% [-5.2%; 0.5%]) and a four days delayed (-3.9% [-6.5%; -1.2%]) decrease in FMD. Moreover, a 0.01 ppm increase in ozone led to a decrease in FMD by -13.5% [-27.0%; 0.04%] with a delay of one day. We observed an immediate decrease in NTGMD by -1.7% [-3.3%; -0.04%] with a 1°C decrease in air temperature. Furthermore, we found a non-significant increase in sICAM-1 in association with a temperature decrement on the same day (1.1% [-0.2; 2.4%]) as well as with a 1°C decrease in the 5-day temperature average (2.6% [-0.2; 5.6%]). However, no association was found between an increase in ozone and NTGMD or sICAM-1.
The association between a 1°C temperature decrease and a four days delayed decrease in FMD was more pronounced with high $PM_{2.5}$ concentrations compared to medium or low $PM_{2.5}$ levels. Immediate temperature effects on NTGMD were also stronger in association with high $PM_{2.5}$ concentrations. Moreover, our results indicated that immediate and one day delayed associations between temperature and FMD were stronger with high ozone concentrations compared to medium or low levels.

6 Discussion and conclusions

This thesis adds to the existing knowledge in the field of short-term effects of air pollution since it reports results on the association between air pollution and (cause-specific) mortality and morbidity from Central and Eastern European cities. Only a few epidemiological studies have investigated the association between UFP and health outcomes so far and especially results from Eastern Europe are rare. Moreover, exposure assessment and statistical analyses were conducted based on a priori fixed and harmonized protocols within the UFIREG project.

This thesis also discusses short-term changes in endothelial function in individuals with T2D in response to temperature decreases and ozone increases since endothelial dysfunction might be one of the possible mechanisms explaining cardiovascular events in association with day-to-day environmental changes.

Our results of the UFIREG study indicated delayed and prolonged effects of UFP exposure on respiratory mortality and hospital admissions. Although our results on UFP and respiratory health outcomes were not statistically significant, we consider these findings as potentially important, and we assume that the short study period might be an explanation for the non-significant associations. Within the UFIREG study, PM was associated with cardiovascular and respiratory health outcomes. Cardiovascular mortality and cardiovascular hospital admissions increased in association with an increment in the $PM_{2.5}$-average of lag 2-5. Moreover, we observed a prolonged association between $PM_{2.5}$ (6-day average exposure) and respiratory hospital admissions. Results of PM_{10} and $PM_{2.5-10}$ were similar compared to $PM_{2.5}$.

Comparing our results on $PM_{2.5}$ and cause-specific mortality to a national U.S. analysis, our findings for a 10 μg/m³ $PM_{2.5}$ increase and cardiovascular mortality (average of lag 2-5: 2.4% [-2.3%; 7.3%]) are comparable to U.S. regions with a dry climate as New Mexico, Arizona and Nevada (lag 0-1: 3.1% [-0.02%; 6.3%]) and to U.S. regions with a dry together with continental climate as Montana, Idaho, Wyoming, Utah and Colorado (lag 0-1: 1.7% [-0.8%; 4.2%]) (34). However, our results showed a stronger association between $PM_{2.5}$ and respiratory hospital admissions compared to results from other European regions and the U.S. (35-37). Stafoggia et al. found a 1.36% [0.23, 2.49] increase in respiratory hospital admissions with a 10 μg/m³ increase in the 6-day average of $PM_{2.5}$ in eight Southern European cities (36). A study on 202 U.S. counties reported an immediate 1.1% [0.3%; 1.8%] increase in respiratory hospital admissions in association with a 10 μg/m³ increase in $PM_{2.5}$ during winter (35). We found a 6.0% [4.0%; 8.2%] increase in respiratory hospital admissions in association with the same increment in the 6-day average of $PM_{2.5}$. Different climate conditions and differences in the compositions of $PM_{2.5}$ might play a role. However, also different life-styles and differences in socioeconomic and behavioral patterns between the regions need to be considered. Due to the short study period of the UFIREG project, we observed effect estimates with relatively large confidence intervals especially for mortality outcomes. For investigating rare death cases and diseases in smaller cities such as Augsburg, Chernivtsi and Ljubljana it is important for future studies to use longer time periods to produce more precise effect estimates. Nevertheless, UFIREG is important, as it is one of the very few multi-city studies investigating the associations between UFP and fine particles and (cause-specific) mortality and hospital admissions including cities from Central and Eastern European countries using UFP measurements based on the same protocols and instruments.

The DEPS panel study showed associations between markers of endothelial function and air temperature and ozone in individuals with T2D. We observed immediate and delayed decreases in FMD in association with a decrease in temperature as well as with an increase in ozone. NTGMD decreased immediately with a temperature decrement, but showed no association with an increase in ozone. Moreover, our results suggest that temperature and $PM_{2.5}$ as well as temperature and ozone might have additive effects on markers of endothelial function. A previous analysis in the same study reported an immediate association between $PM_{2.5}$ increases and decreases in FMD (18). Moreover, NTGMD decreased with a delay of one day in association with a 10 μg/m³ increase in $PM_{2.5}$ (18). Therefore, we assume that the immediate $PM_{2.5}$-effect reported by Schneider et al. (18) and the immediate ozone effect in our study are best explained by a reduced bioavailability of NO. However, our reported immediate temperature effect might be rather explained by an endothelial-independent mechanism on the reactivity of the smooth muscle cells since we found immediate decreases in FMD as well as NTGMD in association with a 1°C temperature decrease. People with T2D are especially vulnerable for endothelial dysfunction and subsequent cardiovascular diseases (32). Due to microvascular and macrovascular abnormalities, regulation of vascular tone is impaired in diabetics (38). Although results on individuals with T2D are not generalizable to the general population, investigating potentially susceptible groups might give better insight into possible underlying biological pathways.

There are several plausible biological mechanisms explaining the association between air pollution and cardio-respiratory diseases (8, 39, 40). Inhaled air pollutants can induce inflammation and oxidative stress in the lungs. Studies have shown that air pollution can accelerate and exacerbate the progression of chronic pulmonary conditions such as chronic obstructive pulmonary disease or asthma. Moreover, pulmonary reflexes are assumed to be affected by air pollution (8, 39, 40). The release of pro-inflammatory markers or vasculoactive molecules from the lung cells may lead to inflammation and systemic oxidative stress beyond the lungs resulting in adverse cardiovascular outcomes such as endothelial dysfunction and promotion of atherosclerosis (41). Constituents of PM and particularly UFP can be translocated from the alveolar space to other organs with the blood stream. PM and UFP in the blood might cause vascular inflammation, impaired vascular function and increased platelet aggregation (8, 39, 40). Systemic oxidative stress and inflammation, imbalance of the autonomic nervous system and endothelial dysfunction can lead to insulin resistance and therefore promote the progression of T2D (42, 43). However, we observed UFP effects rather on the respiratory than cardiovascular system, whereas other studies found an association between UFP and cardiovascular as well as respiratory health outcomes (44-47). The described associations between air temperature and cardiovascular events might be explained by an activation of the sympathetic nervous system in response to temperature decreases. Activation of the sympathetic nervous system further leads to an increase in blood pressure, increased blood flow and heart rate and to vasoconstriction of the blood vessels (48). In the case of low NO bioavailability or arterial stiffness, subsequent vasodilatation of the blood vessels might be impaired. A decrease in temperature was also associated with increases in inflammatory markers, which might promote atherosclerosis (49). Individuals with diabetes are assumed to be especially susceptible to the described mechanisms. Due to the microvascular and macrovascular abnormalities, adaptation to short-term changes in air pollution and air temperature is impaired in diabetics leading to clinical conditions that might not be significant in healthy individuals (50, 51).

The UFIREG study adds to the growing scientific knowledge on health effects of UFP and indicated that it is important to integrate UFP into routine measurement networks in order to provide data for future short- and long-term epidemiological studies. So far, hardly any long-term studies on UFP have been conducted (52). Further studies are needed investigating the association between UFP and (cause-specific) mortality and morbidity at multiple locations using harmonized UFP measurements. Findings of the DEPS study suggest a linear association between temperature decreases or ozone increases and endothelial function in diabetic individuals. We conclude that endothelial dysfunction might be one possible biological pathway explaining the association between environmental changes and cardiovascular events that have been observed worldwide.

7 Dissemination and policy implications within the UFIREG project

Prevention and reduction of air pollution demand action particularly by cities, national and international policy makers in the transport sector as well as energy waste management, buildings and agriculture (1). One of the main objectives of the EU-project UFIREG was to increase the knowledge base on the health effects of UFP and to integrate the results into regional political decision-making. Despite a growing body of scientific literature, evidence on the health effects of UFP is still not sufficient to set recommendations about exposure limits to be considered in European air quality guidelines. It is important to integrate UFP into routine measurement networks worldwide to provide data for further epidemiological studies on the health effects of UFP.

Stakeholders and partner cities were involved in the project in order to support and disseminate relevant information of the project. Stakeholders were invited to participate in partner meetings once a year and were involved in discussions on the progress of the project. Every project partner recruited a partner city where one partner city meeting took place within the project period. I wrote letter of intents in order to recruit partner cities and stakeholders for our study group of the Helmholtz Zentrum München. Together with my colleagues, I organized a partner city meeting in Ulm that took place on 23rd of October 2013. At the partner city meetings, the UFIREG project was introduced and related research questions were discussed with environmental and public health agencies, city administration institutions, environmental and automobile associations, health insurance companies, local politicians and citizens. Dissemination of the relevant information was a very important part of the project in order to strengthen the awareness of the population to the adverse health effects of air pollution. At the end of the project, dissemination material was developed to address stakeholders, policy makers, scientists and the general population. I played a significant role in the development of dissemination material. For example, I developed first drafts of a flyer for stakeholders and policy makers and I wrote the first draft of the handbook, which summarized the main results of the UFIREG project. Moreover, I contributed to all further project reports and wrote a first version of the epidemiological part of the environmental health report, which was the main scientific report of the project showing the most important measurement and epidemiological results.

Stakeholders and policy makers play an important role in encouraging efforts for routine UFP measurements and conduction of further multicenter epidemiological studies such as UFIREG. Moreover, stakeholders and policy makers can help to support mitigation strategies such as low-emission zones, encouragement of physical and public transport and alternative energy sources for vehicles. The potential effects of UFP on health remains an important public health problem that needs further investigation in different regions worldwide. The creation of so-called supersites or special measurement sites at carefully chosen spots in a city, which provide novel exposure parameters and metrics, should be considered (53). Moreover, larger and more specific multi-city studies and longer study periods are needed to produce powerful results.

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9 Publications

9.1 Associations between ultrafine and fine particles and mortality in five central European cities – Results from the UFIREG study

Authors: Stefanie Lanzinger, Alexandra Schneider, Susanne Breitner, Massimo Stafoggia, Ivan Erzen, Miroslav Dostal, Anna Pastorkova, Susanne Bastian, Josef Cyrys, Anja Zscheppang, Tetiana Kolodnitska, Annette Peters for the UFIREG study group

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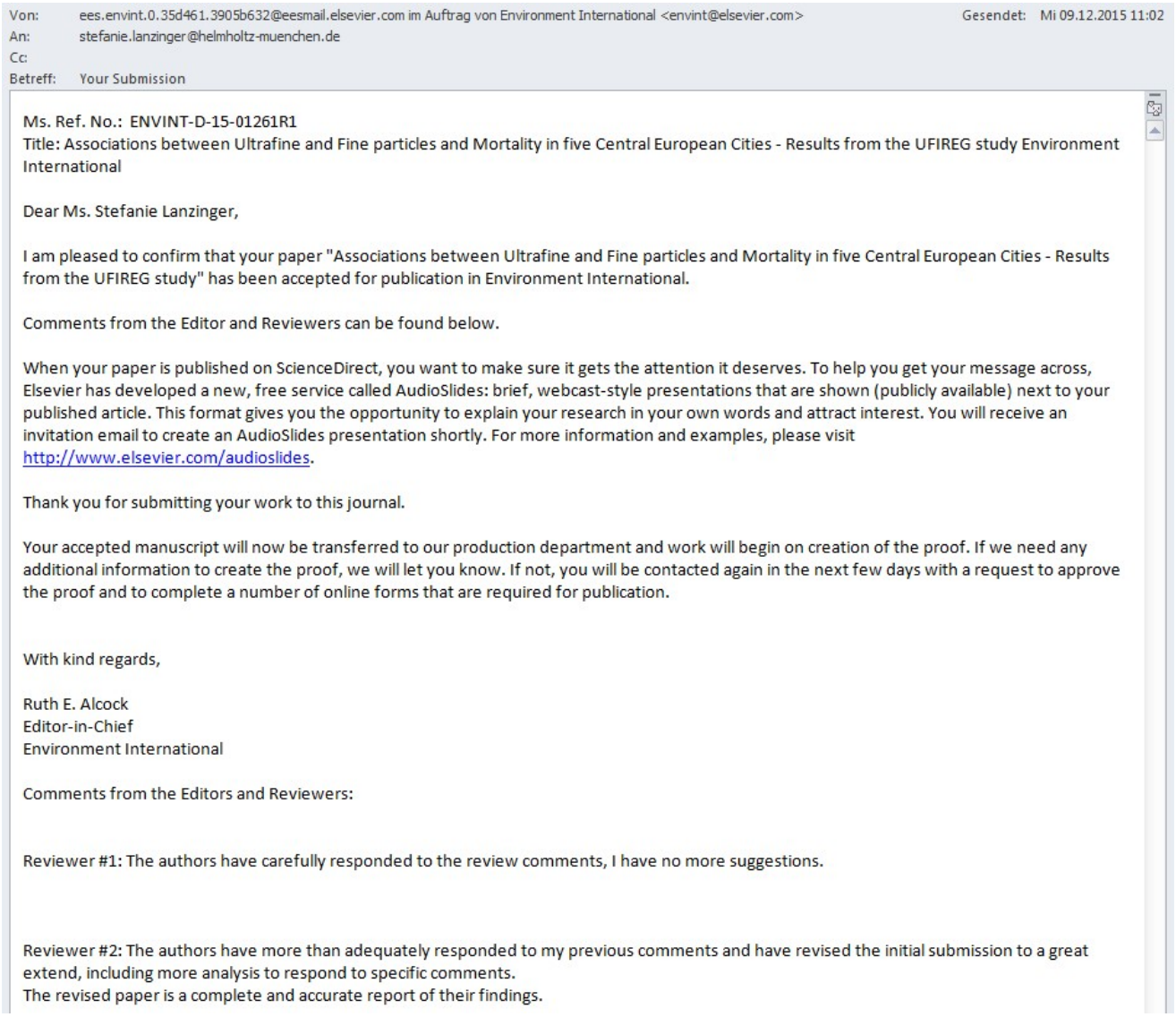

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Dear Ms. Stefanie Lanzinger,

I am pleased to confirm that your paper "Associations between Ultrafine and Fine particles and Mortality in five Central European Cities - Results from the UFIREG study" has been accepted for publication in Environment International.

Comments from the Editor and Reviewers can be found below.

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With kind regards,

Ruth E. Alcock
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Comments from the Editors and Reviewers:

Reviewer #1: The authors have carefully responded to the review comments, I have no more suggestions.

Reviewer #2: The authors have more than adequately responded to my previous comments and have revised the initial submission to a great extend, including more analysis to respond to specific comments.
The revised paper is a complete and accurate report of their findings.

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Associations between ultrafine and fine particles and mortality in five central European cities — Results from the UFIREG study

Stefanie Lanzinger [a,*], Alexandra Schneider [a], Susanne Breitner [a], Massimo Stafoggia [b], Ivan Erzen [c], Miroslav Dostal [d], Anna Pastorkova [d], Susanne Bastian [e], Josef Cyrys [a,f], Anja Zscheppang [g], Tetiana Kolodnitska [h], Annette Peters [a], for the UFIREG study group

[a] Helmholtz Zentrum München–German Research Center for Environmental Health, Institute of Epidemiology II, Neuherberg, Germany
[b] Department of Epidemiology, Lazio Region Health Service, Rome, Italy
[c] National Institute of Public Health Slovenia, Ljubljana, Slovenia
[d] Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[e] Saxon State Office for Environment, Agriculture and Geology, Dresden, Germany
[f] Environmental Science Center, University of Augsburg, Augsburg, Germany
[g] Research Association Public Health Saxony and Saxony-Anhalt, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[h] L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Chernivtsi, Ukraine

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ABSTRACT

Background: Evidence on health effects of ultrafine particles (UFP) is still limited as they are usually not monitored routinely. The few epidemiological studies on UFP and (cause-specific) mortality so far have reported inconsistent results.
Objectives: The main objective of the UFIREG project was to investigate the short-term associations between UFP and fine particulate matter (PM) < 2.5 μm ($PM_{2.5}$) and daily (cause-specific) mortality in five European Cities. We also examined the effects of PM < 10 μm (PM_{10}) and coarse particles ($PM_{2.5-10}$).
Methods: UFP (20–100 nm), PM and meteorological data were measured in Dresden and Augsburg (Germany), Prague (Czech Republic), Ljubljana (Slovenia) and Chernivtsi (Ukraine). Daily counts of natural and cardiorespiratory mortality were collected for all five cities. Depending on data availability, the following study periods were chosen: Augsburg and Dresden 2011–2012, Ljubljana and Prague 2012–2013, Chernivtsi 2013–March 2014. The associations between air pollutants and health outcomes were assessed using confounder-adjusted Poisson regression models examining single (lag 0–lag 5) and cumulative lags (lag 0–1, lag 2–5, and lag 0–5). City-specific estimates were pooled using meta-analyses methods.
Results: Results indicated a delayed and prolonged association between UFP and respiratory mortality (9.9% [95%-confidence interval: −6.3%; 28.8%] increase in association with a 6-day average increase of 2750 particles/cm^3 (average interquartile range across all cities)). Cardiovascular mortality increased by 3.0% [−2.7%; 9.1%] and 4.1% [0.4%; 8.0%] in association with a 12.4 μg/m^3 and 4.7 μg/m^3 increase in the $PM_{2.5}$- and $PM_{2.5-10}$-averages of lag 2–5.
Conclusions: We observed positive but not statistically significant associations between prolonged exposures to UFP and respiratory mortality, which were independent of particle mass exposures. Further multi-centre studies are needed investigating several years to produce more precise estimates on health effects of UFP.

1. Introduction

Epidemiological short-term studies on ultrafine particles (UFP) with a diameter < 100 nm (0.1 μm) and mortality are still rare, whereas a large number of studies investigated the effects of particulate matter (PM) with an aerodynamic diameter < 10 μm (PM_{10}) or <2.5 μm ($PM_{2.5}$, fine particles) (Atkinson et al., 2014; Rückerl et al., 2011). Most of the studies focused on the effects of fine particles on all-cause mortality and mortality due to cardiovascular and respiratory causes (Atkinson et al., 2014; Rückerl et al., 2011). A review by Atkinson et al. (2014) reported a 1.0% [95%-confidence interval: 0.5%; 1.6%] increase in all-cause mortality in association with a 10 μg/m^3 increase in $PM_{2.5}$ based on 23 estimates, but with substantial regional variation. The effect

* Corresponding author at: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology II, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany.
E-mail address: stefanie.lanzinger@helmholtz-muenchen.de (S. Lanzinger).

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estimates associated with $PM_{2.5}$ on respiratory mortality were stronger (1.5% [1.0%; 2.0%]) than estimates for cardiovascular mortality (0.8% [0.4%; 1.3%]).

Due to their small size and little mass the deposition and clearance of UFP in the respiratory tract differ from larger particles (Kreyling et al., 2006). Because of the differences in deposition and the potential for translocation as well as their huge active surface, effects of UFP might be at least partly independent from those of larger particles such as PM_{10} and $PM_{2.5}$ (Brook et al., 2004; HEI, 2013; Peters et al., 2011; Rückerl et al., 2011). So far, experimental studies do not provide sufficient evidence to confirm this hypothesis. Further, there is suggestive, but not consistent epidemiological evidence on the association between short-term exposures to UFP and cardiorespiratory health (HEI, 2013; WHO, 2013a). Moreover, hardly any epidemiological studies of long-term exposures to ambient UFP have been conducted yet (Ostro et al., 2015).

The few epidemiological short-term studies on UFP and (cause-specific) mortality so far have reported inconsistent results (HEI, 2013). One of the first studies on health effects of UFP reported 1-day delayed increases in respiratory mortality (15.5% [5.5%; 26.4%]) and 4-days delayed increases in cardiovascular mortality (5.1% [−1.0%; 11.5%]) in association with an interquartile range (IQR) increase in UFP (12,680 particles/cm^3) (Wichmann et al., 2000). Increases in natural and cardiorespiratory mortality with a delay of at least two days in association with UFP increases were also found in other analyses (Breitner et al., 2009; Breitner et al., 2011; Stolzel et al., 2007). However, shorter time lags were also reported (Atkinson et al., 2010; Forastiere et al., 2005). In a study conducted in London an IQR increase of 10,166 particles/cm^3 in total particle number concentration (PNC) was associated with increases in all-cause mortality (1.4% [0.5%; 2.4%]), cardiovascular mortality (2.2% [0.6%; 3.8%]) and respiratory mortality (2.3% [−0.1%; 4.8%]) with a 1-day delay, while no associations were found for other time lags (Atkinson et al., 2010). Moreover, two studies conducted in Helsinki and Prague studying the association between PNC in different size ranges and (cause-specific) mortality found only weak or no associations (Branis et al., 2010; Halonen et al., 2009).

The project "Ultrafine particles — an evidence based contribution to the development of regional and European environmental and health policy" (UFIREG) had the goal to monitor UFP with the same instrumentation and assess the short-term health effects of ultrafine and fine particles on daily (cause-specific) mortality in time-series analyses. So far, European studies on short-term associations between UFP and mortality were primarily focused on Western European countries (HEI, 2013). However, the UFIREG project involved cities from Central and Eastern European countries using harmonised exposure and epidemiological methodology in all cities. Five cities in four Central and Eastern European countries participated in the study: Augsburg and Dresden (Germany), Chernivtsi (Ukraine), Ljubljana (Slovenia) and Prague (Czech Republic). The UFIREG project started in July 2011 and ended in December 2014. We hypothesised that we would be able to observe independent associations of ultrafine PNC and fine particle mass concentrations on (cause-specific) mortality. Moreover, we also investigated PNC, PM_{10}, coarse particles with an aerodynamic diameter > 2.5 μm and < 10 μm ($PM_{2.5-10}$) and nitrogen dioxide (NO_2) as pollutants of secondary interest.

2. Methods

The study population comprised residents of Augsburg, Chernivtsi, Dresden, Ljubljana and Prague. Daily counts of (cause-specific) deaths were obtained from official statistics for each of the five cities. Only residents of a city who died in that city were considered. Infants younger than one year were excluded from the analyses. The causes of death are based on the International Statistical Classification of Diseases and Related Health Problems (ICD-10). Deaths due to natural causes (ICD-10: A00-R99) and deaths due to cardiovascular (ICD-10: I00-I99) and respiratory diseases (ICD-10: J00-J99) were considered. Mortality data for Augsburg and Dresden were obtained from the Research Data Centres of the Federal Statistical Office and the Statistical Offices of the Free States of Bavaria and Saxony, respectively. For Ljubljana, mortality data were obtained from the National Institute of Public Health in Slovenia. All data for Prague were provided by the Institute of Health Information and Statistics of the Czech Republic. For Chernivtsi, mortality data were provided by the Main Department of Statistics in Chernivtsi Region.

We also obtained information on additional variables for confounding adjustment, including indicator variables for weekdays and holidays, meteorological parameters (air temperature, relative humidity, barometric pressure), and – if available – influenza epidemics. Information on influenza epidemics in Augsburg and Dresden were provided by the German Influenza Working Group of the Robert Koch Institute (https://influenza.rki.de/Default.aspx). Data on influenza epidemics in Prague were obtained from the National Institute of Public Health in Prague and the Hygiene Station of the City of Prague. In Ljubljana, these data were provided by the National Institute of Public Health in Slovenia. No information on influenza epidemics was available in Chernivtsi. Sociodemographic data such as number of inhabitants (per age-group and sex), estimated percentage of smokers, population density or number of newborns and deceased persons was used to describe the population in the cities involved in the project. Data for Augsburg derived from the Statistical Yearbook of Augsburg. For Dresden, data were obtained from the census in 2011 and the Statistical Office of the Free State of Saxony. The Statistical Office of the Republic of Slovenia provided sociodemographic data for Ljubljana. Data for Prague were obtained from the Institute of Health Information and Statistics of the Czech Republic and the Czech statistical office. For Chernivtsi data derived from the Main Statistic Department in Chernivtsi Region.

Air pollution and meteorological parameters were measured on an hourly basis at local fixed measurement sites. The providers of air pollution and meteorological data are described elsewhere (UFIREG-report 2014). The measurement stations in Augsburg, Chernivtsi, Dresden and Ljubljana were located at urban background sites. The monitoring station in Prague was located at a suburban background site. Meteorological parameters included air temperature, relative humidity and barometric pressure. PM_{10}, $PM_{2.5}$, and NO_2 were measured in Augsburg, Dresden, Ljubljana and Prague. However, these parameters were not available in Chernivtsi. In Augsburg, Dresden, Ljubljana and Prague $PM_{2.5}$ and PM_{10} were measured at the same measurement site. $PM_{2.5-10}$ was calculated as the difference between site specific PM_{10} and $PM_{2.5}$. PNC were measured using custom-made mobility particle size spectrometers, either Differential or Scanning Mobility Particle Sizers. They enabled highly size-resolved PNC measurements in the range from 10 to 800 nm, except for Prague, where PNC were measured from 10 to 500 nm. The mobility particle size spectrometers delivered data in a 5- to 20-minute time-resolution. Hourly averages were calculated with a threshold of 75% data availability. The overall availability of PNC data reached more than 75% at all stations (UFIREG-report 2014). Imputation of hourly missing PM data was only possible for Augsburg and Prague where an additional urban background measurement station was available. Imputation was performed using a modified APHEA (Air Pollution and Health: A European Approach) procedure (Berglind et al., 2009; Katsouyanni et al., 1996). Missing hours of one monitor were imputed by a weighted average of the other monitor. If the respective hourly mean value was not available at both monitors, the average of the preceding and the following hourly means was used. Daily 24-hour averages of all air pollutants and meteorological parameters were only calculated if 75% of the hourly values were available.

An extensive quality assurance programme was an essential part of the high standards for data collection. It comprised staff training, an initial comparison of spectrometers in a laboratory, frequent on-site comparisons against reference instruments, remote monitoring, and automated function control units at two sites (Dresden and Chernivtsi).

The quality assurance programme showed that the deviation for particles smaller than 15 nm was between 20% and 60%. Therefore, the size class 10 to 20 nm was excluded from the epidemiological analysis. We investigated UFP in the size range 20 to 100 nm and PNC in the size range 20 to 800 (for Prague 20 to 500 nm) in all cities.

Depending on the start of the measurements and the availability of epidemiological data, the following study periods were chosen for the epidemiological analyses: Augsburg and Dresden: January 2011 to December 2012; Ljubljana and Prague: January 2012 to December 2013; Chernivtsi: January 2013 until March 2014. The period 2011 to 2012 for the German cities was chosen due to German data protection rules; data on (cause-specific) mortality of 2013 was not available by the end of the project period.

2.1. Statistical analysis

Spearman's rank correlation coefficient was used to calculate correlations between air pollution and meteorological parameters. The association between air pollutants and mortality was investigated using Poisson regression models allowing for overdispersion. In a first step, a basic confounder model was set up a priori for all cities based on a review of the current literature. The basic model included long-term time-trend, dummy variables for day of the week (Monday to Sunday), a dummy variable for public holidays referring to single days (holidays vs. non-holidays), a dummy variable for the decrease of the populations present in the city during longer vacation periods with high travel activities for example during school vacation (Christmas, Easter, summer vacation), a dummy variable for influenza epidemics (where available), air temperature (average of lags 0–1 [lag 0: same-day; lag 1: one day before the event] to represent effects of high temperatures and average of lags 2–13 [lag 2: two days prior to the event; lag 13: 13 days prior to the event] to represent effects of low temperatures), and relative humidity (average of lags 0–1 and average of lags 2–13). Penalised regression splines with natural cubic regression splines as smoothing basis were used to allow for non-linear confounder adjustment. The spline for time-trend was fixed to have four degrees of freedom per year to sufficiently represent long-term trend and seasonality since previous studies in Germany reported a negative residual autocorrelation when using more degrees of freedom per year (Peters et al., 2009). Splines for meteorological variables were fixed to three degrees of freedom. We performed single-lag models from lag 0 (same day of the event) up to lag 5 (five days prior to the event) to visually examine the lag structure of the association between particle exposures and health outcomes. Cumulative effect models were used representing immediate (2-day average: lag 0–1), delayed (average of lag 2–5) and prolonged effects (6-day average: lag 0–5).

City-specific effect estimates were combined with random-effects models. For each meta-analytical estimate, a χ^2-test for heterogeneity was performed and the corresponding p-value reported, together with the I^2-statistic, which represents the proportion of total variation in effect estimates that is due to between-cities heterogeneity. Cities were weighted according to the precision of the city-specific effect estimates. For pooling the city-specific estimates the maximum likelihood effects estimator after van Houwelingen was used (van Houwelingen et al., 2002). The analyses were conducted for all ages (increasing the statistical power of the analysis) as well as stratified for deaths among those below 75 years of age and above 75 years. Moreover, we conducted the analyses for females and males separately in order to test effect modification by sex. Effect modification by season (October–March vs. April–September) was analysed by including an interaction term in the model. We estimated two-pollutant models to assess interdependencies of UFP and $PM_{2.5}$ as well as UFP and NO_2 effects.

2.2. Sensitivity analyses

The sensitivity of the air pollution effects was assessed by re-running the above-described analyses with the following variations:

(1) Different values of smoothness for time-trend (6 and 3 degrees of freedom per year) and meteorological variables (5 degrees of freedom) were specified.
(2) Air temperature and relative humidity were replaced by apparent temperature, a combination of both. Apparent temperature was calculated using the following formula (Kalkstein and Valimont, 1986; Steadman, 1979): $at = -2.653 + (0.994 \times temp) + (0.0153 \times dp \times dp)$ with at = apparent temperature, temp = air temperature and dp = dew point temperature (Supplemental Material Formula (1)).
(3) Air pollution effects were adjusted for air temperature by using temperature above the median for heat effects and below the median for cold effects (Stafoggia et al., 2013).
(4) We adjusted for air temperature and relative humidity including the average of lag 0–1 and average of lag 2–5.
(5) Barometric pressure was additionally included in the models.
(6) Effect estimates for Augsburg and Prague were recalculated using a dataset with imputed missing data.
(7) We analysed air pollution effects using distributed lag non-linear models as described by Gasparrini (2011). We assessed up to 7 and 14 lags using a second- and third-degree polynomial. Results of polynomial distributed lag models were pooled according to Gasparrini et al. (2012).

Effects of UFP on mortality are presented as percent changes in mortality outcomes ((relative risk from Poisson regression models − 1) *100) per 2750 particles/cm^3 increase (average IQR across all five cities) in daily UFP. Effects of $PM_{2.5}$ on mortality are presented as percent changes in mortality outcomes per 12.4 μg/m^3 increase (average IQR across Augsburg, Dresden, Ljubljana and Prague) in daily $PM_{2.5}$. As pollutants of secondary interest, we also analysed effects of PNC (20–800 nm [20–500 nm in Prague]), PM_{10}, $PM_{2.5-10}$, and NO_2 on (cause-specific) mortality. Results of secondary pollutants are presented as percent changes in (cause-specific) mortality per 3675 particles/cm^3, 16 μg/m^3, 4.7 μg/m^3, and 15.4 μg/m^3 (average IQRs across all five cities) increase in PNC, PM_{10}, $PM_{2.5-10}$, and NO_2, respectively.

Data management was conducted using SAS statistical package (version 9.3; SAS Institute Inc., Cary, NC). Statistical analyses were performed using R project for statistical computing (version 2.15.3, http://www.r-project.org/) using the "mgcv", "splines", "dlnm","metafor" and "mvmeta" packages.

Table 1
Socio-demographical information of the five UFIREG cities.

City	Year	Population	City area (km^2)	Density of population[a]	Newborns	Deceased persons
Augsburg	2011	266,647	146.9	1815.8	2253	2820
	2012	272,699	146.9	1857.0	2465	2950
Chernivtsi	2013	258,371	153.0	1688.7	2751	2447
Dresden	2011	517,765	328.3	1577.1	5907	4772
	2012	525,105	328.3	1599.4	6001	5040
Ljubljana	2012	280,607	275.0	1020.4	3084	2272
	2013	282,994	275.0	1029.1	2982	2242
Prague	2012	1,246,780	496.2	2512.7	14,176	12,411
	2013	1,243,201	496.2	2505.4	13,867	12,149

[a] Inhabitants/km^2.

3. Results

A description of the UFIREG cities is shown in Table 1 and Supplemental Fig. 1 shows the location of the five cities in Central and Eastern Europe. Prague was the largest of the five cities with about 1.2 million inhabitants and an area of almost 500 km^2. Dresden was the second largest city in the UFIREG project with about 500,000 inhabitants within an area of more than 300 km^2. The number of inhabitants in Augsburg, Ljubljana, and Chernivtsi was comparable and ranged from about 260,000 to 280,000 inhabitants. Ljubljana, however, was larger than Augsburg and Chernivtsi with an area of 275 km^2. In all cities, except for Augsburg, the number of newborns was higher than the number of deceased persons during the respective study periods. The percentages of women and men were similar in all cities with about 52% women and 48% men. In Chernivtsi, 11% of the population were 65 years or older, whereas in the other cities the number of people aged 65 years or older ranged from 18% in Prague and Ljubljana to 20% and 22% in Augsburg and Dresden, respectively.

According to the WHO Report on the Global Tobacco Epidemic 2013, the Czech Republic showed the highest prevalence of tobacco smoking of countries within the study with 36.9% followed by the Ukraine with 28.8% in 2012 (WHO, 2013b). In the same year, the prevalence of tobacco smoking in Germany was 25.7% and in Slovenia 25.4%.

Table 2 shows a description of mortality outcomes by city for each year. In Augsburg, Dresden, Ljubljana, and Prague 40%–50% of natural death cases were due to cardiovascular diseases. In Chernivtsi, almost 70% of natural deaths were due to cardiovascular diseases in 2013. Chernivtsi was excluded from the analysis on respiratory mortality due to an insufficient number of respiratory death cases in the study period. Supplemental Table 1 presents a description of (cause-specific) mortality outcomes per 100,000 inhabitants.

A description of 24-hour averages of air pollution and meteorological variables by city is shown in Table 3. In Prague and Ljubljana UFP measurements started in mid-April 2012; hence, missing values of UFP were higher compared to the other air pollutants in these two cities. UFP were moderately correlated with PM_{10}, $PM_{2.5}$ and $PM_{2.5-10}$ (Spearman's rank correlation coefficient $0.3 \leq r_s \leq 0.5$) in all cities (Supplemental Table 2). Moreover, the correlation between air pollution and meteorological parameters was low to moderate ($r_s < 0.6$) in all cities. High correlations were observed between PM_{10} and $PM_{2.5}$ with $r_s = 0.9$ in Augsburg, Dresden, Ljubljana and Prague. The seasonal variation of UFP and $PM_{2.5}$ for each city separately is shown in Supplemental Fig. 2. Prague and Dresden showed higher UFP concentrations during summer compared to the other cities, whereas, Chernivtsi showed highest concentrations during autumn (Supplemental Fig. 2A). In all cities $PM_{2.5}$ concentrations were higher during winter compared to the other seasons (Supplemental Fig. 2B). Ljubljana showed the highest $PM_{2.5}$ concentrations in winter compared to the other cities.

Table 2
Description of (cause-specific) mortality outcomes by city.

City	Year	Population	Mean daily natural death counts (SD)	Mean daily cardiovascular death counts (SD)	Mean daily respiratory death counts (SD)
Augsburg[a]	2011	266,647	6.9 (2.5)	3.1 (1.7)	0.5 (0.8)
	2012	272,699	7.2 (2.8)	3.1 (1.7)	0.4 (0.6)
Chernivtsi	2013	258,371	6.3 (2.7)	4.3 (2.1)	0.1 (0.4)
Dresden[a]	2011	517,765	11.5 (3.4)	5.3 (2.3)	0.6 (0.9)
	2012	525,105	12.2 (3.7)	5.4 (2.4)	0.6 (0.8)
Ljubljana	2012	280,607	5.8 (2.5)	2.3 (1.5)	0.4 (0.6)
	2013	282,994	5.7 (2.4)	2.3 (1.5)	0.3 (0.5)
Prague	2012	1,246,780	27.1 (5.7)	13.7 (4.1)	1.5 (1.3)
	2013	1,243,201	26.5 (5.9)	12.8 (3.8)	1.7 (1.4)

Outcome definitions: natural causes ICD-10 A00-R99, cardiovascular diseases ICD-10 I00-I99, respiratory diseases ICD-10 J00-J99.
[a] Reference: Research Data Centres of the Federal Statistical Office and the Statistical Offices of the Länder, Death Statistics, 2011–2012, own calculations.

Table 3
Description of 24-hour averages of air pollution and meteorological variables by city.

City (study period)	N	Min	Median	Mean (SD)	Max	IQR[a]
Augsburg (2011–2012)						
Air temperature (°C)	720	−13.4	9.9	10.0 (8.0)	26.8	12.4
Relative humidity (%)	720	39.6	78.3	77.1 (13.0)	100	20.3
PM_{10}[b] (μg/m^3)	725	2.7	17.2	20.0 (12.5)	91.5	14.5
$PM_{2.5}$[c] (μg/m^3)	720	1.7	12.4	14.9 (9.8)	86.3	10.8
$PM_{2.5-10}$[d] (μg/m^3)	714	0.1	5.3	6.0 (4.2)	36.0	5.3
UFP[e] (n/cm^3)	712	1161	5172	5880 (3016)	28,800	3332
PNC[f] (n/cm^3)	712	1369	6409	7239 (3643)	29,470	4124
NO_2[h] (μg/m^3)	718	4.2	26.9	28.0 (11.8)	74.0	16.1
Chernivtsi (2013)						
Air temperature (°C)	291	−7.4	13.9	11.9 (8.2)	27.4	13.8
Relative humidity (%)	291	31.7	74	74.0 (15.6)	100	22.6
PM_{10}[b] (μg/m^3)	.	.	.	.	.	.
$PM_{2.5}$[c] (μg/m^3)	.	.	.	.	.	.
$PM_{2.5-10}$[d] (μg/m^3)						
UFP[e] (n/cm^3)	340	1769	5018	5511 (2614)	19,160	3324
PNC[f] (n/cm^3)	340	2212	6908	7775 (3782)	29,030	4325
NO_2[h] (μg/m^3)	.	.	.	.	.	.
Dresden (2011–2012)						
Air temperature (°C)	731	−13.4	11.7	11.7 (8.2)	29.6	12.8
Relative humidity (%)	731	36	69.6	69.5 (11.1)	94.3	16.7
PM_{10}[b] (μg/m^3)	726	2.2	16.5	20.9 (15.2)	103.5	14.3
$PM_{2.5}$[c] (μg/m^3)	720	1.5	11.6	16.2 (13.8)	95.7	13.1
$PM_{2.5-10}$[d] (μg/m^3)	717	0.0	4.3	4.7 (2.7)	21.6	3.0
UFP[e] (n/cm^3)	639	677	3752	4286 (2338)	14,440	2882
PNC[f] (n/cm^3)	639	855	5446	5851 (2902)	16,710	4068
NO_2[h] (μg/m^3)	719	3.9	20.4	22.3 (10.0)	67.3	12.9
Ljubljana (2012–2013)						
Air temperature (°C)	730	−8.8	12.2	11.7 (8.7)	29.4	14.0
Relative humidity (%)	731	37.8	74.3	73.8 (13.7)	97.5	23.6
PM_{10}[b] (μg/m^3)	682	3.0	20.0	24.9 (16.8)	130.0	18.0
$PM_{2.5}$[c] (μg/m^3)	694	3.4	16.5	20.7 (14.3)	114.8	14.4
$PM_{2.5-10}$[d] (μg/m^3)	646	0.0	3.9	5.0 (5.1)	29.8	5.8
UFP[e] (n/cm^3)	435	855	4400	4693 (1896)	13,920	1935
PNC[f] (n/cm^3)	435	1685	6071	6750 (3121)	24,360	2689
NO_2[h] (μg/m^3)	683	1.8	22.2	25.1 (14.8)	119.4	16.4
Prague (2012–2013)						
Air temperature (°C)	723	−13.7	9	9.2 (8.4)	27.2	13.1
Relative humidity (%)	704	40.8	78.2	77.3 (13.2)	98.9	20.4
PM_{10}[b] (μg/m^3)	681	5.1	22.2	26.2 (15.7)	100.9	17.2
$PM_{2.5}$[c] (μg/m^3)	612	1.6	13.1	16.2 (11.6)	78.8	11.4
$PM_{2.5-10}$[d] (μg/m^3)	579	1.7	9.2	9.8 (4.0)	44.6	4.6
UFP[e] (n/cm^3)	464	960	3797	4197 (2010)	14,960	2278
PNC[g] (n/cm^3)	464	1217	5417	5799 (2537)	16,950	3168
NO_2[h] (μg/m^3)	707	4.5	19.5	21.9 (11.7)	74.2	16.2

[a] Interquartile range.
[b] Particulate matter with a size range of <10 μm in aerodynamic diameter.
[c] Particulate matter with a size range of <2.5 μm in aerodynamic diameter.
[d] Coarse particles with a size range of 2.5–10 μm in aerodynamic diameter.
[e] Ultrafine particles with a size range of 0.02 to 0.1 μm in aerodynamic diameter (20–100 nm).
[f] Particle number concentration with a size range of 0.02 to 0.8 μm in aerodynamic diameter (20–800 nm).
[g] Particle number concentration with a size range of 0.02 to 0.5 μm in aerodynamic diameter (20–500 nm).
[h] Nitrogen dioxide.

3.1. Associations between air pollutants and (cause-specific) mortality

Most of the associations reported in this section were not statistically significant. However, we still interpret these findings as potentially important as results indicated delayed and prolonged associations between UFP and PM and cause-specific mortality. The strongest associations between air pollutants and mortality outcomes were observed for the cumulative lag periods. Table 4 shows percent changes in natural and cause-specific mortality in association with an average IQR increase in air pollutants for the 2-day average, the average of lag 2 to lag 5 and the 6-day average. Results on single time lags are presented in Supplemental Table 3. We observed no associations between UFP and natural

Table 4
Percent changes (95%-CI) in (cause-specific) mortality with each average IQR increase in air pollutants.

Association under investigation	IQR[a]	2-day average (lag 0–1)	Average of lag 2–5	6-day average (lag 0–5)
Natural mortality				
UFP[b] (n/cm^3)	2750	0.1 (−2.0; 2.4)	−1.2 (−4.0; 1.8)	−0.3 (−3.8; 3.2)
PNC[c] (n/cm^3)	3675	−0.2 (−2.4; 2.1)	−1.2 (−4.1; 1.8)	−0.6 (−4.0; 2.9)
$PM_{2.5}$ (μg/m^3)	12.4	−0.5 (−2.2; 1.2)	0.9 (−3.4; 5.4)*	0.3 (−3.7; 4.5)*
PM_{10} (μg/m^3)	16.0	−0.2 (−1.9; 1.5)	0.8 (−3.3; 5.1)*	0.8 (−3.6; 5.3)*
$PM_{2.5-10}$ (μg/m^3)	4.7	1.1 (−0.8; 3.0)	1.2 (−1.6; 4.0)	1.7 (−1.9; 5.4)*
NO_2 (μg/m^3)	15.4	0.5 (−1.6; 2.8)	0.1 (−3.4; 3.7)	0.4 (−3.1; 4.1)
Cardiovascular mortality				
UFP[b] (n/cm^3)	2750	−0.5 (−3.6; 2.8)	−0.5 (−5.3; 4.5)	−0.2 (−5.5; 5.4)
PNC[c] (n/cm^3)	3675	−0.7 (−3.9; 2.5)	−0.1 (−5.5; 5.6)	−0.1 (−5.8; 5.9)
$PM_{2.5}$ (μg/m^3)	12.4	−0.4 (−2.9; 2.2)	3.0 (−2.7; 9.1)*	1.6 (−2.8; 6.2)
PM_{10} (μg/m^3)	16.0	−0.3 (−2.7; 2.1)	3.2 (−2.4; 9.2)*	2.3 (−2.9; 7.8)*
$PM_{2.5-10}$ (μg/m^3)	4.7	0.9 (−2.3; 4.3)	4.1 (0.4; 8.0)	4.2 (−0.6; 9.1)
NO_2 (μg/m^3)	15.4	−1.1 (−4.3; 2.2)	2.2 (−3.4; 8.0)	0.8 (−3.7; 5.5)
Respiratory mortality				
UFP[b] (n/cm^3)	2750	3.7 (−5.8; 14.2)	8.5 (−4.8; 23.7)	9.9 (−6.3; 28.8)
PNC[c] (n/cm^3)	3675	1.5 (−8.0; 12.1)	5.8 (−6.4; 19.7)	5.6 (−8.3; 21.7)
$PM_{2.5}$ (μg/m^3)	12.4	−3.4 (−9.9; 3.6)	−0.9 (−8.1; 6.9)	−2.4 (−10.5; 6.4)
PM_{10} (μg/m^3)	16.0	−3.6 (−9.8; 3.0)	−3.6 (−10.4; 3.6)	−5.1 (−12.6; 3.1)
$PM_{2.5-10}$ (μg/m^3)	4.7	−2.2 (−9.3; 5.4)	−2.1 (−10.3; 6.7)	−4.1 (−13.1; 5.9)
NO_2 (μg/m^3)	15.4	2.7 (−6.1; 12.4)	−1.3 (−13.7; 12.9)	−1.2 (−12.8; 11.9)

[a] Average interquartile range across all cities.
[b] Ultrafine particles with a size range 20–100 nm.
[c] Particle number concentration with a size range 20–800 nm (for Prague 20–500 nm).
* Heterogeneity p-value < 0.1 and I^2 > 50%.

or cardiovascular mortality for all cities combined. However, results indicated delayed and prolonged effects of UFP on respiratory mortality. For example, the pooled effect estimate of respiratory mortality increased by 9.9% [−6.3%; 28.8%] in association with a 2750 particles/cm^3 increase in the 6-day average of UFP. Results of PNC were similar, showing delayed and prolonged effects on respiratory mortality.

We found no changes in natural and respiratory mortality in association with increases in $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$ for all cities combined. However, our findings pointed to delayed increases in cardiovascular mortality in association with IQR increases in the averages of lag 2 to lag 5 of $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$, respectively. A 12.4 μg/m^3 increase in the $PM_{2.5}$-average of lag 2 to lag 5 was associated with a 3.0% [−2.7%; 9.1%] increase in cardiovascular mortality. Results of PM_{10} were similar. However, we also found heterogeneity in the pooled effect estimates of $PM_{2.5}$ as well as PM_{10} and cardiovascular mortality. A 4.7 μg/m^3 increase in the $PM_{2.5-10}$ average of lag 2 to lag 5 led to a significant increase in cardiovascular mortality by 4.1% [0.4%; 8.0%]. There was no association between increases in NO_2 and mortality outcomes.

City-specific and pooled effect estimates of respiratory mortality with increases in the 6-day average of UFP and PNC for Augsburg, Dresden, Ljubljana and Prague are presented in Fig. 1A. All cities except Ljubljana showed (slight) increases in the relative risk of respiratory mortality in association with UFP; Dresden showed the strongest effect. Results of PNC and respiratory mortality were similar.

Fig. 1B shows city-specific and pooled effect estimates for the $PM_{2.5}$-, PM_{10}- and $PM_{2.5-10}$-averages of lag 2 to lag 5 and cardiovascular mortality. Chernivtsi was excluded here since PM data were not available. Augsburg showed a statistically significant increase in deaths due to cardiovascular diseases in association with increases in $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$; whereas Dresden showed decreases in the relative risk of

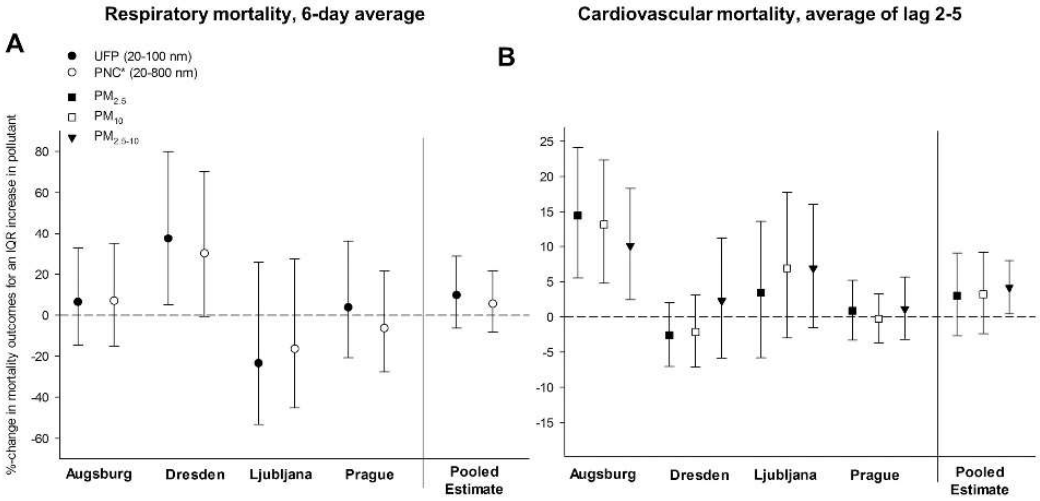

Fig. 1. A) Percent change in the city-specific and pooled effect estimates of respiratory mortality with each IQR increase in UFP and PNC, 6-day average. B) Percent change in the city-specific and pooled effect estimates of cardiovascular mortality with each IQR increase in $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$, average of lag 2–5. *Prague: PNC 20–500 nm.

cardiovascular mortality with increases in $PM_{2.5}$ and PM_{10}. Non-significant but positive effect estimates were found for Ljubljana. Prague showed no association between $PM_{2.5}$, $PM_{2.5-10}$ or PM_{10} and cardiovascular mortality.

Moreover, city-specific effect estimates for Augsburg showed a significant five-days delayed increase in cardiovascular mortality by 6.0% [1.0%; 11.4%] in association with an IQR increase in UFP (Supplemental Fig. 3).

Effect modification by age, sex and season were only investigated for respiratory mortality and the 6-day average of UFP and for cardiovascular mortality and the $PM_{2.5}$-average of lag 2 to lag 5 since UFP and $PM_{2.5}$ were of primary interest and strongest effects were observed for these cumulative lags. Effects of UFP and $PM_{2.5}$ were not significantly modified by age, sex and season (Supplemental Table 4). However, effects of UFP as well as $PM_{2.5}$ seemed to be driven by the older age group and females. But, due to limited power the results of effect modification analyses should be interpreted with caution.

3.2. Sensitivity analyses and two-pollutant models

Table 5 shows the results of the sensitivity analyses for respiratory mortality and the 6-day average of UFP and cardiovascular mortality and the $PM_{2.5}$-average of lag 2 to lag 5.

(1) Increasing the degrees of freedom for the smooth function of trend decreased the pooled effect estimate for UFP and respiratory mortality and to a lower extent the pooled effect estimate for $PM_{2.5}$ and cardiovascular mortality. Using fewer degrees of freedom for the trend did not change the effect estimates.
(2) Increasing the degrees of freedom for smooth functions of air temperature and relative humidity slightly increased the pooled effect estimate for UFP and respiratory mortality, whereas weakened the association between $PM_{2.5}$ and cardiovascular mortality.
(3) Replacing air temperature and relative humidity by apparent temperature in the model slightly decreased the pooled effects of UFP on respiratory mortality and marginally increased the pooled effects of $PM_{2.5}$ on cardiovascular mortality.
(4) Adjusting for air temperature by using temperature above the median for heat effects and below the median for cold effects strengthened the association between UFP and respiratory mortality. Pooled effect estimates for $PM_{2.5}$ and cardiovascular mortality decreased when using this method.
(5) Adjusting for air temperature and relative humidity average of lag 0–1 and average of lag 2–5 strengthened the association between UFP and respiratory mortality; whereas, weakened the association between $PM_{2.5}$ and cardiovascular mortality.
(6) Additionally adjusting for barometric pressure decreased the pooled effect estimate of respiratory mortality in association with UFP increases. The association between $PM_{2.5}$ and cardiovascular mortality slightly increased when barometric pressure was included in the model.
(7) Effect estimates for Augsburg and Prague did not change when the data set with imputed missing data was used (data not shown).
(8) Results of second- and third-degree polynomial distributed lag models supported delayed and prolonged associations between UFP and $PM_{2.5}$ and cardio-respiratory mortality (Supplemental Figs. 4 and 5).

Table 5
Sensitivity analyses, percent change (95%-CI) in respiratory mortality per IQR increase in UFP and percent change in cardiovascular mortality per IQR increase in $PM_{2.5}$.

Sensitivity analysis	UFP[a] and respiratory mortality (6-day average)	$PM_{2.5}$[b] and cardiovascular mortality (average of lag 2–5)
Original model	9.9 (−6.3; 28.8)	3.0 (−2.7; 9.1)
More DF[c] (DF = 6 df/year) for smooth function of trend	5.6 (−9.0; 22.4)	2.6 (−3.9; 9.7)
Fewer DF[c] (DF = 3 df/year) for smooth function of trend	9.9 (−9.3; 33.1)	2.9 (−2.7; 8.9)
More DF[c] (DF = 5) for smooth functions of meteorological variables	10.9 (−5.9; 30.6)	1.9 (−2.5; 6.5)
Use of apparent temperature	7.5 (−10.7; 29.4)	3.4 (−2.6; 9.7)
Adjusting for air temperature by using temperature above the median for heat effects and below the median for cold effects	14.1 (−2.1; 32.9)	1.3 (−2.1; 4.8)
Adjusting for air temperature and relative humidity average of lag 0–1 and average of lag 2–5	11.8 (−6.7; 34.0)	2.1 (−3.9; 8.4)
Inclusion of barometric pressure	4.1 (−14.7; 27.0)	3.2 (−3.4; 10.2)

Average interquartile range for UFP across all cities: 2750 particles/cm^3.
Average interquartile range for $PM_{2.5}$ across all cities: 12.4 $\mu g/m^3$.
[a] Ultrafine particles with a size range of 0.02 to 0.1 μm in aerodynamic diameter (20–100 nm).
[b] Particulate matter with a size range of <2.5 μm in aerodynamic diameter.
[c] Degrees of freedom.

We analysed two-pollutant models for $PM_{2.5}$ and UFP as well as for UFP and NO_2 in order to test interdependencies of the pollutants. UFP and $PM_{2.5}$ were moderately correlated in all cities with $r_s \leq 0.4$ (Supplemental Table 2). The correlation between UFP and NO_2 was also moderate in all cities with $r_s \leq 0.5$. Since we did not find an association between $PM_{2.5}$ or UFP and natural mortality, we only calculated two-pollutant models for cause-specific mortality. The association between UFP and cause-specific mortality was similar in single- and two-pollutant models with $PM_{2.5}$ (Fig. 2). Including NO_2 strengthened the decrease in cardiovascular and the increase in respiratory mortality in association with UFP. The association between $PM_{2.5}$ and cause-specific mortality was similar in single- and two-pollutant models with UFP.

4. Discussion

4.1. Summary

We observed positive but not statistically significant associations between exposures to UFP and respiratory mortality. Our results indicated a delayed and prolonged association between UFP and respiratory mortality (9.9% [−6.3%; 28.8%] increase in association with a 2750 particles/cm^3 increase in the 6-day average of UFP). Effect estimates for PNC and respiratory mortality were weaker, but consistent. Moreover, findings pointed to a delayed increase in cardiovascular mortality (3.0% [−2.7%; 9.1%]) per 12.4 $\mu g/m^3$ increment in the $PM_{2.5}$-average of lag 2 to lag 5. Results of PM_{10} were similar, but we observed heterogeneity in the pooled effect estimates of $PM_{2.5}$ as well as PM_{10} and cardiovascular mortality. A 4.7 $\mu g/m^3$ increase in the $PM_{2.5-10}$-average of lag 2 to lag 5 led to a significant increase by 4.1% [0.4%; 8.0%] in cardiovascular mortality.

4.2. Associations between air pollutants and (cause-specific) mortality

Short-term exposure to fine particles has been shown to be associated with natural all-cause and cause-specific mortality (Rückerl et al., 2011; WHO, 2013a). For example, studies conducted in 112 U.S. cities (Zanobetti and Schwartz, 2009) or in 10 areas of the European Mediterranean Region (Samoli et al., 2013) found increases in natural mortality of 1.0% and 0.6% per 10 $\mu g/m^3$ increment in $PM_{2.5}$, respectively. A recent meta-analysis by Atkinson et al. (2014) based on estimates from single-city and multi-city studies worldwide confirmed previous findings. They reported increases of 1.0% in all-cause and 1.5% in respiratory

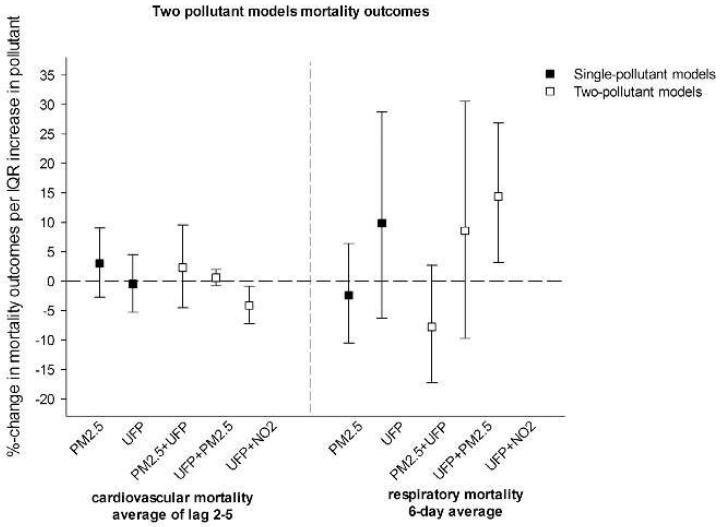

Fig. 2. Percent change in cause-specific mortality per IQR increase in air pollutants using single- and two-pollutant models. $PM_{2.5}$: main effects of $PM_{2.5}$, UFP: main effects of UFP, $PM_{2.5}$ + UFP: effects of $PM_{2.5}$ adjusted for UFP, UFP + $PM_{2.5}$: effects of UFP adjusted for $PM_{2.5}$, UFP + NO_2: effects of UFP adjusted for NO_2.

mortality in association with a 10 μg/m^3 increase in $PM_{2.5}$. In contrast to these studies we did not observe an association between $PM_{2.5}$ and natural or respiratory mortality as other single-city studies conducted in Prague or Erfurt, Germany, reported (Branis et al., 2010; Peters et al., 2009). However, it is important to point out that the confidence intervals are wide and do include the estimates obtained in meta-analyses.

Moreover, Atkinson et al. (2014) reported a summary increase in cardiovascular mortality by 2.3% [1.2%; 3.3%] per 10 μg/m^3 increment in $PM_{2.5}$ for the European Region based on estimates from studies conducted in Austria, the Czech Republic, France, Spain and the UK. We observed a similar increase in cardiovascular mortality (pooled effect estimate of 2.4% [−2.3%; 7.3%]) in association with the same increment in $PM_{2.5}$. However, studies conducted in Prague or London, UK, reported no evidence of associations between $PM_{2.5}$ and cardiovascular mortality (Atkinson et al., 2010; Branis et al., 2010). Comparing our results to a national U.S. analysis, our findings for $PM_{2.5}$ and cardiovascular mortality are comparable to U.S. regions with a dry climate as New Mexico, Arizona and Nevada (lag 0–1: 3.1% [−0.02%; 6.3%]) and to U.S. regions with a dry together with continental climate as Montana, Idaho, Wyoming, Utah and Colorado (lag 0–1: 1.7% [−0.8%; 4.2%]) (Zanobetti and Schwartz, 2009). However, we have found strongest associations for the $PM_{2.5}$-average exposure of lag 2–5, whereas no association for the 2-day average of lag 0–1. Other studies have found an association for the 2-day average (Zanobetti and Schwartz, 2009), but also for the average of lag 2–5 and the 6-day average exposure (Samoli et al., 2013). For example Samoli et al. (2013) observed an increase in the association between $PM_{2.5}$ and cardiovascular mortality with longer cumulative lag periods. Furthermore, we observed an increase in cardiovascular mortality with an increase in the $PM_{2.5-10}$-average of lag 2–5. Zanobetti and Schwartz (2009) also reported an association between $PM_{2.5-10}$ and cardiovascular mortality in 47 U.S. cities and Samoli et al. (2013) observed a non-significant but positive association between $PM_{2.5-10}$ and cardiovascular mortality in the European Mediterranean Region. Moreover, in general there is growing evidence on the association between $PM_{2.5-10}$ and natural and cause-specific mortality (Adar et al., 2014).

Evidence from epidemiological studies on UFP and mortality is still limited. A small number of studies on UFP and cause-specific mortality also reported increases in the risk of respiratory mortality (Atkinson et al., 2010; Stolzel et al., 2007; Wichmann et al., 2000). Wichmann et al. (2000) observed a significant increment of 15.5% [5.5%; 26.4%] in respiratory mortality per IQR increase in UFP of 12,690 particles/cm^3 with a delay of one day in Erfurt. Associations between UFP and respiratory mortality in Erfurt were also shown by Stolzel et al. (2007) for an extended period of the previous study by Wichmann et al. (2000). An IQR increase of 9748 particles/cm^3 in UFP led to an immediate increase of 5.0% [−1.9%; 12.3%] and to a one-day delayed increase of 5.3% [−1.4%; 12.4%] in the relative risk of respiratory mortality. However, in our study the association between UFP and respiratory mortality was more delayed compared to previous studies. We observed a 9.9% [−6.3%; 28.8%] increase in respiratory mortality in association with a 2750 particles/cm^3 increase in the 6-day average of UFP. Studies also reported increases in all-cause or natural as well as cardiovascular deaths in association with increases in UFP or different size ranges of PNC (Atkinson et al., 2010; Breitner et al., 2009; Breitner et al., 2011; Forastiere et al., 2005; Stolzel et al., 2007; Wichmann et al., 2000). In contrast to previous studies, our pooled effect estimates did not show any associations. However, city-specific effect estimates for Augsburg showed five-days delayed effects of UFP on cardiovascular mortality (6.0% [1.0%; 11.4%]). We assume that our non-significant results for the other cities might be at least partly due to missing data and insufficient statistical power. However, Branis et al. (2010) also found no associations between PNC and total, cardiovascular and respiratory mortality in Prague. Moreover, a Finish study conducted in Helsinki reported only weak associations between PNC in the size range 30–100 nm and cause-specific mortality (Halonen et al., 2009).

We found heterogeneous effects of $PM_{2.5}$ and PM_{10} on cardiovascular mortality between the cities, in particular between Augsburg and Dresden. While Augsburg showed a significant increase in the relative risk of cardiovascular mortality with increases in $PM_{2.5}$ and PM_{10}, Dresden showed negative effect estimates. The negative effects in Dresden were more pronounced during April–September but were also there during the colder period from October–March. There are no plausible biological mechanisms explaining a protective effect of $PM_{2.5}$ and PM_{10} on the cardiovascular system, the heterogeneous findings might be due to different sources and compositions of $PM_{2.5}$ and PM_{10}. $PM_{2.5}$ and PM_{10} might be influenced by a local source that could be more pronounced in Dresden compared to the other cities. Additional analyses on the source apportionment are necessary to support this assumption. Moreover, the air mass origin might also play a role in the heterogeneity

of the results. Nevertheless, we cannot exclude uncontrolled residual confounding or chance as possible explanations especially since we observed no heterogeneous effects of UFP.

However, the seasonal variation of UFP differed between the cities. New particle formation events during summer were observed particularly in Dresden and Prague which might be an explanation for the high UFP concentrations during summer in these two cities. Moreover, leaf burning might lead to the high UFP levels in autumn in Chernivtsi. The high $PM_{2.5}$ concentration in Ljubljana during winter might be explained by temperature inversion.

4.3. Plausible biological mechanisms

Fine and ultrafine particles can cause oxidative stress in the lungs which can further lead to lung inflammation (Brook et al., 2010; Newby et al., 2015; Rückerl et al., 2011). Oxidative stress has also been suggested to play a role in the development of certain lung diseases as asthma (Mazzoli-Rocha et al., 2010). In general, the extrapulmonary effects of PM and UFP are explained by three pathways. First, systemic oxidative stress and inflammation may be caused by the release of pro-inflammatory mediators or vasculoactive molecules from lung cells upon fine and UFP exposure. This may lead to a change in vascular tone (endothelial dysfunction), adverse cardiac outcomes, and a pro-coagulation state with thrombus formation and ischemic response as well as promotion of atherosclerotic lesions as suggested by Utell et al. (2002). Second, an imbalance of the autonomic nervous system or heart rhythm may occur due to fine and UFP deposited in the pulmonary tree. These effects can be either triggered directly, by stimulating pulmonary neural reflexes (Widdicombe and Lee, 2001), or indirectly, by provoking oxidative stress and inflammation in the lung. Third, translocation of UFP and PM constituents into the blood may cause endothelial dysfunction and vasoconstriction, increased blood pressure and platelet aggregation (Brook et al., 2010; Rückerl et al., 2011). Once in the circulation, UFP might also have direct effects on the heart and other organs.

It is assumed that the three pathways do not act independently. Moreover, it is very likely that UFP are also linked to different biological pathways than fine particles because of the different deposition pattern and the fact that UFP are not well recognized and cleared by the immune system and can escape natural defence mechanisms. In contrast to larger particles, UFP have a higher biological reactivity and surface area and by reaching the bloodstream UFP can be transported to other organs (Brook et al., 2004; HEI, 2013; Rückerl et al., 2011; WHO, 2013a). However, we observed UFP effects rather on the respiratory than cardiovascular system, whereas other studies found an association between UFP and cardiovascular health as well as UFP and respiratory health outcomes (Breitner et al., 2009; Breitner et al., 2011; Stolzel et al., 2007; Wichmann et al., 2000).

4.4. Strengths and limitations

The study presented here was a prospective planned multi-centre effort with the aim to provide two years of continuous time-series data. Within the framework of the UFIREG project the association between UFP and (cause-specific) mortality was investigated at multiple locations using the same UFP measurement device.

Compared to earlier studies in European cities we observed slightly lower PNC values, especially for Prague (Borsos et al., 2012; von Bismarck-Osten et al., 2013). However, it has to be mentioned that we excluded the size range 10–20 nm due to measurement uncertainties. Furthermore, a decreasing trend in PNC in European regions was observed during the last decades (Asmi et al., 2013; Birmili et al., 2015). In contrast to the other cities, the monitoring station in Prague was located at a suburban background site. In all the five cities, exposure was measured at one fixed monitoring site with the same instrumentation following joint standard operating procedures and quality measures. Exposure misclassification might be possible especially for UFP as it was shown that the spatial variability of UFP was higher than for fine particles. However, PNC showed high temporal correlations across different sites in the city area of Augsburg despite differing magnitudes in space (Cyrys et al., 2008). Birmili et al. (2013) reported low spatial variability in PNC among urban background stations in Dresden. Therefore, it is suggested that UFP exposure of the average population might be adequately characterized by one monitoring site in short-term effect studies like UFIREG if the fixed urban background station is chosen carefully (Cyrys et al., 2008). However, the real location of the stations is always a compromise between the requirements on the type of station and real possibilities as requirements for operation and connection to electricity. Moreover, UFP does not penetrate indoors well and hence indoor exposures may not be well presented (HEI, 2013).

There might be differences in coding of the primary causes of death that might explain the differences in death counts between the countries. For example, it might be possible that in Chernivtsi respiratory diseases were often coded as cardiovascular diseases explaining the low number of deaths due to respiratory diseases during the study period in Chernivtsi. With regard to health effects, different lifestyles might also play an important role. The prevalence of tobacco smoking was higher in Czech Republic and Ukraine compared to Germany and Slovenia (WHO, 2013b). It might be possible that air pollution plays a smaller role with regard to health effects in countries with higher smoking prevalence. Air pollution is responsible for 3.7 million premature deaths worldwide, whereas, tobacco causes 6 million deaths per year (WHO, 2013b).

Due to different starting dates of UFP measurements and because of a delayed availability of health data in Germany it was not possible to use the same analysing periods for all five cities. Moreover, for Chernivtsi only one full year could be investigated due to limited data availability. Due to the short study period we observed effect estimates with relatively large confidence intervals. Especially for investigating rare death cases in smaller cities such as Augsburg, Chernivtsi and Ljubljana it is important for future studies to use longer time periods to produce more precise effect estimates. In our study the small power is partly compensated by the multi-cities and meta-analysis design. Nevertheless, UFIREG was one of the very few multi-centre studies investigating the associations between UFP and fine particles and (cause-specific) mortality including cities from Central and Eastern European countries since most research activities so far were concentrated on Western European countries (HEI, 2013).

5. Conclusions

We observed positive but not statistically significant associations between prolonged exposures to UFP and respiratory mortality, which where independent of particle mass exposures. Effects of $PM_{2.5}$ on cardiovascular mortality were comparable to results from other European regions and U.S. regions with a dry climate and a dry together with continental climate (Atkinson et al., 2014; Zanobetti and Schwartz, 2009).

The study adds to the growing scientific literature on health effects of UFP and indicates that dedicated efforts are needed allowing time-series data collections over extended periods. Therefore, it is important to integrate UFP into routine measurement networks in order to provide data for future epidemiological studies especially those which will consider spatio-temporal variation in UFP.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.12.006.

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SUPPLEMENTAL MATERIAL

Dew point temperature (dp) was calculated as follows:

$$dp = \frac{1}{\frac{1}{temp + 241.413} - \frac{\log_{10}(\frac{rh}{100})}{1838.675}} - 241.413 \quad (1)$$

with temp= air temperature and rh=relative humidity.

Supplemental Table 1. Description of (cause-specific) mortality outcomes per 100,000 inhabitants.

City	Year	Population	Daily mean (SD)/100,000 inhabitants		
			Natural death counts	Cardiovascular death counts	Respiratory death counts
Augsburg	2011	266,647	2.6 (0.9)	1.2 (0.6)	0.2 (0.3)
	2012	272,699	2.6 (1.0)	1.1 (0.6)	0.1 (0.2)
Chernivtsi	2013	258,371	2.4 (1.0)	1.7 (0.8)	0.04 (0.2)
Dresden	2011	517,765	2.2 (0.7)	1.0 (0.4)	0.1 (0.2)
	2012	525,105	2.3 (0.7)	1.0 (0.5)	0.1 (0.2)
Ljubljana	2012	280,607	2.1 (0.9)	0.8 (0.5)	0.1 (0.2)
	2013	282,994	2.0 (0.8)	0.8 (0.5)	0.1 (0.2)
Prague	2012	1,246,780	2.2 (0.5)	1.1 (0.3)	0.1 (0.1)
	2013	1,243,201	2.1 (0.5)	1.0 (0.3)	0.1 (0.1)

outcome definitions:

natural causes ICD-10 A00-R99, cardiovascular diseases ICD-10 I00-I99, respiratory diseases ICD-10 J00-J99

Supplemental Table 2. Spearman's rank correlation coefficients for meteorological and air pollution parameters.

Augsburg (2011-2012)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}^{+}$ (µg/m³)	$PM_{2.5\text{-}10}$ (µg/m³)	UFP§ (n/cm³)	PNC# (n/cm^3)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.57	-0.19	-0.33	0.17	0.06	0.01	-0.53
Relative humidity (%)	-0.57	1.00	-0.03	0.11	-0.30	-0.37	-0.33	0.19
PM_{10}† (µg/m³)	-0.19	-0.03	1.00	0.93	0.78	0.43	0.54	0.70
$PM_{2.5}^{+}$ (µg/m³)	-0.33	0.11	0.93	1.00	0.53	0.37	0.49	0.73
$PM_{2.5\text{-}10}$" (µg/m³)	0.17	-0.30	0.78	0.53	1.00	0.45	0.50	0.44
UFP§ (n/cm³)	0.06	-0.37	0.43	0.37	0.45	1.00	0.99	0.51
PNC# (n/cm^3)	0.01	-0.33	0.54	0.49	0.50	0.99	1.00	0.58
NO_2& (µg/m³)	-0.53	0.19	0.70	0.73	0.44	0.51	0.58	1.00
Chernivtsi (2013)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}^{+}$ (µg/m³)	$PM_{2.5\text{-}10}$ (µg/m³)	UFP§ (n/cm³)	PNC# (n/cm^3)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.55	.	.	.	0.08	0.01	.
Relative humidity (%)	-0.55	1.00	.	.	.	-0.29	-0.19	.
PM_{10}† (µg/m³)	.	.	.	.	.	.	.	.
$PM_{2.5}^{+}$ (µg/m³)	.	.	.	.	.	.	.	.
$PM_{2.5\text{-}10}$" (µg/m³)	.	.	.	.	.	.	.	.
UFP§ (n/cm³)	0.08	-0.29	.	.	.	1.00	0.97	.
PNC# (n/cm^3)	0.01	-0.19	.	.	.	0.97	1.00	.
NO_2& (µg/m³)	.	.	.	.	.	.	.	.
Dresden (2011-2012)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}^{+}$ (µg/m³)	$PM_{2.5\text{-}10}$ (µg/m³)	UFP§ (n/cm³)	PNC# (n/cm^3)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.50	-0.28	-0.37	0.17	0.29	0.19	-0.42
Relative humidity (%)	-0.50	1.00	0.06	0.14	-0.28	-0.42	-0.35	0.28
PM_{10}† (µg/m³)	-0.28	0.06	1.00	0.97	0.58	0.37	0.56	0.68
$PM_{2.5}^{+}$ (µg/m³)	-0.37	0.14	0.97	1.00	0.40	0.30	0.50	0.68

$PM_{2.5-10}$" (µg/m³)	0.17	-0.28	0.58	0.40	1.00	0.51	0.58	0.37
UFP§ (n/cm³)	0.29	-0.42	0.37	0.30	0.51	1.00	0.96	0.33
PNC# (n/cm^3)	0.19	-0.35	0.56	0.50	0.58	0.96	1.00	0.45
NO_2& (µg/m³)	-0.42	0.28	0.68	0.68	0.37	0.33	0.45	1.00

Ljubljana (2012-2013)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}$‡ (µg/m³)	$PM_{2.5-10}$ (µg/m³)	UFP§ (n/cm³)	PNC# (n/cm^3)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.45	-0.44	-0.53	-0.06	-0.17	-0.22	-0.54
Relative humidity (%)	-0.45	1.00	0.06	0.16	-0.14	0.08	0.13	0.38
PM_{10}† (µg/m³)	-0.44	0.06	1.00	0.95	0.67	0.36	0.59	0.57
$PM_{2.5}$‡ (µg/m³)	-0.53	0.16	0.95	1.00	0.43	0.27	0.50	0.55
$PM_{2.5-10}$" (µg/m³)	-0.06	-0.14	0.67	0.43	1.00	0.46	0.55	0.40
UFP§ (n/cm³)	-0.17	0.08	0.36	0.27	0.46	1.00	0.95	0.54
PNC# (n/cm^3)	-0.22	0.13	0.59	0.50	0.55	0.95	1.00	0.62
NO_2& (µg/m³)	-0.54	0.38	0.57	0.55	0.40	0.54	0.62	1.00

Prague (2012-2013)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}$‡ (µg/m³)	$PM_{2.5-10}$ (µg/m³)	UFP§ (n/cm³)	PNC$ (n/cm^3)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.52	-0.19	-0.25	0.14	0.31	0.15	-0.46
Relative humidity (%)	-0.52	1.00	0.00	0.12	-0.16	-0.31	-0.18	0.35
PM_{10}† (µg/m³)	-0.19	0.00	1.00	0.96	0.77	0.29	0.54	0.68
$PM_{2.5}$‡ (µg/m³)	-0.25	0.12	0.96	1.00	0.61	0.25	0.50	0.66
$PM_{2.5-10}$" (µg/m³)	0.14	-0.16	0.77	0.61	1.00	0.40	0.56	0.43
UFP§ (n/cm³)	0.31	-0.31	0.29	0.25	0.40	1.00	0.94	0.26
PNC$ (n/cm^3)	0.15	-0.18	0.54	0.50	0.56	0.94	1.00	0.46
NO_2& (µg/m³)	-0.46	0.35	0.68	0.66	0.43	0.26	0.46	1.00

†particulate matter with a size range of <10 µm in aerodynamic diameter

‡particulate matter with a size range of <2.5 µm in aerodynamic diameter

"coarse particles with a size range of 2.5-10 µm in aerodynamic diameter

§ultrafine particles with a size range of 0.02 to 0.1µm in aerodynamic diameter (20-100 nm)

#particle number concentration with a size range of 0.02 to 0.8µm in aerodynamic diameter (20-800 nm)

$particle number concentration with a size range of 0.02 to 0.5µm in aerodynamic diameter (20-500 nm)

&nitrogen dioxide

Supplemental Table 3. Percent changes (95%-CI) in (cause-specific) mortality with each average IQR increase in air pollutants, single lags.

Association under investigation	IQR†	lag 0	lag 1	lag 2	lag 3	lag 4	lag 5
Natural mortality							
UFP (n/cm^3)	2,750	-0.8 (-2.6; 0.9)	0.9 (-0.8; 2.7)	-0.3 (-2.0; 1.4)	-0.6 (-2.9; 1.8)	-0.1 (-1.7; 1.6)	-0.4 (-2.0; 1.3)
PNC (n/cm3)	3,675	-0.9 (-2.8; 0.9)	0.6 (-1.2; 2.5)	-0.4 (-2.1; 1.4)	-0.5 (-2.8; 1.8)	0.0 (-1.7; 1.8)	-0.4 (-2.1; 1.3)
$PM_{2.5}$ (µg/m³)	12.4	-0.4 (-1.9; 1.2)	-0.3 (-1.9; 1.4)	0.3 (-2.1; 2.7)*	0.5 (-2.3; 3.4)*	0.1 (-2.3; 2.6)*	0.1 (-2.7; 3.0)*
PM_{10} (µg/m³)	16.0	0.1 (-1.6; 1.8)	-0.4 (-1.9; 1.2)	0.0 (-2.1; 2.1)	0.6 (-2.1; 3.4)*	0.0 (-2.1; 2.1)	0.4 (-2.7; 3.5)*
$PM_{2.5-10}$ (µg/m³)	4.7	1.2 (-0.4; 2.8)	0.3 (-1.3, 2.0)	0.7 (-0.9; 2.2)	0.2 (-1.4; 1.9)	0.7 (-1.1; 2.4)	1.1 (-1.4; 3.8)*
NO_2 (µg/m³)	15.4	0.6 (-1.3; 2.6)	0.5 (-1.4; 2.5)	-0.2 (-2.1; 1.7)	0.7 (-1.9; 3.4)	-0.8 (-2.9; 1.4)	0.0 (-2.2; 2.3)
Cardiovascular mortality							
UFP (n/cm^3)	2,750	-0.8 (-3.3; 1.7)	0.2 (-2.3; 2.9)	-0.2 (-2.7; 2.3)	0.3 (-2.4; 3.1)	0.2 (-2.2; 2.7)	0.1 (-3.2; 3.6)
PNC (n/cm3)	3,675	-1.1 (-3.7; 1.6)	0.0 (-2.6; 2.8)	-0.1 (-2.6; 2.5)	0.4 (-2.7; 3.7)	0.6 (-1.9; 3.2)	0.2 (-3.6; 4.1)*
$PM_{2.5}$ (µg/m³)	12.4	-0.7 (-2.9; 1.6)	0.2 (-2.0; 2.5)	1.3 (-1.2; 3.9)	1.7 (-1.0; 4.4)	1.5 (-2.2; 5.4)*	1.2 (-3.8; 6.4)*
PM_{10} (µg/m³)	16.0	-0.5 (-2.7; 1.7)	-0.2 (-2.2; 2.0)	0.5 (-1.5; 2.6)	2.2 (-0.9; 5.3)	1.4 (-1.8; 4.8)	1.5 (-3.3; 6.5)*
$PM_{2.5-10}$ (µg/m³)	4.7	0.3 (-2.0; 2.7)	0.8 (-2.1; 3.7)	0.9 (-1.4; 3.2)	2.3 (0.1; 4.6)	2.1 (-0.1; 4.4)	3.1 (-0.4; 6.7)*
NO_2 (µg/m³)	15.4	-0.7 (-3.5; 2.2)	-0.4 (-4.0; 3.4)	-0.0 (-2.8; 2.9)	1.9 (-1.3; 5.1)	0.1 (-4.0; 4.5)	1.3 (-2.9; 5.7)
Respiratory mortality							
UFP (n/cm^3)	2,750	0.2 (-7.3; 8.2)	4.5 (-4.1; 13.8)	-2.2 (-13.4; 10.4)*	3.3 (-4.3; 11.6)	5.5 (-1.7; 13.2)	5.5 (-1.6; 13.2)
PNC (n/cm3)	3,675	-0.4 (-8.0; 7.9)	2.5 (-5.9; 11.6)	-2.5 (-10.8; 6.5)	2.7 (-5.6; 11.7)	4.5 (-3.0; 12.6)	5.3 (-2.2; 13.4)
$PM_{2.5}$ (µg/m³)	12.4	-1.9 (-7.8; 4.3)	-3.1 (-9.0; 3.2)	-1.1 (-6.8; 5.0)	1.0 (-4.7; 7.1)	-3.1 (-8.6; 2.8)	0.9 (-4.9; 6.9)
PM_{10} (µg/m³)	16.0	-2.3 (-8.2; 3.9)	-4.2 (-9.7; 1.7)	-2.2 (-7.7; 3.6)	-0.9 (-6.5; 5.1)	-4.4 (-9.8; 1.2)	-1.3 (-6.8; 4.6)
$PM_{2.5-10}$ (µg/m³)	4.7	0.0 (-5.9; 6.2)	-2.5 (-8.4; 3.7)	-0.8 (-6.6; 5.4)	0.0 (-6.4; 6.8)	-0.8 (-6.6; 5.3)	-2.8 (-8.6; 3.5)
NO_2 (µg/m³)	15.4	2.3 (-5.4; 10.6)	2.2 (-5.7; 10.8)	-4.4 (-11.9; 3.6)	2.6 (-6.3; 12.4)	-1.8 (-11.0; 8.4)	-2.4 (-9.7; 5.4)

†average interquartile range across all cities, *heterogeneity p-value<0.1 and I^2>50%

Supplemental Table 4. Percent change (95%-CI) in respiratory mortality per IQR increase in UFP and percent change in cardiovascular mortality per IQR increase in $PM_{2.5}$, effect modification by age, sex and season.

	UFP** and respiratory mortality (6-day average)	$PM_{2.5}$† and cardiovascular mortality (average of lag2-5)
Main effect	9.9 (-6.3; 28.8)	3.0 (-2.7; 9.1)
Age		
<75 years	insufficient cases	0.2 (-5.8; 6.6)
≥75 years	8.3 (-11.6; 32.8)	3.1 (-2.5; 9.1)*
Sex		
females	8.8 (-28.1; 64.9)*	3.5 (-3.5; 11.0)*
males	1.9 (-14.9; 22.1)	0.3 (-3.9; 4.7)
Season		
October-March	5.2 (-9.2; 21.9)	2.8 (-2.9; 8.8)*
April-September	3.7 (-27.6; 48.7)*	3.2 (-4.0; 11.0)

Average interquartile range for UFP: 2,750 particles/cm^3

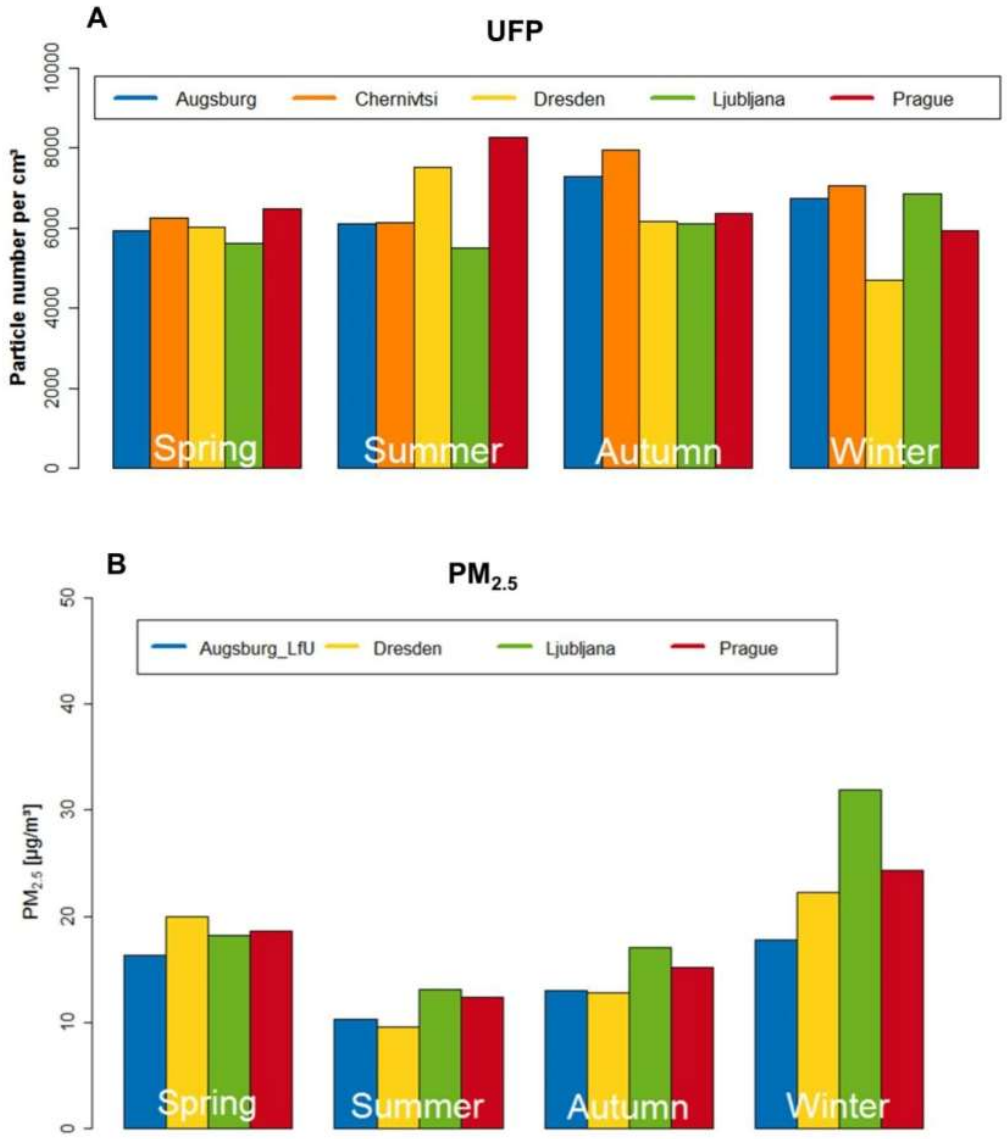
Average interquartile range for $PM_{2.5}$: 12.4 µg/m^3

*heterogeneity p-value<0.1 and I^2>50%

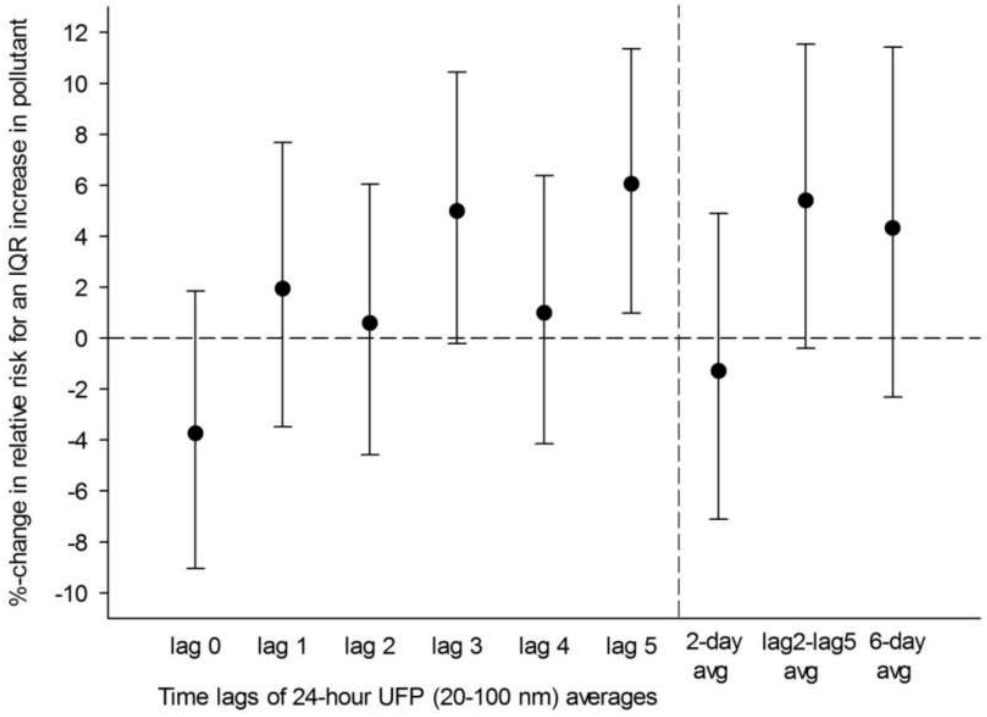
**ultrafine particles with a size range of 0.02 to 0.1µm in aerodynamic diameter (20-100 nm)

†particulate matter with a size range of <2.5 µm in aerodynamic diameter

Supplemental Figure 1. Location of the five cities in Central and Eastern Europe.

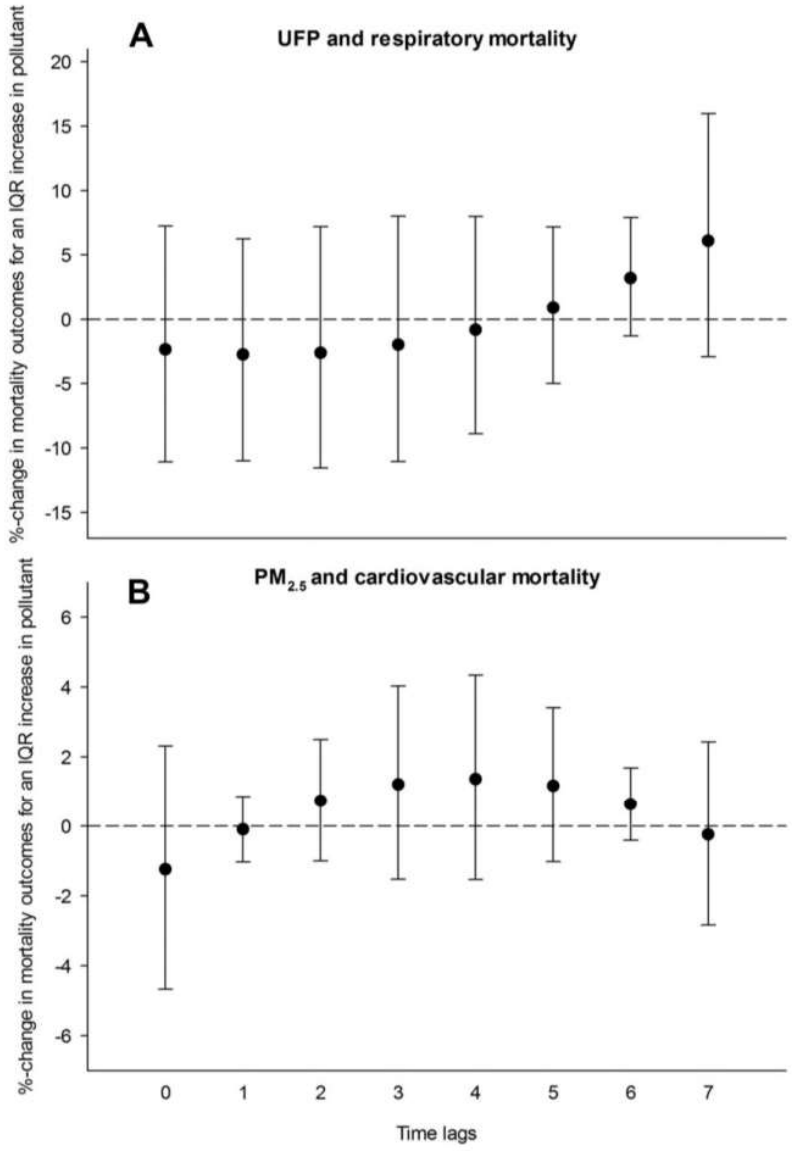


Supplemental Figure 2. Seasonal variation of A) UFP and B) $PM_{2.5}$ by city.

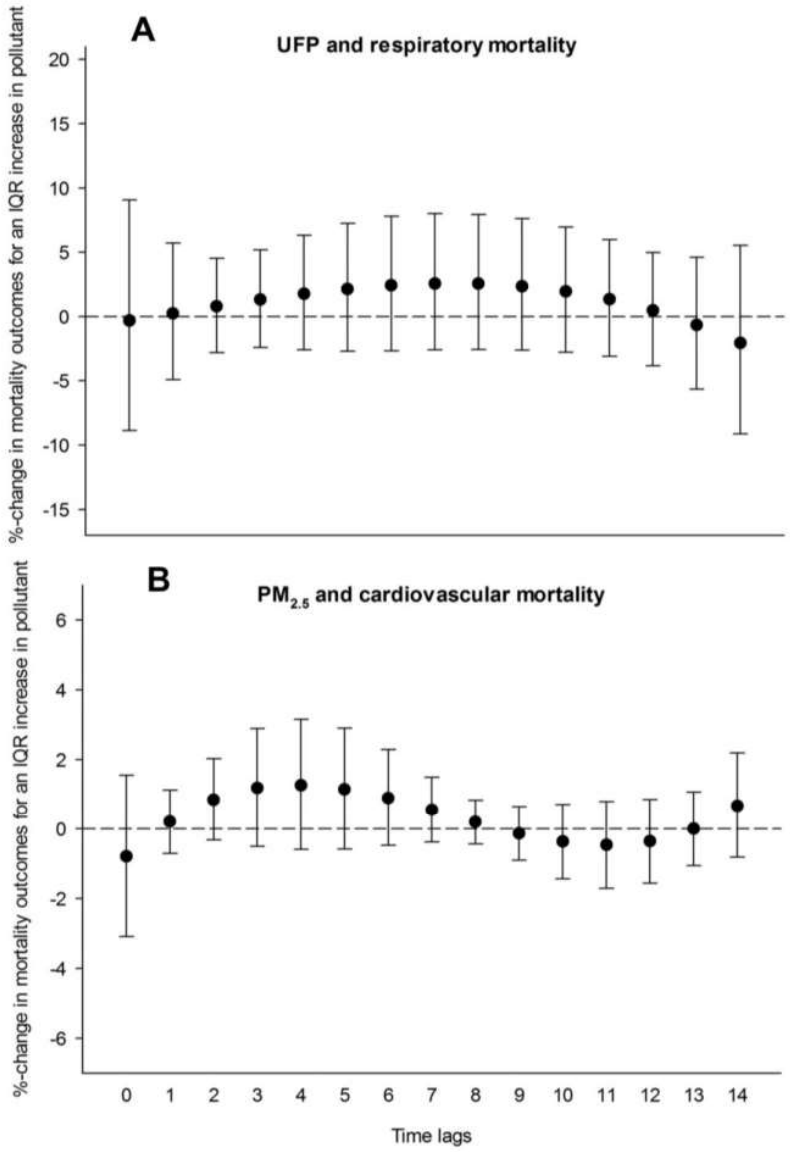


Supplemental Figure 3. Percent change in the relative risk of cardiovascular mortality per IQR increase in UFP in Augsburg.

IQRs: 3,332 particles/cm^3 for single lags, 2,915 particles/cm^3 for 2-day average, 2,328 particles/cm^3 for avg of lag 2-5, 2,162 for 6-day average.



Supplemental Figure 4. Results of second degree polynomial distributed lag models presented as percent changes in A) respiratory mortality per IQR increase in UFP and B) cardiovascular mortality per IQR increase in $PM_{2.5}$, lag 0 to 7.



Supplemental Figure 5. Results of third degree polynomial distributed lag models presented as percent changes in A) respiratory mortality per IQR increase in UFP and B) cardiovascular mortality per IQR increase in $PM_{2.5}$, lag 0 to 14.

9.2 Ultrafine and fine particles and hospital admissions in Central Europe, results from the UFIREG study

Authors: Stefanie Lanzinger, Alexandra Schneider, Susanne Breitner, Massimo Stafoggia, Ivan Erzen, Miroslav Dostal, Anna Pastorkova, Susanne Bastian, Josef Cyrys, Anja Zscheppang, Tetiana Kolodnitska, Annette Peters for the UFIREG study group

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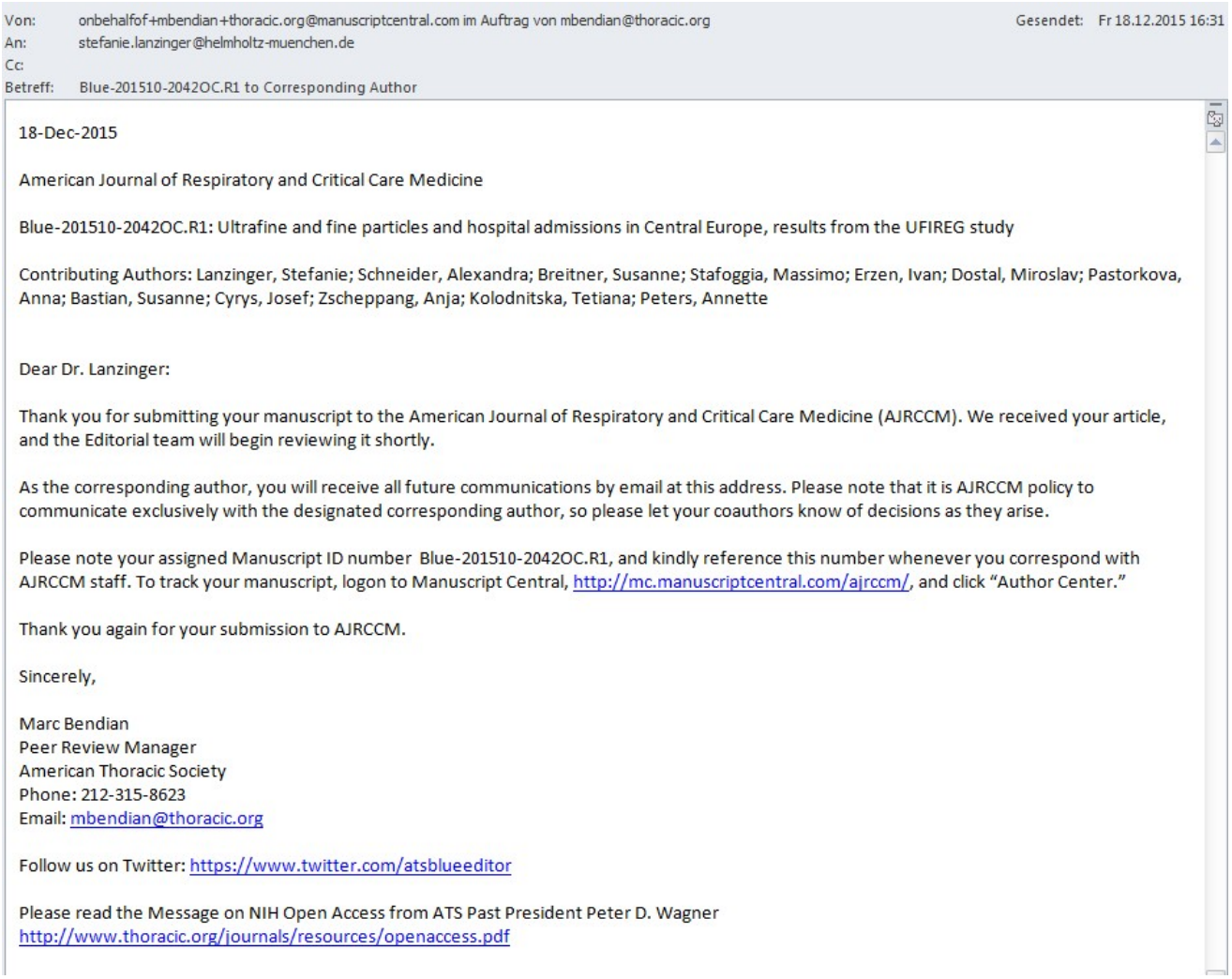

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An: stefanie.lanzinger@helmholtz-muenchen.de
Cc:
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Contributing Authors: Lanzinger, Stefanie; Schneider, Alexandra; Breitner, Susanne; Stafoggia, Massimo; Erzen, Ivan; Dostal, Miroslav; Pastorkova, Anna; Bastian, Susanne; Cyrys, Josef; Zscheppang, Anja; Kolodnitska, Tetiana; Peters, Annette

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Ultrafine and fine particles and hospital admissions in Central Europe, results from the UFIREG study

Stefanie Lanzinger[1], Alexandra Schneider[1], Susanne Breitner[1], Massimo Stafoggia[2], Ivan Erzen[3], Miroslav Dostal[4], Anna Pastorkova[4], Susanne Bastian[5], Josef Cyrys[1,6], Anja Zscheppang[7], Tetiana Kolodnitska[8], Annette Peters[1] for the UFIREG study group

[1]Helmholtz Zentrum München–German Research Center for Environmental Health, Institute of Epidemiology II, Neuherberg, Germany;

[2]Department of Epidemiology, Lazio Region Health Service, Rome, Italy;

[3]National Institute of Public Health Slovenia, Ljubljana, Slovenia;

[4]Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic;

[5]Saxon State Office for Environment, Agriculture and Geology, Dresden, Germany;

[6]Environmental Science Center, University of Augsburg, Augsburg;

[7]Research Association Public Health Saxony and Saxony-Anhalt, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany;

[8]L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Chernivtsi, Ukraine (State enterprise)

Correspondence

Stefanie Lanzinger

Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology II

Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany

Telephone: +49-89-3187-3260

Fax: +49-89-3187-338

Email: stefanie.lanzinger@helmholtz-muenchen.de

Author contributions: S.L. contributed to data collection, data management, data analysis, manuscript writing and editing; A.S. was the chair of the epidemiological work group, contributed to data management, data analysis, manuscript drafting and editing; S.Br. contributed to data management, data analysis and manuscript editing; M.S. contributed to data analysis and manuscript editing; I.E., M.D. and A.P. contributed to data collection, data management and manuscript editing; S.Ba. was the chair of the exposure work group, contributed to data collection, data management and manuscript editing; J.C. contributed to data collection, data management and manuscript editing; A.Z. was part of the lead partner of the project, contributed to data collection, data management and manuscript editing; T.K. contributed to data collection, data management and manuscript editing; A.P. coordinated the project for the Munich project partners, contributed to data analysis, manuscript drafting and editing.

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At a Glance Commentary

Scientific Knowledge on the Subject: The association between particulate matter (PM_{10} and $PM_{2.5}$) and cardiorespiratory hospital admissions is well established. However, few short-term studies have investigated the association between ultrafine particles and morbidity, and results are inconsistent.

What This Study Adds to the Field: This study investigated short-term effects of ultrafine and fine particles on cause-specific hospital admissions in five Central and Eastern European cities. It is one of the very few multi-city studies in this field and includes cities from Eastern Europe. Further, exposure assessment and statistical analyses were conducted based on a priori fixed and harmonized protocols. Our findings indicated a delayed and prolonged association between UFP and respiratory hospital admissions. Moreover, we found delayed and prolonged associations between $PM_{2.5}$ and hospital admissions due to cardiovascular and respiratory diseases.

Abstract

Rationale: Evidence on short-term effects of ultrafine particles (UFP) on health is still inconsistent and few multi-center studies have been conducted so far especially in Europe.

Objectives: Within the UFIREG project, we investigated the short-term effects of UFP and fine particulate matter <2.5 µm ($PM_{2.5}$) on daily cause-specific hospital admissions in five Central and Eastern European cities using harmonized protocols for measurements and analyses.

Methods: Daily counts of cause-specific hospital admissions were obtained for Augsburg and Dresden (Germany) 2011-2012, Chernivtsi (Ukraine) 2013-March 2014, Ljubljana (Slovenia) and Prague (Czech Republic) 2012-2013 focusing on cardiovascular and respiratory diseases. Air pollution and meteorological data were measured at fixed monitoring sites in all cities. We analyzed city-specific associations using confounder-adjusted Poisson regression models and pooled the city-specific effect estimates using meta-analyses methods.

Main Results: A 2,750 particles/cm^3 increase (average interquartile range (IQR) across all cities) in the 6-day average of UFP indicated a delayed and prolonged increase in the pooled relative risk of respiratory hospital admissions (3.4% [95%-confidence interval:-1.7%;8.8%]). We also found increases in the pooled relative risk of cardiovascular (exposure average of lag 2-5: 1.8% [0.1%;3.4%]) and respiratory (6-day average exposure: 7.5% [4.9%;10.2%]) admissions per 12.4 µg/m^3 increase (average IQR) in $PM_{2.5}$.

Conclusions: Our findings indicated a delayed and prolonged association between UFP and respiratory hospital admissions in Central and Eastern Europe. Cardiovascular and respiratory hospital admissions increased in association with an increase in $PM_{2.5}$. Further multi-center

studies are needed using harmonized UFP measurements to draw definite conclusions on health effects of UFP.

Word count for the abstract: 250 words

Key Words: Ultrafine particles, particulate matter, hospital admissions, respiratory, cardiovascular

Introduction

Many epidemiological studies investigated the association between particulate matter (PM) with an aerodynamic diameter <10 µm (PM_{10}) or <2.5 µm ($PM_{2.5}$) and (emergency) hospital admissions especially due to cardiovascular and respiratory diseases (1-4). For example, Atkinson et al. (5) found increases in hospital admissions due to cardiovascular (0.9% [0.3%;1.5%]) and respiratory diseases (1.0% [-0.6%;2.6%]) in association with a 10 µg/m^3 increase in $PM_{2.5}$ in a combined analysis of the American, European, South East Asian and Western Pacific Region.

Only a few studies investigated the association between ultrafine particles (UFP) and hospital admissions world-wide. Moreover, European research on the health effects of UFP was primarily conducted in Western European Countries (6, 7). UFP might contribute to the reported health effects through different biological mechanisms compared to larger particles such as PM_{10} and $PM_{2.5}$. The deposition and clearance in the respiratory tract differ between UFP and larger particles. While larger and fine particles deposit mainly in the upper and lower respiratory tract, UFP can penetrate deeply into the pulmonary alveoli and can be translocated with the blood stream to other organs (6, 8). Due to the important differences in deposition, the potential for translocation and their large active surface it is assumed that UFP might have at least partly independent health effects compared to larger particles (6, 9-11). However, the few studies investigating short-term effects of UFP on cardiovascular and respiratory diseases showed inconsistent results (6, 7).

Branis et al. (12) reported strongest associations for accumulation mode particles in the size range 205-487 nm. A 1,000 particles/cm^3 increase in the 7-day moving average of this particle size class was associated with increases in cardiovascular (16.4% [5.2%;28.7%]) and respiratory (33.4% [12.6%;57.9%]) hospital admissions in Prague. However, Atkinson and colleagues (13) only found weak associations between total particle number concentration

(PNC) and emergency hospital admissions for cardiovascular and respiratory causes in London. A study carried out in Copenhagen found significant associations between hospital admissions for respiratory diseases and an interquartile range (IQR) increase in the 5-day average of PNC in the size range 6-700 nm; however, associations diminished after additional adjustment for PM_{10} or $PM_{2.5}$ (14).

Within the framework of the project "Ultrafine particles – an evidence based contribution to the development of regional and European environmental and health policy", we investigated the association between UFP, PM and cause-specific hospital admissions in five Central European cities (Augsburg and Dresden, Germany; Chernivtsi, Ukraine; Ljubljana, Slovenia and Prague, Czech Republic). As a secondary objective we examined the short-term effects of PNC, PM_{10}, coarse particles with an aerodynamic diameter 2.5-10 µm ($PM_{2.5-10}$), and nitrogen dioxide (NO_2) on cause-specific hospital admissions in these five cities. This study is one of the very few multi-city studies in this field and further includes cities from Eastern Europe. Moreover, exposure assessment and statistical analyses were conducted based on a priori fixed and harmonized protocols.

Methods

For Augsburg, Dresden, Ljubljana and Prague daily counts of cause-specific hospital admissions were obtained from official statistics. Hospital admission data for Chernivtsi were collected directly from the hospitals. Infants younger than one year were excluded from the analyses. Only ordinary (no day-hospital) and acute (no scheduled) hospitalizations were considered. Moreover, only the primary diagnosis was considered for the identification of the outcomes.

The primary diagnoses were defined according to the International Statistical Classification of Diseases and Related Health Problems (ICD-10). We investigated hospital admissions due to cardiovascular (ICD-10: I00-I99) and respiratory diseases (ICD-10: J00-J99). Moreover, we investigated hospital admissions due to diabetes (ICD-10: E10-E14) as an outcome of secondary interest. No informed consent by the patients was needed as data were anonymous and collected as daily counts. In Chernivtsi, Ljubljana and Prague data was restricted to people living in the city and hospitalized within the city. However, for Augsburg and Dresden, it was difficult to exclude all scheduled hospital admissions and to restrict to people living in the city and hospitalized in the city. Due to the German data protection rules, we only could restrict to people living in Augsburg and hospitalized in Bavaria and people living in Dresden and hospitalized in Saxony, respectively. Regarding respiratory hospital admissions, the categories J33 (nasal polyp), J34 (other disorders of nose and nasal sinuses) and J35 (chronic diseases of tonsils and adenoids) were excluded by hand for Augsburg and Dresden as the number of hospitalizations in these categories were very high, and only acute hospitalizations were considered.

Hourly data of air pollutants and meteorological variables (air temperature, relative humidity and barometric pressure) were measured at local fixed measurement sites in each city. All measurement sites were located in the urban background; for Prague, the monitoring station

was located in a suburban background region. PNC in the range from 10-800 nm (for Prague from 10-500 nm) were measured using differential or scanning mobility particle size spectrometers (15). PM_{10}, $PM_{2.5}$ and NO_2 were measured in Augsburg, Dresden, Ljubljana and Prague, but were not available in Chernivtsi. $PM_{2.5-10}$ was calculated by subtracting $PM_{2.5}$ from PM_{10}. Daily 24-hour averages of air pollutants and meteorological parameters were only calculated if 75% of the hourly values were available. Due to measurement uncertainties in the size range 10 to 20 we investigated UFP in the size range 20 to 100 nm and PNC in the size range 20 to 800 (for Prague 20 to 500 nm) in all cities.

Hospital admission statistics for Augsburg and Dresden of 2013 were not available by the end of the project. Hence, due to the start of the measurements and the availability of epidemiological data, the following study periods were chosen: Augsburg and Dresden: January 2011 to December 2012; Ljubljana and Prague: January 2012 to December 2013; Chernivtsi: January 2013 until March 2014.

Statistical analysis

In a first step, we used Poisson regression models allowing for overdispersion to investigate the association between air pollutants and cause-specific hospital admissions for each city separately. The same confounder model was used for all cities and confounders were chosen based on a review of the current literature. The confounder model included date order (representing time-trend), dummy variables for day of the week, a dummy variable for holidays, a dummy variable for the decrease of the populations present in the city during vacation periods (Christmas, Easter, summer vacation), a dummy variable for influenza epidemics (in Augsburg, Dresden, Ljubljana and Prague), air temperature (average of lags 0-1 [lag 0: same-day; lag 1: one day before the event] to represent effects of high temperatures and average of lags 2-13 [lag 2: two days prior to the event; lag 13: 13 days prior to the event] to represent effects of low temperatures), and relative humidity (average of lags 0-1 and

average of lags 2-13). Penalized regression splines with natural cubic regression splines as smoothing basis were used to allow for non-linear confounder adjustment. The spline for date order was fixed to have four degrees of freedom per year to sufficiently represent long-term trend and seasonality. Splines for meteorological variables were fixed to three degrees of freedom. We investigated single-lags from same day of the event (lag 0) up to five days prior to the event (lag 5). Moreover, we estimated cumulative effect models to represent immediate (2-day average: lag 0-1), delayed (average of lag 2-5) and prolonged effects (6-day average: lag 0-5).

In a second stage, city-specific effect estimates were combined using random-effects models. For each meta-analytical estimate, a χ^2-test for heterogeneity was performed and the corresponding p-value and I^2-statistic was reported. Cities were weighted according to the precision (standard error) of the city-specific effect estimates. For pooling the city-specific estimates the maximum likelihood effects estimator after van Houwelingen was used (16).

We investigated effect modification by age (<75 years vs. ≥75 years) and sex (females vs. males) in stratified analyses. Effect modification by season (October-March vs. April-September) was analyzed by including an interaction term in the model. Two-pollutant models were calculated in order to assess interdependencies of UFP and $PM_{2.5}$ effects as well as interdependencies of UFP and NO_2 effects. We conducted several sensitivity analyses on the confounder model to test the robustness of our results (see online data supplement).

Results

Daily means of cause-specific hospital admissions per 100,000 inhabitants differed between the cities as presented in Table 1. A description of air pollution and meteorological

parameters by city is presented in Table E1 in the online data supplement. Mean PM_{10} values ranged from 20.0 $\mu g/m^3$ in Augsburg to 26.2 $\mu g/m^3$ in Prague. Ljubljana showed highest $PM_{2.5}$ values with a mean of 20.7 $\mu g/m^3$, whereas highest UFP concentrations were observed in Augsburg with a mean of 5,880 particles/cm^3. UFP were moderately correlated with PM_{10}, $PM_{2.5}$ and $PM_{2.5-10}$ (Spearman's rank correlation coefficient $0.3 \leq r_s \leq 0.5$) in all cities (Table E2). The correlation between PNC and PM_{10}, $PM_{2.5}$ as well as $PM_{2.5-10}$ was slightly higher with r_s between 0.5 and 0.6. Meteorological parameters were low to moderately correlated to air pollution parameters ($r_s<0.6$) in all cities and high correlations were observed between PM_{10} and $PM_{2.5}$ with $r_s=0.9$ in Augsburg, Dresden, Ljubljana and Prague.

Strongest associations between air pollutants and cardiovascular and respiratory hospital admissions were found for the cumulative lag periods. Table 2 shows percent changes in relative risk (RR) of cause-specific hospital admissions together with 95%-confidence intervals (CIs) for the 2-day average, the average of lag 2-5 and the 6-day average. The associations for single time lags are presented in Table E3. We observed no association between UFP, PNC and NO_2 and cardiovascular hospital admissions in pooled (Table 2) and city-specific analyses (Figure 1A). However, we observed a delayed and prolonged association between an IQR increase in $PM_{2.5}$ and cardiovascular hospital admissions (Table 2, Figure 1B). Strongest effect estimates were observed for Augsburg showing a 4.6% [0.6%;8.7%] increase in cardiovascular admissions with a 12.4 $\mu g/m^3$ increase in the $PM_{2.5}$-average of lag 2-5. Associations between PM_{10} and $PM_{2.5-10}$ and cardiovascular hospital admissions were similar but weaker compared to $PM_{2.5}$.

For respiratory hospital admissions strongest associations were found for the 6-day averages of air pollutants. A 2,750 particles/cm^3 increase in the 6-day average of UFP was associated with a 3.4% [-1.7%;8.8%] increase in the pooled RR of respiratory hospital admissions (Table 2). A 2,750 particles/cm^3 increase in UFP was associated with significant increases in the

city-specific RRs of respiratory hospital admissions in Augsburg (7.3% [1.4%;13.6%]) and Dresden (8.8% [1.8%;16.3%]) (Figure 2A). Chernivtsi showed a weak positive association and non-significant decreases in respiratory hospital admissions were found for Ljubljana and Prague. Results of PNC were similar. We observed increases in the pooled (Table 2) and city-specific (Figure 2B) RR of respiratory hospital admissions with increases in $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$. Strongest effect estimates were observed for the $PM_{2.5}$-6-day average and respiratory hospital admissions showing a 7.5% [4.9%;10.2%] increased pooled RR. Moreover, we observed a significant increase in respiratory admissions in association with a 15.4 $\mu g/m^3$ increase in the NO_2-average of lag 2-5. Our results indicated that delayed and prolonged exposure to UFP, PNC, $PM_{2.5}$ and PM_{10} increase the pooled RR of diabetes hospital admissions (Table E3).

Associations between UFP and $PM_{2.5}$ and cardiovascular hospital admissions remained stable in single- and two-pollutant models (Figure 3A). In Augsburg, Dresden and Prague effect estimates of $PM_{2.5}$ and respiratory hospital admissions were also similar in single- and two-pollutant models (Figure 3B). In Ljubljana, the association between $PM_{2.5}$ and respiratory hospital admissions strengthened when adjusting for UFP. Adjusting for $PM_{2.5}$ weakened the association between UFP and respiratory hospital admissions in Augsburg and to a lesser extent in Dresden. Moreover, UFP and respiratory hospital admission showed a negative association when adjusting for $PM_{2.5}$ in Ljubljana. Two-pollutant models of UFP and NO_2 showed no association with respiratory hospital admissions in Augsburg.

Effects of UFP on respiratory hospital admissions were stronger in people ≥75 years compared to the younger age group (Table E4). The older age group also showed a slightly stronger increased pooled relative risk of cardiovascular hospital admissions with $PM_{2.5}$. We observed no effect modification by sex. The association between UFP and respiratory hospital admissions was similar during October-March and April-September. However, we found an

increase in cardiovascular hospital admissions with a 12.4 $\mu g/m^3$ increase in $PM_{2.5}$ during the cold period; whereas, no association during the warm period. Effects of $PM_{2.5}$ on respiratory hospital admissions were slightly stronger during the cold period compare to the warm period. Sensitivity analyses were only conducted for cumulative lags periods showing the strongest associations. Table E5 shows the results of sensitivity analyses for respiratory hospital admissions and the 6-day average of UFP, for cardiovascular and respiratory hospital admissions and $PM_{2.5}$, average of lag 2-5 and 6-day average, respectively.

1) Increasing the degrees of freedom per year for the smooth function of trend decreased the pooled effect estimates for UFP and $PM_{2.5}$ on cause-specific hospital admissions compared to the original model. Whereas, using fewer degrees of freedom per year for the trend did not influence the pooled effect estimates.

Increasing the degrees of freedom for smooth functions of air temperature and relative humidity weakened the association between UFP and $PM_{2.5}$ and cause-specific hospital admissions.

2) Replacing air temperature and relative humidity by apparent temperature slightly increased the pooled effect estimate of UFP on respiratory hospital admissions. Effects of $PM_{2.5}$ on cardiovascular hospital admissions remained nearly unchanged and the association between $PM_{2.5}$ and respiratory hospital admissions decreased a bit when apparent temperature was used.

3) Adjusting for air temperature by using temperature above the median for heat effects and below the median for cold effects strengthened the association between UFP and respiratory hospital admissions. Effects of $PM_{2.5}$ on cardiovascular hospital admissions remained similar to the original model and effects of $PM_{2.5}$ on respiratory hospital admissions weakened slightly.

4) Adjusting for air temperature and relative humidity including the average of lag 0-1 and the average of lag 2-5 strengthened the effect estimates of UFP as well as $PM_{2.5}$ and cause-

specific hospital admissions.

5) Additionally adjusting for barometric pressure decreased the effect estimates of UFP and $PM_{2.5}$ on respiratory hospital admissions; whereas, the association between $PM_{2.5}$ and hospital admissions due to cardiovascular diseases remained nearly unchanged.

6) Effect estimates for Augsburg and Prague did not change significantly when the data set with imputed missing data was used (data not shown).

7) Results of second degree polynomial distribute lag models support a delayed and prolonged association between $PM_{2.5}$ increases and hospital admissions due cardiovascular and especially respiratory diseases (Figure E1). The association between UFP and respiratory hospital admissions was positive but not significant for all time lags.

8) Using city-specific confounder models (Table E6) showed positive but weaker effect estimates of UFP and respiratory hospital admissions compared to the a priori defined confounder model. Using city-specific confounder models also weakened the association between $PM_{2.5}$ and cause-specific hospital admissions. However, effect estimates of $PM_{2.5}$ and respiratory hospital admissions remained significant. Overall, the directions of the city-specific effect estimates were similar to the a priori defined confounder model (data not shown).

Discussion

Within the UFIREG project, we investigated the association between daily air pollution concentrations and cause-specific hospital admissions in Augsburg, Chernivtsi, Dresden, Ljubljana and Prague. Our findings indicated a delayed and prolonged association between

exposure to UFP and PNC and increases in respiratory hospital admissions (6-day average of UFP: 3.4% [-1.7%;8.8%] and PNC: 4.3% [-0.9%;9.8%]). Moreover, we found a delayed and prolonged association between $PM_{2.5}$ and cause-specific hospital admissions. A 12,4 $\mu g/m^3$ increase in the $PM_{2.5}$-average of lag 2-5 was associated with a 1.8% [0.1%;3.4%] increase in the pooled RR of cardiovascular hospital admissions. Increases in the 6-day average of $PM_{2.5}$were associated with increases in respiratory hospital admissions by 7.5% [4.9%;10.2%]. We observed an association between NO_2 and respiratory hospital admissions. Moreover, hospital admissions due to diabetes increased in association with exposure to UFP, PNC and PM. Effects of PM_{10} and $PM_{2.5-10}$ were similar but weaker compared to $PM_{2.5}$.

$PM_{2.5}$ has been shown to be associated with increases in hospital admissions especially due to cardiovascular and respiratory diseases (5, 11). For example, Stafoggia et al. (3) reported immediate (2-day average: 0.5% [0.1%;0.9%]) and prolonged (6-day average: 0.5% [0.0%;1.0%]) increases in cardiovascular hospital admissions and prolonged increases in respiratory hospital admissions (6-day average: 1.4% [0.2%; 2.5%]) in eight Southern European Regions. We also observed a delayed increase in cardiovascular hospital admissions (average of lag 2-5: 1.5% [0.1%;2.7%] per 10 $\mu g/m^3$ increment in $PM_{2.5}$). Our effect estimates of $PM_{2.5}$ and respiratory hospital admissions (6-day average: 6.0% [4.0%;8.2%]) were stronger compared to results from other European regions and the U.S. (3, 4, 17). In contrast to our study, Stafoggia et al. (3) and Zanobetti et al. (4) analyzed emergency hospital admissions. However, our association between $PM_{2.5}$ and respiratory hospital admissions was also stronger compared to a U.S. study conducted by Bell et al. (17) focusing not only on emergency hospital admissions. Our effect estimates for $PM_{2.5}$ and respiratory hospital admissions are comparable to results of $PM_{2.5}$ and hospital admissions due to chronic obstructive pulmonary disease in the U.S. Southeast Region involving 35 counties which were examined in a large study on altogether 204 U.S. urban counties (18). In our study increases in respiratory hospital admissions were also associated with exposure to NO_2, showing

strongest effects for the average of lag 2-5. There is a growing literature on the health effects of NO_2 (19). For example, a review by Mills and colleagues (19) reported increases in respiratory hospital admissions by 0.6% [0.3%; 0.8%] based on estimates of the WHO American, European, South East Asian and Western Pacific Region. In contrast to our study the authors also reported an association between NO_2 and cardiovascular hospital admissions (19).

Findings of our study pointed to a five-days delayed increase of 3.6% [1.0%;6.3%] in hospital admissions due to diabetes per 10 $\mu g/m^3$ $PM_{2.5}$ increment. Increases of the same magnitude in diabetes hospital admissions (2.7% [1.3%;4.2%] and 1.1% [0.6%;1.7%] per 10 $\mu g/m^3$ increase, respectively) were also reported in two studies conducted in the U.S., however, for the 2-day average of $PM_{2.5}$ (4, 20).

We found an increase in cardiovascular hospital admissions with an average IQR increase in $PM_{2.5}$ during the cold period from October-March; whereas, no association was observed during the warm period from April-September. Bell et al. (17) also observed higher effect estimates for $PM_{2.5}$ and cardiovascular hospital admissions during winter in 202 U.S. counties. However, a multi-city study in Southern European regions found stronger effects from April-September compared to the colder period (3). Different climate conditions and also different lifestyle patterns could be possible explanations. Moreover, differences in $PM_{2.5}$ compositions between regions should be considered.

A small number of studies also reported associations between cardiovascular or respiratory hospital admissions and PNC of different size ranges (1, 12, 14). Atkinson and colleagues (13) found only weak associations between total PNC and emergency hospital admissions for cardiovascular and respiratory causes in London. Nevertheless, the results indicated a four-days delayed increase in respiratory hospital admissions in association with a 10,166 particles/cm^3 increase in PNC especially in people older than 65 years (13). A German study

found significant two to seven days delayed associations between UFP and cardiovascular hospital admissions, especially for hypertensive crisis, in Leipzig (2). Our pooled effect estimates on cardiovascular hospital admissions did not show any association. However, our results pointed to delayed (average of lag 2-5: 2.2% [-0.9%;5.3%]) and prolonged increases (6-day average: 3.4% [-1.7%;8.8%]) in respiratory hospital admissions per 2,750 particles/cm^3 increment in UFP.

The change in effect estimates for single- and two-pollutant models on respiratory hospital admissions in Ljubljana might be an indication for a remaining collinearity between $PM_{2.5}$ and UFP in Ljubljana. Although, the Spearman correlation coefficient was moderate with r_s=0.3. In Augsburg the association between UFP and respiratory hospital admission vanished after adjusting for NO_2. There might be also a remaining collinearity between UFP and NO_2 in Augsburg since the correlation between UFP and NO_2 was higher in Augsburg and Ljubljana (r_s=0.5) compared to the other cities ($r_s \leq 0.3$). The association between UFP and respiratory hospital admissions weakened in Augsburg and Dresden after adjusting for $PM_{2.5}$. However, it has been demonstrated statistically that when there are two risk factors in a regression model and one has a higher level of precision, the one with more precision will dominate the prediction (21).

Plausible biological mechanisms

Oxidative stress in the lungs and lung inflammation caused by air pollutants, especially by fine and UFP, are one of the potential biological mechanisms leading to respiratory diseases (11, 22, 23). Three biological pathways that are assumed to not acting independently are discussed to be associated with effects beyond the lung. 1) Inhalation of particles may lead to a release of pro-inflammatory mediators or vasculoactive molecules from lung cells causing systemic oxidative stress and inflammation. This may further cause endothelial dysfunction, adverse cardiac outcomes, and a pro-coagulation state with thrombus formation and ischemic

response as well as promotion of atherosclerotic lesions (24). 2) Particles deposited in the pulmonary tree may be associated with imbalance of the autonomic nervous system or heart rhythm either caused by stimulating pulmonary neural reflexes (25) or by provoking oxidative stress and inflammation in the lung. 3) UFP and PM constituents can be translocated into the blood causing endothelial dysfunction and vasoconstriction, increased blood pressure and platelet aggregation, and might also directly affect the heart and other organs (11, 22).

Moreover, systemic oxidative stress and inflammation, imbalance of the autonomic nervous system and endothelial dysfunction can lead to insulin resistance and therefore can promote the progression of type-2 diabetes (26, 27).

Because of their small size UFP are not well recognized and cleared by the immune system and can escape natural defence mechanisms. UFP have a higher biological reactivity and surface area than larger particles and can be transported to other organs (6, 7, 9, 11). Therefore, it is suggested that UFP might also be linked to different biological mechanism than larger particles.

Strengths and Limitations

One of the strengths of the UFIREG project was that the associations between ultrafine and fine particles and cause-specific hospital admissions were studied in multiple cities in Central and Eastern Europe. So far many studies were conducted including Western European countries. In all the five cities UFP was measured using harmonized measurement devices. Epidemiological analyses were conducted according to a common analysis plan in order to produce comparable results. Therefore, comparable city-specific effect estimates could be examined leading to adequate pooled effect estimates for the involved Central and Eastern European Countries.

Our study is limited by a short study period of two and in case of Chernivtsi only one full year. It is recommended to use longer time periods for future studies to get powerful results. However, despite the short periods we found significant effect estimates for $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$ on hospital admission outcomes. Moreover our results indicated a delayed and prolonged association between exposure to UFP and respiratory diseases. For respiratory hospital admissions we observed the strongest associations with increases in the 6-day average of UFP and $PM_{2.5}$. The chance of uncontrolled confounding increases with longer time lags. However, previous studies also reported delayed associations between air pollutants and respiratory hospital admissions (3, 28). For example, Stafoggia and colleagues (3) reported strongest effects estimates for the 6-day average increase in air pollutants and respiratory hospital admission in Southern Europe.

Difficulties in the exclusion of scheduled hospital admissions and in the restriction to people living in the city and hospitalized in the city might have caused differences between the cities concerning daily counts of hospital admissions. Moreover, differences in coding the primary diagnosis and different health care systems need to be considered. Only the primary diagnosis of hospital admissions was considered therefore, the number of cases might be underestimated. For example, the number of hospital admissions due to diabetes might be higher when including also the secondary diagnosis.

UFP have been shown to have a higher spatial variability than fine particles. Therefore exposure misclassification might be a bigger issue than with $PM_{2.5}$ or PM_{10}. A study conducted in Augsburg reported high temporal correlations in PNC across different sites in the city area of Augsburg despite differing magnitudes in space (29). Moreover, another German study showed low spatial variability in PNC among urban background stations in Dresden (30). It was suggested that short-term UFP exposure of the average population might

be adequately represented by a fixed urban background station if the location is chosen carefully (29).

Conclusions

Our findings indicated a delayed and prolonged association between exposure to UFP and respiratory hospital admissions in Central and Eastern Europe. Cardiovascular and respiratory hospital admissions increased in association with an increase in $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$ showed similar results. Effects of $PM_{2.5}$ on respiratory hospital admissions were stronger compared to results from other European regions and the U.S. (3, 4, 17). Moreover, respiratory hospital admissions increased in association with exposure to NO_2 and we observed an increase in hospital admissions due to diabetes in association with increases in air pollutants.

Based on our experience with the UFIREG study, we suggest integrating UFP into routine measurement networks to provide data for further short- and long-term studies on health effects of UFP. So far, hardly any long-term studies on UFP have been conducted (31). Further studies are needed investigating the association between UFP and morbidity at multiple locations using harmonized UFP measurements.

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Figure Legends

Figure 1. Percent change in the city-specific and pooled relative risk of cardiovascular hospital admissions with each IQR increase in A) UFP and PNC, average of lag 2-5; B) $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$, average of lag 2-5.

*Prague: PNC 20-500 nm

Figure 2. Percent change in the city-specific and pooled relative risk of respiratory hospital admissions with each IQR increase in A) UFP and PNC, 6-day average; B) $PM_{2.5}$, PM_{10} and $PM_{2.5-10}$, 6-day average.

*Prague: PNC 20-500 nm

Figure 3. Percent change in the city-specific and pooled relative risk of A) cardiovascular hospital admissions per IQR increase in pollutant and B) respiratory hospital admissions per IQR increase in pollutant using single- and two-pollutant models.

$PM_{2.5}$: main effects of $PM_{2.5}$, UFP: main effects of UFP, $PM_{2.5}$+UFP: effects of $PM_{2.5}$ adjusted for UFP, UFP+$PM_{2.5}$: effects of UFP adjusted for $PM_{2.5}$, UFP+NO_2: effects of UFP adjusted for NO_2.

Tables

Table 1. Socio-demographical information and cause-specific hospital admissions per 100,000 inhabitants by city.

City	Year	Population	City area (km^2)	Daily mean (SD)/100,000 inhabitants of hospital admissions		
				Cardiovascular	Respiratory	Diabetes
Augsburg*	2011	266,647	147	7.3 (3.2)	3.1 (1.5)	0.8 (0.7)
	2012	272,699	147	7.2 (3.2)	3.2 (1.6)	0.8 (0.6)
Chernivtsi	2013	258,371	153	4.7 (2.2)	2.0 (1.3)	0.3 (0.4)
Dresden*	2011	517,765	328	6.5 (2.5)	2.2 (0.9)	0.6 (0.4)
	2012	525,105	328	6.5 (2.5)	2.2 (0.9)	0.6 (0.4)
Ljubljana	2012	280,607	275	4.1 (2.0)	3.0 (1.7)	0.2 (0.3)
	2013	282,994	275	3.6 (1.7)	2.6 (1.3)	0.2 (0.3)
Prague	2012	1,246,780	496	1.8 (0.7)	0.6 (0.3)	0.1 (0.1)
	2013	1,243,201	496	2.0 (0.7)	0.8 (0.4)	0.1 (0.1)

*Reference: Research Data Centers of the Federal Statistical Office and the Statistical Offices of the Länder, Hospital Admission Statistics, 2011-2012, own calculations

Table 2. Percent changes in the pooled relative risk (95%-CI) of cause-specific hospital admissions with each average IQR increase in air pollutants.

Association under investigation	IQR†	2-day average	average of lag 2-5	6-day average
Cardiovascular hospital admissions				
UFP (n/cm^3)	2,750	-0.6 (-2.4; 1.1)	0.3 (-1.7; 2.4)	-0.1 (-2.6; 2.4)
PNC (n/cm^3)	3,675	-0.6 (-2.3; 1.3)	0.8 (-1.3; 2.9)	0.4 (-2.1; 3.0)
$PM_{2.5}$ (µg/m³)	12.4	0.5 (-1.2; 2.3)	1.8 (0.1; 3.4)	1.7 (-0.1; 3.6)
PM_{10} (µg/m³)	16.0	-0.2 (-2.5; 2.1)*	1.0 (-0.9; 2.9)	0.8 (-1.9; 3.5)*
$PM_{2.5-10}$ (µg/m³)	4.7	-0.3 (-2.2; 1.6)	1.4 (-0.5; 3.4)	1.0 (-1.1; 3.2)
NO_2 (µg/m³)	15.4	-0.8 (-2.8; 1.2)	0.0 (-2.3; 2.3)	-0.8 (-3.5; 2.0)
Respiratory hospital admissions				
UFP (n/cm^3)	2,750	1.5 (-3.4; 6.7)*	2.2 (-0.9; 5.3)	3.4 (-1.7; 8.8)
PNC (n/cm^3)	3,675	1.9 (-3.2; 7.3)*	3.1 (-0.1; 6.5)	4.3 (-0.9; 9.8)
$PM_{2.5}$ (µg/m³)	12.4	3.5 (0.3; 6.7)*	6.4 (4.1; 8.8)	7.5 (4.9; 10.2)
PM_{10} (µg/m³)	16.0	4.1 (1.1; 7.1)	6.0 (3.3; 8.6)	7.3 (4.4; 10.3)
$PM_{2.5-10}$ (µg/m³)	4.7	3.4 (0.9; 5.8)	4.9 (2.2; 7.6)	6.3 (3.2; 9.5)
NO_2 (µg/m³)	15.4	2.7 (-2.1; 7.8)*	5.1 (0.7; 9.7)	6.8 (-0.2; 14.2)*
Diabetes hospital admissions				
UFP (n/cm^3)	2,750	0.4 (-4.7, 5.7)	2.8 (-3.2; 9.1)	2.9 (-4.5; 10.9)
PNC (n/cm^3)	3,675	0.6 (-4.7; 6.3)	3.8 (-2.4; 10.5)	3.9 (-3.7; 12.1)
$PM_{2.5}$ (µg/m³)	12.4	1.2 (-3.3; 5.9)	4.5 (-0.6; 9.8)	4.1 (-1.5; 9.9)
PM_{10} (µg/m³)	16.0	0.5 (-4.0; 5.2)	4.7 (-0.4; 10.1)	3.9 (-1.7; 9.8)
$PM_{2.5-10}$ (µg/m³)	4.7	-1.2 (-6.2; 4.1)	2.0 (-3.9; 8.3)	0.7 (-5.9; 7.6)
NO_2 (µg/m³)	15.4	0.3 (-5.8; 6.7)	-1.9 (-8.9; 5.7)	-0.8 (-9.1; 8.2)

†average interquartile range across all cities

*heterogeneity p-value<0.1 and I^2>50%

Figures

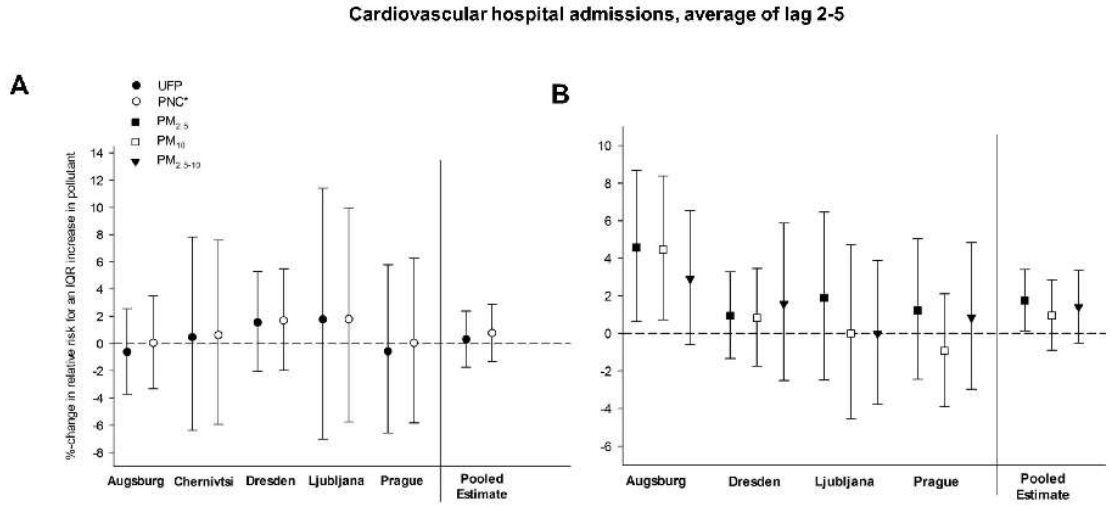

Figure 1

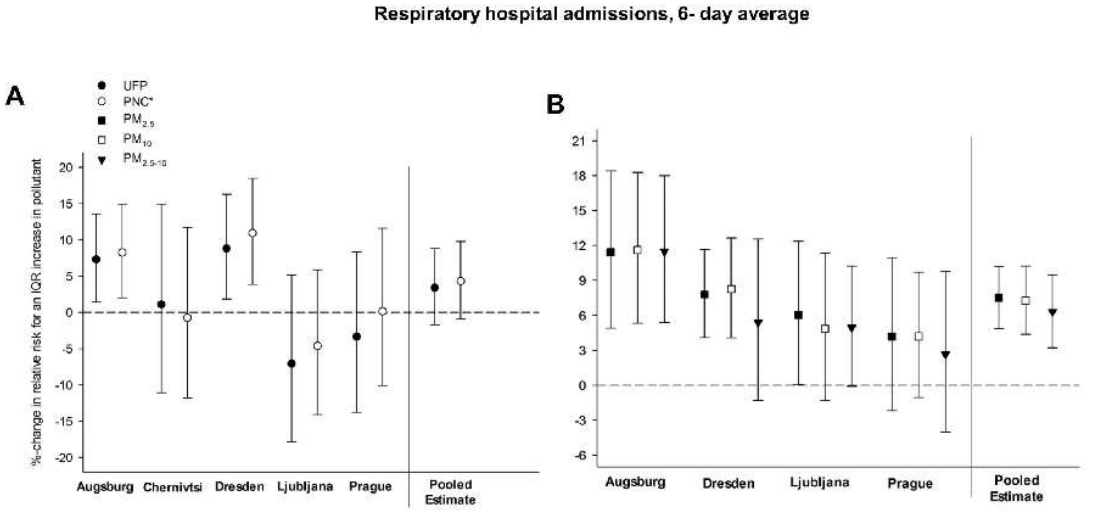

Figure 2

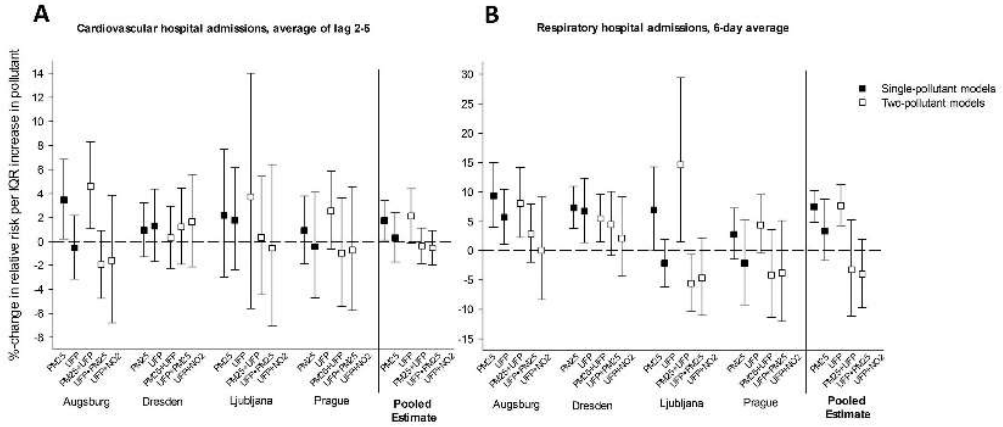
A
Cardiovascular hospital admissions, average of lag 2-5
B
Respiratory hospital admissions, 6-day average
%-change in relative risk per IQR increase in pollutant
Single-pollutant models
Two-pollutant models
Augsburg
Dresden
Ljubljana
Prague
Pooled Estimate

Figure 3

Ultrafine and Fine particles and hospital admissions in Central Europe, Results from the UFIREG study

Stefanie Lanzinger, Alexandra Schneider, Susanne Breitner, Massimo Stafoggia, Ivan Erzen, Miroslav Dostal, Anna Pastorkova, Susanne Bastian, Josef Cyrys, Anja Zscheppang, Tetiana Kolodnitska, Annette Peters for the UFIREG study group

Online Data Supplement

Methods

Data collection

Hospital admission statistics for Augsburg and Dresden were obtained from the Research Data Centres of the Federal Statistical Office and the Statistical Offices of the Free States of Bavaria and Saxony, respectively. For Ljubljana, hospital admission data were obtained from the National Institute of Public Health in Slovenia. The Institute of Health Information and Statistics of the Czech Republic provided hospital admissions statistics for Prague.

Information on influenza epidemics in Augsburg and Dresden were provided by the German Influenza Working Group of the Robert Koch Institute (https://influenza.rki.de/Default.aspx). Data on influenza epidemics in Prague were obtained from the National Institute of Public Health in Prague and the Hygiene Station of the City of Prague. In Ljubljana, these data were provided by the National Institute of Public Health in Slovenia. No information on influenza epidemics was available in Chernivtsi. Sociodemographic information for Augsburg derived from the Statistical Yearbook of Augsburg. For Dresden, data were obtained from the census in 2011 and the Statistical Office of the Free State of Saxony. The Statistical Office of the Republic of Slovenia provided socio-demographic data for Ljubljana. Data for Prague were obtained from the Institute of Health Information and Statistics of the Czech Republic and the Czech statistical office. For Chernivtsi data derived from the Main Statistic Department in Chernivtsi Region.

PNC measurements were performed using differential or scanning mobility particle size spectrometers (DMPS/SMPS). They enable highly size-resolved PNC measurements in the range from 10 to 800 nm (except in Prague: 10 to 500 nm) with particle number concentrations between 100 to 100,000 particles per cm^3. Data processing (so-called inversion) of the electrical mobility distribution (measured by the spectrometer) into the true particle number size distribution included the multiple charge correction according to Pfeifer et al. (1), coincidence

correction of the condensation particle counter (CPC) and the correction of the counting efficiency of the CPC. Particle losses due to diffusion in the inlet system and the spectrometer were also quantified using theoretical functions in the data evaluation software (2). PM_{10} and $PM_{2.5}$ mass concentrations were either determined with a tapered element oscillating microbalance/filter dynamics measurement system (TEOM/FDMS in Dresden and Ljubljana), high volume samplers (HVS in Dresden and Ljubljana) or via ß-absorption (in Augsburg and Prague). More information on the measurement instruments can be found elsewhere (3). Imputation of missing particulate matter data was possible for Augsburg and Prague where an additional urban background measurement station was available. We imputed missing data according to a modified APHEA (Air Pollution and Health: A European approach) procedure (4). Missing hours of one monitor were imputed by a weighted average of the other monitor. If the respective hourly mean value was not available at both monitors, the average of the preceding and the following hourly means was used.

Sensitivity analyses

To test the robustness of our results we conducted the following sensitivity analyses:

1) We tested different values of smoothness (less and more degrees of freedom (df) for time-trend and meteorological variables.

2) Apparent temperature was included in the model replacing air temperature and relative humidity. Apparent temperature was calculated based on the following formula (5, 6): $at = -2.653 + (0.994 \times temp) + (0.0153 \times dp \times dp)$

with at= apparent temperature, temp=air temperature and dp=dew point temperature.

Dew point temperature (dp) was calculated as follows:

$$dp = \frac{1}{\frac{1}{temp + 241.413} - \frac{\log_{10}(\frac{rh}{100})}{1838.675}} - 241.413$$

with temp= air temperature and rh=relative humidity.

3) We adjusted for air temperature by using temperature above the median for heat effects and below the median for cold effects as shown by Stafoggia et al. (7).

4) We adjusted for air temperature and relative humidity including the average of lag 0-1 and the average of lag 2-5.

5) Air pollution effects were additionally adjusted for barometric pressure.

6) Air pollution effects for Augsburg and Prague were recalculated using a dataset of imputed missing particulate matter data.

7) Distributed lag non-linear models as described by Gasparrini et al. (8) were used to analyse the association between air pollutants and cause-specific hospital admissions. We estimated up to 7 lags using a second degree polynomial and pooled the results according to Gasparrini et al. (9).

8) We reanalyzed the air pollution effects using a city-specific confounder model. We used the absolute value of the sum of the partial autocorrelation function for the selection of df for time trend. Model selection for the other variables (day of the week, holidays, vacation periods, influenza, air temperature and relative humidity) was carried out by minimizing Akaike's Information criterion (AIC). The best cumulative lag for air temperature and relative humidity was chosen and meteorological variables were included linearly or smoothly depending on the AIC value.

Effects of UFP on cause-specific hospital admissions are presented as percent changes in relative risk per 2,750 particles/cm^3 increase (average interquartile range (IQR) across all five

cities) in daily UFP. Effects of $PM_{2.5}$ are presented as percent changes in relative risk per 12.4 $\mu g/m^3$ increase (average IQR across Augsburg, Dresden, Ljubljana and Prague) in daily $PM_{2.5}$. PNC (20-800nm (20-500nm in Prague)), PM_{10}, $PM_{2.5-10}$, NO_2 and SO_2 were investigated as pollutants of secondary interest. Results of secondary pollutants are presented as percent changes in relative risk per 3,675 particles/cm^3, 16 $\mu g/m^3$, 4.7 $\mu g/m^3$, 15.4 $\mu g/m^3$ and 2.8 $\mu g/m^3$ increase in PNC, PM_{10}, $PM_{2.5-10}$and NO_2, respectively.

Data management was conducted using SAS statistical package (version 9.3; SAS Institute Inc, Cary, NC). Statistical analyses were performed using R project for statistical computing (version 2.15.3, http://www.r-project.org/) using the "mgcv", "splines", "dlnm" ,"metafor" and "mvmeta" packages.

Results

Effect modification

Effect modification by age, sex and season were analyzed for the association between UFP and respiratory hospital admissions, 6-day average, $PM_{2.5}$ and cardiovascular hospital admissions, average of lag 2-5 and $PM_{2.5}$ and respiratory hospital admissions, 6-day average since we observed the strongest associations for those cumulative lags.

Table E1. Description of air pollution and meteorological parameters by city.

City (study period)	N	min	median	mean (SD)	max	IQR*
Augsburg (2011-2012)						
Air temperature (°C)	720	-13.4	9.9	10.0 (8.0)	26.8	12.4
Relative humidity (%)	720	39.6	78.3	77.1 (13.0)	100	20.3
PM_{10}† (µg/m³)	725	2.7	17.2	20.0 (12.5)	91.5	14.5
$PM_{2.5}$‡ (µg/m³)	720	1.7	12.4	14.9 (9.8)	86.3	10.8
$PM_{2.5-10}$" (µg/m³)	714	0.1	5.3	6.0 (4.2)	36.0	5.3
UFP§ (n/cm³)	712	1,161	5,172	5,880 (3,016)	28,800	3,332
PNC# (n/cm^3)	712	1,369	6,409	7,239 (3,644)	29,470	4,124
NO_2& (µg/m³)	718	4.2	26.9	28.0 (11.8)	74.0	16.1
Chernivtsi (2013)						
Air temperature (°C)	291	-7.4	13.9	11.9 (8.2)	27.4	13.8
Relative humidity (%)	291	31.7	74	74.0 (15.6)	100	22.6
PM_{10}† (µg/m³)	.	.	.	.	.	.
$PM_{2.5}$‡ (µg/m³)	.	.	.	.	.	.
$PM_{2.5-10}$" (µg/m³)						
UFP§ (n/cm³)	340	1,769	5,018	5,511 (2,615)	19,160	3,324
PNC# (n/cm^3)	340	2,212	6,908	7,775 (3,782)	29,030	4,325
NO_2& (µg/m³)	.	.	.	.	.	.
Dresden (2011-2012)						
Air temperature (°C)	731	-13.4	11.7	11.7 (8.2)	29.6	12.8
Relative humidity (%)	731	36	69.6	69.5 (11.1)	94.3	16.7
PM_{10}† (µg/m³)	726	2.2	16.5	20.9 (15.2)	103.5	14.3
$PM_{2.5}$‡ (µg/m³)	720	1.5	11.6	16.2 (13.8)	95.7	13.1
$PM_{2.5-10}$" (µg/m³)	717	0.0	4.3	4.7 (2.7)	21.6	3.0
UFP§ (n/cm³)	639	677	3,752	4,286 (2,339)	14,440	2,882
PNC# (n/cm^3)	639	855	5,446	5,851 (2,902)	16,710	4,068
NO_2& (µg/m³)	719	3.9	20.4	22.3 (10.0)	67.3	12.9
Ljubljana (2012-2013)						
Air temperature (°C)	730	-8.8	12.2	11.7 (8.7)	29.4	14.0
Relative humidity (%)	731	37.8	74.3	73.8 (13.7)	97.5	23.6
PM_{10}† (µg/m³)	682	3.0	20.0	24.9 (16.8)	130.0	18.0
$PM_{2.5}$‡ (µg/m³)	694	3.4	16.5	20.7 (14.3)	114.8	14.4
$PM_{2.5-10}$" (µg/m³)	646	0.0	3.9	5.0 (5.1)	29.8	5.8
UFP§ (n/cm³)	435	855	4,400	4,693 (1,897)	13,920	1,935
PNC# (n/cm^3)	435	1,685	6,071	6,750 (3,122)	24,360	2,689
NO_2& (µg/m³)	683	1.8	22.2	25.1 (14.8)	119.4	16.4
Prague (2012-2013)						
Air temperature (°C)	723	-13.7	9	9.2 (8.4)	27.2	13.1
Relative humidity (%)	704	40.8	78.2	77.3 (13.2)	98.9	20.4
PM_{10}† (µg/m³)	681	5.1	22.2	26.2 (15.7)	100.9	17.2
$PM_{2.5}$‡ (µg/m³)	612	1.6	13.1	16.2 (11.6)	78.8	11.4
$PM_{2.5-10}$" (µg/m³)	579	1.7	9.2	9.8 (4.0)	44.6	4.6
UFP§ (n/cm³)	464	960	3,797	4,197 (2,010)	14,960	2,278

PNC$ (n/cm^3)	464	1,217	5,417	5,799 (2,537)	16,950	3,168
NO_2& (μg/m³)	707	4.5	19.5	21.9 (11.7)	74.2	16.2

*interquartile range

†particulate matter with a size range of <10 μm in aerodynamic diameter

‡particulate matter with a size range of <2.5 μm in aerodynamic diameter

"coarse particles with a size range of 2.5-10 μm in aerodynamic diameter

§ultrafine particles with a size range of 0.02 to 0.1 μm in aerodynamic diameter (20-100 nm)

#particle number concentration with a size range of 0.02 to 0.8 μm in aerodynamic diameter (20-800 nm)

$particle number concentration with a size range of 0.02 to 0.5 μm in aerodynamic diameter (20-500 nm)

&nitrogen dioxide

Table E2. Spearman's rank correlation coefficients for meteorological and air pollution parameters.

Augsburg (2011-2012)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}$‡ (µg/m³)	$PM_{2.5-10}$ (µg/m³)	UFP§ (n/cm³)	PNC# (n/cm³)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.57	-0.19	-0.33	0.17	0.06	0.01	-0.53
Relative humidity (%)		1.00	-0.03	0.11	-0.30	-0.37	-0.33	0.19
PM_{10}† (µg/m³)			1.00	0.93	0.78	0.43	0.54	0.70
$PM_{2.5}$‡ (µg/m³)				1.00	0.53	0.37	0.49	0.73
$PM_{2.5-10}$" (µg/m³)					1.00	0.45	0.50	0.44
UFP§ (n/cm³)						1.00	0.99	0.51
PNC# (n/cm³)							1.00	0.58
NO_2& (µg/m³)								1.00
Chernivtsi (2013)	Air temperature (°C)		PM_{10}† (µg/m³)	$PM_{2.5}$‡ (µg/m³)	$PM_{2.5-10}$ (µg/m³)	UFP§ (n/cm³)	PNC# (n/cm³)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.55	.	.	.	0.08	0.01	.
Relative humidity (%)		1.00	.	.	.	-0.29	-0.19	.
PM_{10}† (µg/m³)			.	.	.	.	.	.
$PM_{2.5}$‡ (µg/m³)				.	.	.	.	.
$PM_{2.5-10}$" (µg/m³)					.	.	.	.
UFP§ (n/cm³)						1.00	0.97	.
PNC# (n/cm³)							1.00	.
NO_2& (µg/m³)								.
Dresden (2011-2012)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}$‡ (µg/m³)	$PM_{2.5-10}$ (µg/m³)	UFP§ (n/cm³)	PNC# (n/cm³)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.50	-0.28	-0.37	0.17	0.29	0.19	-0.42
Relative humidity (%)		1.00	0.06	0.14	-0.28	-0.42	-0.35	0.28
PM_{10}† (µg/m³)			1.00	0.97	0.58	0.37	0.56	0.68
$PM_{2.5}$‡ (µg/m³)				1.00	0.40	0.30	0.50	0.68

$PM_{2.5-10}$" (µg/m³)					1.00	0.51	0.58	0.37
UFP§ (n/cm³)						1.00	0.96	0.33
PNC# (n/cm^3)							1.00	0.45
NO_2& (µg/m³)								1.00
Ljubljana (2012-2013)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}$‡ (µg/m³)	$PM_{2.5-10}$ (µg/m³)	UFP§ (n/cm³)	PNC# (n/cm^3)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.45	-0.44	-0.53	-0.06	-0.17	-0.22	-0.54
Relative humidity (%)		1.00	0.06	0.16	-0.14	0.08	0.13	0.38
PM_{10}† (µg/m³)			1.00	0.95	0.67	0.36	0.59	0.57
$PM_{2.5}$‡ (µg/m³)				1.00	0.43	0.27	0.50	0.55
$PM_{2.5-10}$" (µg/m³)					1.00	0.46	0.55	0.40
UFP§ (n/cm³)						1.00	0.95	0.54
PNC# (n/cm^3)							1.00	0.62
NO_2& (µg/m³)								1.00
Prague (2012-2013)	Air temperature (°C)	Relative humidity (%)	PM_{10}† (µg/m³)	$PM_{2.5}$‡ (µg/m³)	$PM_{2.5-10}$ (µg/m³)	UFP§ (n/cm³)	PNC$ (n/cm^3)	NO_2& (µg/m³)
Air temperature (°C)	1.00	-0.52	-0.19	-0.25	0.14	0.31	0.15	-0.46
Relative humidity (%)		1.00	0.00	0.12	-0.16	-0.31	-0.18	0.35
PM_{10}† (µg/m³)			1.00	0.96	0.77	0.29	0.54	0.68
$PM_{2.5}$‡ (µg/m³)				1.00	0.61	0.25	0.50	0.66
$PM_{2.5-10}$" (µg/m³)					1.00	0.40	0.56	0.43
UFP§ (n/cm³)						1.00	0.94	0.26
PNC$ (n/cm^3)							1.00	0.46
NO_2& (µg/m³)								1.00

†particulate matter with a size range of <10 µm in aerodynamic diameter

‡particulate matter with a size range of <2.5 µm in aerodynamic diameter

"coarse particles with a size range of 2.5-10 µm in aerodynamic diameter

§ultrafine particles with a size range of 0.02 to 0.1µm in aerodynamic diameter (20-100 nm)

#particle number concentration with a size range of 0.02 to 0.8µm in aerodynamic diameter (20-800 nm)

$particle number concentration with a size range of 0.02 to 0.5µm in aerodynamic diameter (20-500 nm)

&nitrogen dioxide

Table E3. Percent changes in the pooled relative risk (95%-CI) of cause-specific hospital admissions with each average IQR increase in air pollutants, single lags.

Association under investigation	IQR†	lag 0	lag 1	lag 2	lag 3	lag 4	lag 5
Cardiovascular hospital admissions							
UFP (n/cm^3)	2,750	-0.9 (-2.2; 0.5)	0.1 (-1.3; 1.5)	0.3 (-1.0; 1.7)	0.1 (-1.6; 1.9)	0.5 (-0.8; 1.9)	-0.2 (-1.6; 1.1)
PNC (n/cm^3)	3,675	-0.9 (-2.4; 0.5)	0.1 (-1.4; 1.6)	0.5 (-1.0; 1.9)	0.1 (-1.7; 1.8)	0.7 (-0.7; 2.2)	0.2 (-1.2; 1.6)
$PM_{2.5}$ (µg/m³)	12.4	0.5 (-1.0; 2.0)	0.6 (-0.8; 2.0)	0.8 (-0.5; 2.0)	1.0 (-0.2; 2.3)	1.0 (-0.3; 2.3)	1.6 (0.1; 3.1)
PM_{10} (µg/m³)	16.0	-0.3 (-1.9; 1.4)	0.2 (-1.7; 2.1)	0.1 (-1.8; 2.1)*	0.5 (-0.8; 1.8)	0.6 (-0.7; 1.9)	1.2 (-0.1; 2.5)
$PM_{2.5-10}$ (µg/m³)	4.7	-0.6 (-2.0; 0.8)	0.5 (-0.9; 1.9)	0.7 (-0.9; 2.4)	0.5 (-0.8; 1.8)	0.5 (-0.9; 1.8)	1.0 (-0.3; 2.3)
NO_2 (µg/m³)	15.4	-0.2 (-1.9; 1.5)	-1.1 (-3.5; 1.4)	-1.3 (-3.0; 0.5)	-0.3 (-2.0; 1.4)	0.2 (-1.5; 1.9)	1.0 (-0.7; 2.7)
Respiratory hospital admissions							
UFP (n/cm^3)	2,750	0.5 (-3.1; 4.3)*	1.4 (-2.6; 5.6)*	0.6 (-1.5; 2.8)	1.3 (-0.6; 3.3)	0.4 (-1.6; 2.3)	1.2 (-0.8; 3.1)
PNC (n/cm^3)	3,675	0.6 (-3.3; 4.6)*	2.0 (-2.3; 6.4)*	1.0 (-1.3; 3.3)	2.1 (0.0; 4.2)	0.9 (-1.2; 3.0)	1.5 (-0.7; 3.7)
$PM_{2.5}$ (µg/m³)	12.4	1.6 (-1.8; 5.1)*	4.2 (2.2; 6.2)	3.5 (1.8; 5.4)	4.3 (2.6; 6.1)	3.5 (1.6; 5.5)	3.8 (1.9; 5.7)
PM_{10} (µg/m³)	16.0	2.2 (-0.8; 5.3)*	4.6 (2.6; 6.6)	3.7 (1.9; 5.5)	4.2 (2.3; 6.0)	3.2 (1.3; 5.1)	3.3 (1.1; 5.5)
$PM_{2.5-10}$ (µg/m³)	4.7	1.6 (-0.7; 4.0)	3.0 (1.1; 4.8)	2.2 (0.4; 4.1)	3.3 (1.4; 5.2)	2.0 (0.1; 3.9)	2.2 (0.2; 4.3)
NO_2 (µg/m³)	15.4	1.7 (-2.6; 6.2)*	2.4 (-1.1; 6.1)*	1.0 (-1.4; 3.5)	2.7 (-0.4; 5.9)	3.0 (0.5; 5.4)	3.6 (1.3; 6.1)
Diabetes hospital admissions							
UFP (n/cm^3)	2,750	0.4 (-3.5; 4.5)	0.2 (-3.8; 4.5)	-0.4 (-4.3; 3.6)	-2.8 (-6.9; 1.4)	7.4 (1.9; 13:3)	1.7 (-2.4; 5.9)
PNC (n/cm^3)	3,675	0.4 (-3.8; 4.9)	0.2 (-4.2; 4.8)	-0.5 (-4.7; 3.9)	-2.0 (-6.5; 2.7)	6.3 (2.0; 10.8)	3.1 (-2.4; 8.9)
$PM_{2.5}$ (µg/m³)	12.4	0.8 (-3.3; 5.0)	0.9 (-3.1; 5.0)	1.4 (-2.5; 5.5)	1.7 (-2.2; 5.8)	2.1 (-1.9; 6.3)	5.6 (1.6; 9.8)
PM_{10} (µg/m³)	16.0	0.1 (-4.0; 4.3)	0.5 (-3.5; 4.7)	0.3 (-4.1; 4.9)	1.6 (-2.3; 5.8)	2.6 (-1.5; 6.8)	6.6 (2.2; 11.1)
$PM_{2.5-10}$ (µg/m³)	4.7	-0.6 (-5.4; 4.4)	-1.5 (-5.8; 3.1)	-1.1 (-5.3; 3.3)	-0.6 (-4.7; 3.7)	1.7 (-2.4; 6.0)	4.0 (-0.2; 8.4)
NO_2 (µg/m³)	15.4	0.2 (-5.1; 5.8)	0.4 (-5.0; 6.0)	-2.3 (-7.5; 3.2)	-3.5 (-8.6; 1.9)	-0.5 (-5.7; 5.0)	2.9 (-2.3; 8.5)

†average interquartile range across all cities

*heterogeneity p-value<0.1 and I^2>50%

Table E4. Effect modification by age, sex and season of the association between air pollutants and cause-specific hospital admissions.

	UFP** and respiratory hospital admissions (6-day average)	$PM_{2.5}$† and cardiovascular hospital admissions (average of lag 2-5)	$PM_{2.5}$† and respiratory hospital admissions (6-day average)
Main effect	3.4 (-1.7; 8.8)	1.8 (0.1;3.4)	7.5 (4.9; 10.2)
Age			
<75 years	3.8 (-0.7; 8.4)	1.1 (-1.0; 3.2)	7.7 (4.0; 11.6)
≥75 years	7.0 (0.3; 14.1)	2.5 (0.3; 4.8)	7.0 (2.4;11.7)
Sex			
females	3.0 (-5.4; 12.2)*	1.7 (-0.4; 3.9)	7.2 (3.4; 11.1)
males	4.6 (-1.0; 10.5)	1.7 (-0.4; 3.9)	8.1 (3.7; 12.7)
Season			
October-March	4.6 (-2.6; 12.4)*	1.9 (0.3; 3.6)	7.5 (4.8; 10.3)
April-September	4.3 (-0.8; 9.5)	-0.1 (-3.3; 3.2)	6.6 (1.2; 12.3)

Average interquartile range for UFP: 2,750 particles/cm^3

Average interquartile range for $PM_{2.5}$: 12.4 $\mu g/m^3$

*heterogeneity p-value<0.1 and I^2>50%

**Ultrafine particles with a size range of 0.02 to 0.1μm in aerodynamic diameter (20-100 nm)

†Particulate matter with a size range of <2.5 μm in aerodynamic diameter

Table E5. Sensitivity analyses, percent change in the pooled relative risk (95%-CI) of respiratory hospital admissions per IQR increase in UFP and percent change in the pooled relative risk of cardiovascular and respiratory hospital admissions per IQR increase in $PM_{2.5}$.

Sensitivity Analysis	UFP* and respiratory hospital admissions (6-day average)	$PM_{2.5}$† and cardiovascular hospital admissions (average of lag 2-5)	$PM_{2.5}$† and respiratory hospital admissions (6-day average)
Original Model	3.4 (-1.7; 8.8)	1.8 (0.1; 3.4)	7.5 (4.9; 10.2)
More DF‡ (DF = 6 per year) for smooth function of trend	1.4 (-2.5; 5.4)	0.9 (-0.8; 2.7)	5.7 (2.9; 8.6)
Fewer DF‡ (DF = 3 per year) for smooth function of trend	3.7 (-1.4; 9.1)	1.9 (0.1; 3.7)	7.1 (4.5; 9.7)
More DF‡ (DF = 5) for smooth functions of meteorological variables	2.4 (-3.4; 8.6)	1.4 (-0.2; 3.1)	6.5 (3.8; 9.2)
Use of apparent temperature	3.9 (0.5; 7.4)	1.8 (0.3; 3.4)	7.1 (4.6; 9.7)
Adjusting for air temperature by using temperature above the median for heat effects and below the median for cold effects	4.4 (-0.2; 9.1)	1.9 (0.3; 3.6)	7.1 (4.6; 9.6)
Adjusting for air temperature and relative humidity average of lag 0-1 and average of lag 2-5	4.3 (-1.4; 10.4)	2.5 (0.2; 4.9)	9.3 (6.7; 12.1)
Inclusion of barometric pressure	2.3 (-3.4; 8.4)	1.7 (0.0; 3.5)	6.8 (4.0; 9.7)
Using a city-specific confounder model	0.9 (-2.9; 4.8)	0.7 (-1.1; 2.5)	4.4 (1.8; 7.0)

Average interquartile range for UFP: 2,750 particles/cm^3

Average interquartile range for $PM_{2.5}$: 12.4 µg/m^3

*Ultrafine particles with a size range of 0.02 to 0.1µm in aerodynamic diameter (20-100 nm)

†Particulate matter with a size range of <2.5 µm in aerodynamic diameter

‡Degrees of freedom

Table E6. City-specific confounder models.

	time trend	day of the week	vacation periods	holidays	influenza	air temperature	relative humidity
Cardiovascular hospital admissions							
Augsburg	df*=11	x	x	x	x	2-day average (df*=2), average of lag 2-5 (df*=3)	average of lag 2-13 (df*=2)
Dresden	df*=8	x	x	x	x	average of lag 0-5 linearly, average of lag 2-13 linearly	2-day average linearly, average of lag 2-13 (df*=4)
Ljubljana	df*=6	x	x	x	x	2-day average (df*=5), average of lag 2-13 (df*=5)	average of lag 2-5 linearly, average of lag 2-13 (df*=4)
Prague	df*=11	x	x	x		2-day average linearly, average of lag 2-13 (df*=2)	2-day average (df*=2), average of lag 2-13 linearly
Respiratory hospital admissions							
Augsburg	df*=9	x	x	x		2-day average (df*=2), average of lag 2-13 (df*=2)	average of lag 2-5 (df*=2), average of lag 2-13 (df*=2)
Chernivtsi	df*=2	x	x	x	not available	2-day average (df*=2), average of lag 2-13 linearly	average of lag 2-5 (df*=3)
Dresden	df*=8	x		x		2-day average (df*=2), average of lag 2-13 (df*=3)	
Ljubljana	df*=8	x	x	x		2-day average linearly, average of lag 2-13 (df*=7)	
Prague	df*=10	x	x			2-day average (df*=2), average of lag 2-13 (df*=2)	averalge of lag 2-5 linearly, average of lag 2-13 linearly

*degrees of freedom

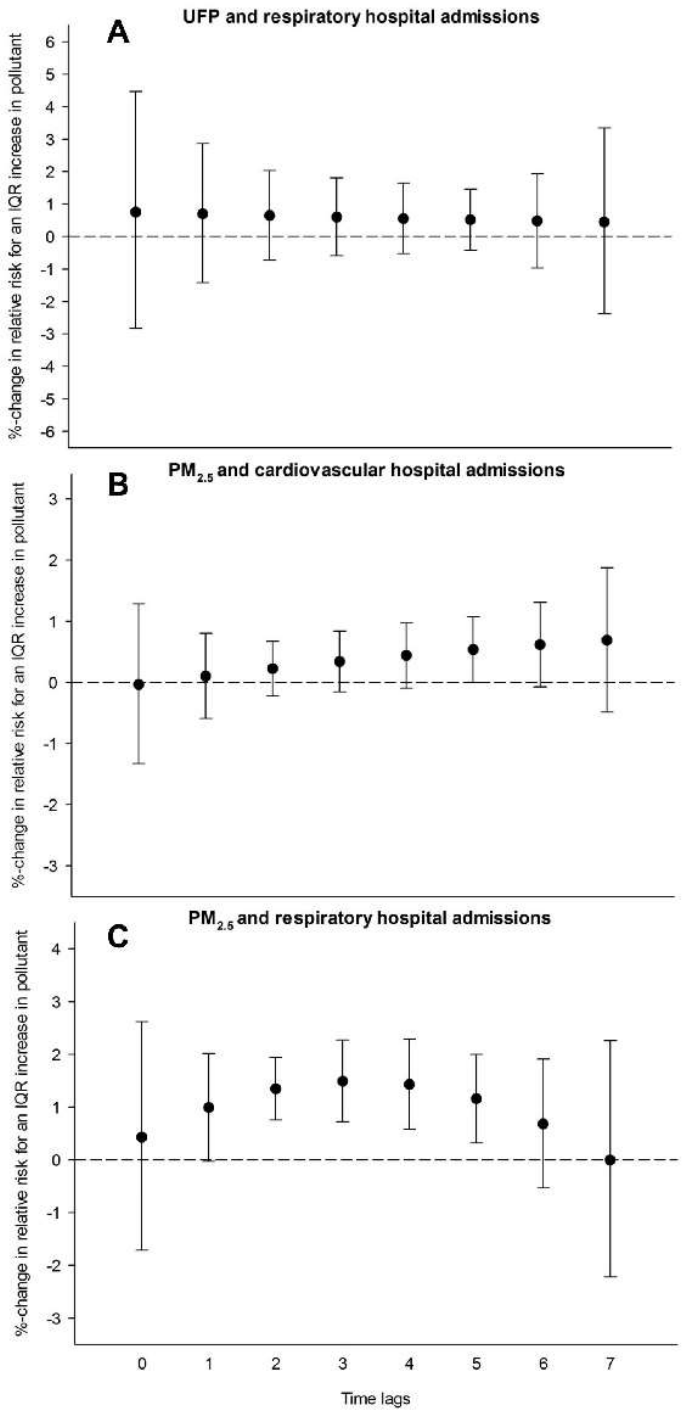

Figure E1. Results of second degree polynomial distributed lag models presented as percent changes in the pooled relative risks of A) respiratory hospital admissions per IQR increase in UFP and B) cardiovascular and C) respiratory hospital admissions per IQR increase in $PM_{2.5}$, lag 0 to 7.

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9.3 The impact of decreases in air temperature and increases in ozone on markers of endothelial function in individuals having type-2 diabetes

Authors: Stefanie Lanzinger, Susanne Breitner, Lucas Neas, Wayne Cascio, David Diaz-Sanchez, Alan Hinderliter, Annette Peters, Robert B. Devlin, Alexandra Schneider

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The impact of decreases in air temperature and increases in ozone on markers of endothelial function in individuals having type-2 diabetes

Stefanie Lanzinger [a,*], Susanne Breitner [a], Lucas Neas [b], Wayne Cascio [b], David Diaz-Sanchez [b], Alan Hinderliter [c], Annette Peters [a], Robert B. Devlin [b], Alexandra Schneider [a]

[a] Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology II, Neuherberg, Germany
[b] Environmental Public Health Division, National Health and Environmental Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, NC, USA
[c] University of North Carolina, School of Medicine, Chapel Hill, NC, USA

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ABSTRACT

Several studies have reported an association between air pollution and endothelial dysfunction, especially in individuals having diabetes. However, very few studies have examined the impact of air temperature on endothelial function. The objective of this analysis was to investigate short-term effects of temperature and ozone on endothelial function in individuals having diabetes. Moreover, we investigated interactive effects between air temperature and air pollution on markers of endothelial function. Between November 2004 and December 2005 flow-mediated dilatation (FMD), nitroglycerin-mediated dilatation (NTGMD) and several blood markers representing endothelial function were measured using brachial artery ultrasound on four consecutive days in 22 individuals with type-2 diabetes mellitus in Chapel Hill, North Carolina (USA). Daily measurements of meteorological parameters, ozone and particulate matter with an aerodynamic diameter $\leq 2.5\,\mu m$ ($PM_{2.5}$) were obtained from fixed monitoring sites. We used additive mixed-models adjusting for time trend, day of the week, relative humidity and barometric pressure to assess temperature and ozone associations with endothelial function. A 1 °C decrease in the 24-h temperature average was associated with a decrease in mean FMD on the same day (−2.2% (95%-confidence interval:[−4.7;0.3%])) and with a delay of one and four days. A temperature decrement also led to an immediate (−1.7%[−3.3;−0.04]) decrease in NTGMD. Moreover, we observed an immediate (−14.6%[−26.3;−2.9%]) and a one day delayed (−13.5% [−27.0; 0.04%]) decrease in FMD in association with a 0.01 ppm increase in the maximum 8-h moving average of ozone. Temperature effects on FMD strengthened when $PM_{2.5}$ and ozone concentrations were high. The associations were similar during winter and summer. We detected an association between temperature decreases and ozone increases on endothelial dysfunction in individuals having diabetes. We conclude that endothelial dysfunction might be a possible mechanism explaining cardiovascular events in association with environmental stimuli.

1. Introduction

Air pollution and air temperature have been shown to be associated with cardiovascular events (Brook et al., 2010, 2011; Henrotin et al., 2010; Rückerl et al., 2011; Wolf et al., 2009). Individuals with diabetes are especially susceptible to environmental stimuli (Schwartz, 2005; Stafoggia et al., 2010; Zanobetti and Schwartz, 2001) due to vascular abnormalities such as an impaired function of the vascular endothelium (Calles-Escandon and Cipolla, 2001). Observational studies have shown that endothelial dysfunction is associated with the occurrence of

Abbreviations: AIC, Akaike's Information Criterion; BMI, body mass index; CI, confidence interval; ELISA, enzyme-linked immunosorbent assays; EPA, Environmental Protection Agency; EPHD, Environmental Public Health Division; E-Selectin, soluble endothelial-leukocyte adhesion molecule; FMD, flow-mediated dilatation; IQR, interquartile range; NO, nitric oxide; NTGMD, nitroglycerin-mediated dilatation; $PM_{2.5}$, particulate matter with an aerodynamic diameter $\leq 2.5\,\mu m$; P-splines, penalized splines; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; T2D, type-2 diabetes; UNC, University of North Carolina; vWF, von Willebrand factor.

* Corresponding author. Tel: +49-89-3187-3260, Fax: +49 89 3187 338.
E-mail address: stefanie.lanzinger@helmholtz-muenchen.de (S. Lanzinger).

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cardiovascular events (Deanfield et al., 2007; Lerman and Zeiher, 2005; Vita and Keaney, 2002; Widlansky et al., 2007). Moreover, it has been suggested that endothelial dysfunction might be one of the biological mechanisms explaining cardiovascular events in association with environmental changes (Nawrot et al., 2005; Widlansky et al., 2007). Several studies have reported an association between air pollution and endothelial dysfunction (Briet et al., 2007; Brook, 2002; Hashemi et al., 2012; O'Neill et al., 2005; Schneider et al., 2008). However, only a few studies have examined the impact of air temperature on endothelial function (Nawrot et al., 2005; Widlansky et al., 2007; Zanobetti et al., 2014), and little is known about the interaction between air temperature and air pollution (Burkart et al., 2013; Roberts, 2004; Stafoggia et al., 2008).

Flow-mediated dilatation (FMD) of the brachial artery – quantified as the percent change in diameter induced by reactive hyperemia – is a marker of endothelial function. FMD occurs predominantly through the release of nitric oxide (NO) by endothelial cells and reflects the bioavailability of NO (Corretti et al., 2002; Deanfield et al., 2007; Faulx et al., 2003; Gokce et al., 2003). Soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble endothelial-leukocyte adhesion molecule (E-Selectin) are specific markers for endothelial cell activation, and von Willebrand factor (vWF) is a largely endothelium derived blood glycoprotein involved in platelet adhesion (Deanfield et al., 2007). Increased levels of sICAM-1 and sVCAM-1 have been shown to be associated with cardiovascular events (Ballantyne and Entman, 2002; Deanfield et al., 2007).

The objective of this analysis was to investigate the short-term effects of air temperature and ozone on FMD, nitroglycerin-mediated dilatation (NTGMD), sICAM-1, sVCAM-1, E-Selectin and vWF in type-2 diabetes (T2D). Moreover, we aimed to evaluate interactive effects between air temperature and air pollution on markers of endothelial function. In this context, we examined interactive effects between air temperature and ozone as well as air temperature and particulate matter with an aerodynamic diameter $\leq 2.5\ \mu m$ ($PM_{2.5}$).

Studies so far reported an immediate (same day) decrease in FMD in association with changes in air pollution and air temperature (Nawrot et al., 2005; Schneider et al., 2008). Therefore, we also expected an immediate effect of air temperature decreases and ozone increases on endothelial function. Moreover, the association between air temperature and endothelial function is assumed to be similar during winter and summer as it was shown for the association between air temperature and myocardial infarction (Wolf et al., 2009). Nawrot et al. reported a linear relationship between air temperature and endothelial function (Nawrot et al., 2005). Moreover, a study conducted by Halonen et al. showed a linear relationship between air temperature and blood markers of endothelial function (Halonen et al., 2010). Therefore, we expected a linear response relationship between decreases in air temperature and changes in endothelial function.

2. Methods

2.1. Study design and study population

Between November 2004 and December 2005, a prospective panel study was conducted in individuals with T2D in Chapel Hill, North Carolina (USA). Persons with T2D aged 48 to 78 years were identified through the University of North Carolina (UNC) Diabetes Care Center as well as newspaper advertisements. The following inclusion criteria had to be fulfilled for each participant: 1) T2D, but without insulin treatment; 2) a stable medication regimen throughout their participation in the study; and 3) normal sinus rhythm. Individuals with the following characteristics were excluded from the study: 1) smoking or recent past-smoking, defined as more than one pack of cigarettes within the year before enrollment; 2) hematocrit < 36%; 3) medical history or health problems precluding participation, decided by the study physician, like pacemaker or implanted cardioverter defibrillator, history of atrial fibrillation, history of solid organ transplant, dialysis therapy, cancer or history of cancer within the last five years, hepatitis B or C, unstable angina, hypersensibility to nitroglycerin/nitrates/nitrites, or respiratory tract infection within the preceding four weeks; 4) a recent vascular event or intervention less than six months or a year ago, depending on the intervention, (e.g. coronary artery graft bypass surgery or percutaneous coronary intervention); or 5) pregnancy. Participants were asked to refrain from vigorous exercise on study mornings as well as to refrain from taking antioxidants (e.g. vitamins C and E), fish oil, niacin, arginine, over-the-counter vasoactive agents (e.g. decongestants), anti-inflammatory agents (e.g. ibuprofen or aspirin) unless it was prescribed as a daily medication (in which case it was continued), for the week before and the week of the study. Moreover, participants were asked to refrain from the usage of phosphodiesterase enzyme inhibitors during the week of study.

The study was conducted at the U.S. Environmental Protection Agency's (EPA) National Health and Environmental Effects Research Laboratory, Environmental Public Health Division (EPHD) in Chapel Hill, North Carolina. Data on demographics, medication, health and smoking status were obtained at baseline as well as at follow-up visits via questionnaires. All participants gave written informed consent. The University of North Carolina Human Studies Biomedical Institutional Review Board and the U.S. EPA approved the study protocol.

2.2. Clinical measurements

Participants visited the study site on five consecutive weekdays starting on Monday morning of each examination week. On the first day, the participants completed a baseline questionnaire. On each of the next four consecutive mornings, the participants came to the study site having fasted since midnight and without having taken diabetes medication. FMD and NTGMD of the brachial artery were measured using ultrasound (HDI 5000 ATL ultrasound machine equipped with a 12.5 MHz transducer (Philips, Bothell, WA, USA)) based on the guidelines published by Corretti et al. (2002). Moreover, blood pressure was measured at rest. FMD measures the endothelium-dependent increase in blood vessel diameter in response to reactive hyperemia, as previously described by Schneider et al. (2008). After baseline images of the artery were recorded, reactive hyperemia was induced through inflation of a pneumatic tourniquet positioned proximal to the antecubital fossa to 50 mmHg above systolic blood pressure for 5 min. After abrupt deflation of the tourniquet images of the brachial artery were recorded once more for 90 s. FMD was quantified as the percent change in brachial artery diameter. NTGMD, an endothelium-independent index of vascular reactivity, was then measured. A second baseline image was acquired after a rest of 15 min. Five minutes after administration of 400 μg sublingual nitroglycerin spray, a final image of the artery was acquired. NTGMD was calculated as the percent change in blood vessel diameter in response to nitroglycerin.

At each visit blood was collected in citrated tubes, and plasma isolated and stored at −80 °C until the end of the study. sICAM-1, sVCAM-1, E-Selectin, and vWF were measured in blood. Moreover, sICAM-1, sVCAM-1 and E-Selectin were quantified using commercially available enzyme-linked immunosorbent assays (ELISA) kits purchased from Lincoplex (Linco Research, Inc., St. Charles, MO) and run on a Luminex 100 Multiplex system. ELISA assays were developed using paired antibodies for vWF purchased from Diagnostica Stago (Asnieres-Sur-Seine, France) as described by Schneider et al. (2010).

2.3. Meteorological and air pollution measurements

Continuous 2-min measurements of air temperature, relative humidity, and barometric pressure were obtained from the EPHD rooftop approximately 30 m above ground level. We calculated 24-h averages for the meteorological parameters if at least 66% of the 2-min measurements were available on a day. Daily 24-h concentrations (midnight to midnight) of $PM_{2.5}$ network data were obtained from a monitoring site located approximately 44 km (27 miles) east of the EPHD. In addition, 9 a.m. to 9 a.m. concentrations of $PM_{2.5}$ measured at the EPHD rooftop were available. The Spearman correlation between both measurements was 0.85 and rooftop $PM_{2.5}$ data was used to impute 3 days of missing network data based on a linear regression model. Rooftop data for meteorological data was complete. Hourly means of ozone concentration were measured at monitoring sites in Wake and Durham Counties, NC, located 42 km (26 miles) and 20 km (12 miles) away from the EPHD. The Spearman correlation between the two sites in Wake County and Durham County was 0.90. Ozone data obtained from Durham County were used to impute missing data in Wake County based on a linear regression model. We calculated maximum 8-h moving averages to analyze effects of ozone concentration. Furthermore, we considered the following time lags for meteorological and air pollution parameters in order to assess immediate, delayed or cumulative effects: 24-h averages of the examination day (lag 0), averages of 24–47 h (lag 1), 48–71 h (lag 2), 72–95 h (lag 3), 96-119 h (lag 4) before the visits and a 5-day average (lag 04).

2.4. Statistical analyses

Spearman's rank correlation coefficient was used to calculate correlations between different health outcomes as well as between meteorological and air pollution parameters. Additive mixed models with a random participant intercept and a compound symmetry covariance structure were used to assess temperature and ozone associations with endothelial function. Confounder models were established for each outcome separately and without consideration of the exposure variables (temperature and ozone). Confounders were selected by minimizing Akaike's Information Criterion (AIC). Potential confounders were included linearly and smoothly as penalized splines (P-splines) depending on the value of AIC. Long-term time trend (counts of the days during the study period) to control for seasonal variations, day of the week and relative humidity were forced into each confounder model. Barometric pressure was included only if the model fit improved based on the AIC value. With regard to relative humidity and barometric pressure all individual time lags were considered; but only the lag showing the strongest effect on the respective outcome based on the AIC value was included into the model. Temperature effects were estimated linearly as well as smoothly using P-splines. Effects are presented as percent changes of the outcome mean per 1 °C decrease in air temperature together with 95%-confidence intervals.

When analyzing ozone effects, the same confounder models were used as for the analysis of temperature effects. However, temperature was also forced into the model using the lag showing the strongest effect on the respective outcome. Ozone effects were estimated linearly and are presented as percent changes of the outcome mean per 0.01 ppm increases in ozone concentration together with 95%-confidence intervals.

For analyzing interactive effects between air temperature and air pollution, we divided $PM_{2.5}$ and ozone concentrations into low ($<25\%$-quartile), medium (25–74%) and high ($\geq 75\%$-quartile) concentrations and included an interaction term into the model. For categorized $PM_{2.5}$ and ozone, we included the same lag as the analyzed temperature lag, respectively.

Analyses were conducted using SAS statistical package (version 9.3; SAS Institute Inc, Cary,NC).

2.5. Sensitivity analyses

We conducted several sensitivity analyses to test the robustness of the associations: 1) we adjusted for $PM_{2.5}$ using the same lag as the analyzed temperature or ozone lag; 2) we adjusted for season; 3) trend was included smoothly using P-splines instead of linearly into all models; 4) we included all confounders using the same lag as the analyzed temperature or ozone lag; 5) temperature and ozone effects were recalculated using an autoregressive covariance structure instead of a compound symmetry covariance structure, given that there might be an autocorrelation between the repeated individual observations of the respective outcomes; 6) in order to assess the individual response of the participants we calculated random slopes for the temperature as well as ozone effects; 7) we checked effect estimates of the effects of relative humidity adjusting for long-term trend, day of the week and air temperature. Moreover, we conducted the following sensitivity analyses only for temperature effects: 8) we adjusted for ozone using the same lag as the analyzed temperature lag; 9) temperature effects were estimated using apparent temperature instead of air temperature. Apparent temperature was calculated using the following formula (Kalkstein and Valimont, 1986; Steadman, 1979): $at = -2.653 + (0.994 \times temp) + (0.0153 \times dp \times dp)$ with at= apparent temperature, temp=air temperature and dp=dew point temperature (Supplemental Material Eq. (A.1)).

3. Results

3.1. Study area and study population

The Raleigh/Durham/Chapel Hill metropolitan area in which the study participants reside has a population of more than 1,000,000 people. It has a temperate climate, with summer highs averaging 29.4–32.2 °C and summer lows averaging 18.3–21.1 °C. Winter highs average 7.2–10 °C and winter lows −1.1–1.7 °C. Twenty-two people with T2D aged 48 to 78 years were identified and volunteered to take part in the study. Each participant visited the EPHD on four consecutive days (Tuesday–Friday) for collection of clinical data; hence as many as four repeated measurements per person were available for the analyses. Nearly 60% of the visits took place between April and October. In Table 1, the characteristics of the study population are summarized. Participants had a mean age of 61 years, and two thirds of them were males. Half of the participants were obese with a body mass index (BMI) ≥ 30 kg/m^2, and almost 60% had systolic blood pressure ≥ 140 mmHg. Half of the individuals were diagnosed with T2D at least 5 years prior to participation in the study. More than 60% of the individuals were taking medication for diabetes such as metformin. Over half of the study population was taking statins and nearly two thirds took aspirin regularly, which could interfere with measurement of vascular inflammation.

3.2. Clinical measurements

A description of the repeated measurements of the study population for FMD, NTGMD, sICAM-1, sVCAM-1, E-Selectin and vWF is shown in Table 2. Depending on the health outcome, a maximum of 83 and a minimum of 78 measurements were available. On average, FMD was 5.9% and brachial artery diameter increased by 13.4% with nitroglycerin. Spearman's rank correlation coefficient between FMD and NTGMD was $r_s=0.52$, indicating a medium correlation. Correlations between blood parameters were very low to moderate with rank correlation coefficients ranging from $r_s=0.04$ between vWF and E-Selectin and $r_s=0.55$ between sVCAM-1 and vWF (Supplemental Table A.1).

3.3. Meteorological and air pollution measurements

A description of the meteorological and air pollution measurements throughout the study period is shown in Table 3. The $PM_{2.5}$ mean over the study period was between the annual primary and secondary U.S. National Ambient Air Quality Standard of 12 μg/m^3 and 15 μg/m^3, respectively (U.S. EPA 2011). Meteorological parameters were low to moderately correlated ($0.18 \leq |r_s| \leq 0.42$). Temperature was moderately correlated with $PM_{2.5}$ ($r_s=0.40$) and ozone ($r_s=0.59$). The correlation between $PM_{2.5}$ and ozone was also moderate with $r_s=0.33$. Moreover, there was a low correlation between relative humidity or barometric pressure and $PM_{2.5}$ with $r_s<0.30$ (Supplemental Table A.2). Fig. 1 by Schneider et al. (2008) shows the course of patient visits, air temperature and $PM_{2.5}$ over the study period.

3.4. Effects of air temperature on markers of endothelial function

Temperature effects were estimated linearly since no deviation from linearity was found. When analyzing temperature effects, sICAM-1 and E-Selectin were log transformed in order to provide a normal distribution of the model residuals. Information on confounder models for each outcome variable is presented in Supplemental table A.3.

No associations between decreases in air temperature and sVCAM-1, E-Selectin, or vWF were found. However, we observed associations between air temperature decreases and FMD, NTGMD and sICAM-1 (Fig. 1). A 1 °C decrease in air temperature was associated with an immediate (lag 0: −2.2% (95%-confidence interval:[−4.7;0.3%]) as well as delayed (e.g. lag 4: (−3.9%[−6.5; −1.2%]) decrease in FMD.

NTGMD also decreased immediately (−1.7%[−3.3; 0.04%]) in relation to a 1 °C decrease in the preceding 24-h temperature average. Furthermore, we found a non-significant trend for an immediate increase (1.1%[−0.2;2.4%]) as well as an increase in the 5-day temperature average (2.6%[−0.2;5.6%]) in sICAM-1 associated with a 1 °C decrease in air temperature.

3.5. Effects of ozone on markers of endothelial function

For all outcomes, except NTGMD where temperature was included smoothly, temperature was included linearly into the model. A 0.01 ppm increase in ozone was associated with an immediate decrease in FMD of 14.6%[−26.3; −2.9%] and with a decrease in FMD of 13.5%[−27.0; 0.04%] with a delay of one day (Fig. 2). We observed no changes in NTGMD, sICAM-1, sVCAM-1,

E-Selectin or vWF in association with an increase in ozone concentration.

3.6. Interactive effects between air temperature and air pollution

Since no temperature effects were found for sVCAM-1, E-Selectin and vWF, interactive effects between air temperature and $PM_{2.5}$ were only analyzed for FMD, NTGMD and sICAM-1. Increasing $PM_{2.5}$ concentration strengthened the association between temperature and FMD with a delay of four days (Table 4C). Temperature effects on FMD were more pronounced in association with high (−8.7% [−13.0; −4.4%]) $PM_{2.5}$ concentrations compared to medium (−1.8% [−4.8; 1.2%]) or low (−1.8%[−5.5; 1.8%]) concentrations with a delay of four days (p-value of the interaction term: 0.01).

Table 1
Description of the study population ($n=22$).

Characteristics	Mean ± SD
Age (years)	61 ± 8
Body mass index (kg/m^2)	33 ± 7
Gender	***n* (%)**
Female	8 (36)
Male	14 (64)
Age (years)	
< 60	9 (41)
≥ 60	13 (59)
Body mass index (kg/m^2)	
< 30	10 (46)
≥ 30	12 (55)
Systolic blood pressure (mmHg)	
< 140	9 (41)
≥ 140	13 (59)
Adiponectin (ng/mL)	
< 3700	11 (52)
≥ 3700	10 (48)
Season when visits occurred	
November–March	9 (41)
April–October	13 (59)
Diseases	***n* (%)**
Duration of diabetes ≥ 5 years	11 (50)
Coronary artery disease	4 (18)
Peripheral vascular disease	3 (14)
Nephropathy	8 (36)
Medication	***n* (%)**
Metformin	14 (64)
Thiazolidinediones	6 (27)
Sulfonylureas	10 (45)
Statins	12 (55)
Aspirin	14 (64)
Beta-adrenergic receptor blockers	9 (41)
Angiotensin converting enzyme-inhibitors	12 (55)
Diuretics	8 (36)
Angiotensin II-receptor blocker	3 (14)
Estrogen	2 (9)

We did not observe significant interactive effects between air temperature and $PM_{2.5}$ on FMD for lag 0. Immediate temperature effects were stronger for NTGMD and high $PM_{2.5}$ concentrations than for medium and low $PM_{2.5}$ levels (Table 4C). However, the p-value of the interaction term was not statistically significant (0.17). No interactive effects between air temperature and $PM_{2.5}$ on sICAM-1 were found (data not shown).

Interactive effects between air temperature and ozone were only analyzed for FMD since we did not observe effects of ozone on the other outcomes. Immediate as well as one day delayed temperature effects on FMD were stronger together with high ozone concentration compared to medium or low ozone levels (p-value of the interaction term lag 0: 0.34, lag1: 0.19) (Table 5C).

3.7. Sensitivity analyses

Sensitivity analyses on temperature effects were conducted for FMD, NTGMD and sICAM-1. Overall, effects of air temperature were quite robust when conducting several sensitivity analyses (Supplemental Fig. A.1).

The immediate temperature effect on FMD slightly strengthened when adjusted for $PM_{2.5}$ and ozone as well as when using apparent instead of air temperature. When additionally adjusting for $PM_{2.5}$ the immediate effect of temperature decreases on NTGMD weakened and was not statistically significant. However, the immediate association between temperature and NTGMD remained similar when conducting other sensitivity analyses. Results were also similar when including confounders using the same lag as the analyzed temperature or ozone lag, respectively (data not shown).

Effects of apparent temperature on FMD, sICAM-1 and NTGMD were similar to those of air temperature (Supplemental Fig. A.1). When evaluating effects of relative humidity no significant effects were found (data not shown). Sensitivity analyses on ozone effects were conducted for FMD only since we did not find ozone effects on the other outcomes. Ozone effects on FMD remained nearly unchanged when we conducted sensitivity analyses supporting the robustness of the reported associations (Supplemental Fig. A.2). Random slopes for temperature effects were calculated for FMD time lag 0 and lag 4, for ozone effects for time lag 0 and lag 1, respectively. Furthermore, we analyzed subject-specific responses to temperature decreases for NTGMD lag 0 and for sICAM-1 considering the 5-day average since strongest effects were shown for those time lags.

Random slopes for FMD and NTGMD showed that individual responses of the participants to temperature were similar across all participants. The effects of temperature decreases were similar during summer and winter showing an immediate decrease in FMD and NTGMD for all participants (Supplemental Fig. A.3A).

However, for sICAM-1 subject-specific responses were slightly heterogeneous (Supplemental Fig. A.3A). Three individuals showed a decrease in sICAM-1 in association with a 1 °C decrease in air temperature; whereas, the other participants showed an increase in sICAM-1. Those three individuals did not differ from the others with respect to age, BMI, medication and disease status. However, all

Table 2
Description of health parameters.

	n	Mean ± SD	Minimum	Maximum
Flow-mediated dilatation[a] (%)	83	5.9 ± 3.9	0.4	16.3
Nitroglycerin-mediated dilatation[a] (%)	78	13.4 ± 7.4	3.8	32.1
Soluble intercellular adhesion molecule-1[a] (ng/ml)	80	186.4 ± 191.1	61.8	794.1
Soluble vascular cell adhesion molecule-1[a] (ng/ml)	80	836.8 ± 156.5	584.2	1125.3
Soluble endothelial-leukocyte adhesion molecule[a] (ng/ml)	80	35.7 ± 17.8	15.1	82.1
Von Willebrand factor[a] (%)	80	106.7 ± 14.1	82.7	130.8

[a] Mean of the patient means for as many as four measurements from 22 individuals.

Table 3
Description of meteorological parameters, ozone and $PM_{2.5}$ throughout the study period (19 November 2004 to 09 December 2005).

	n	mean ± SD	min	25%	median	75%	max	IQR[c]
Air temperature (°C)	385	15.9 ± 8.5	−6.5	9.6	16.4	23.5	31.5	13.9
Relative humidity (%)	385	62.5 ± 16.6	25.1	50.2	65.1	75.5	97.7	25.3
Barometric pressure (hPa)	386	1001.2 ± 6.5	981.4	997.9	1001.9	1004.8	1021.9	6.9
Ozone[a] (ppm)	377	0.041 ± 0.016	0.005	0.027	0.039	0.052	0.082	0.025
$PM_{2.5}$[b] ($\mu g/m^3$)	383	13.6 ± 7.0	2.0	8.4	12.6	17.7	38.9	9.3

[a] Maximum 8-h moving averages.
[b] Particulate matter with a size range of $< 2.5\ \mu m$ in aerodynamic diameter.
[c] Interquartile range.

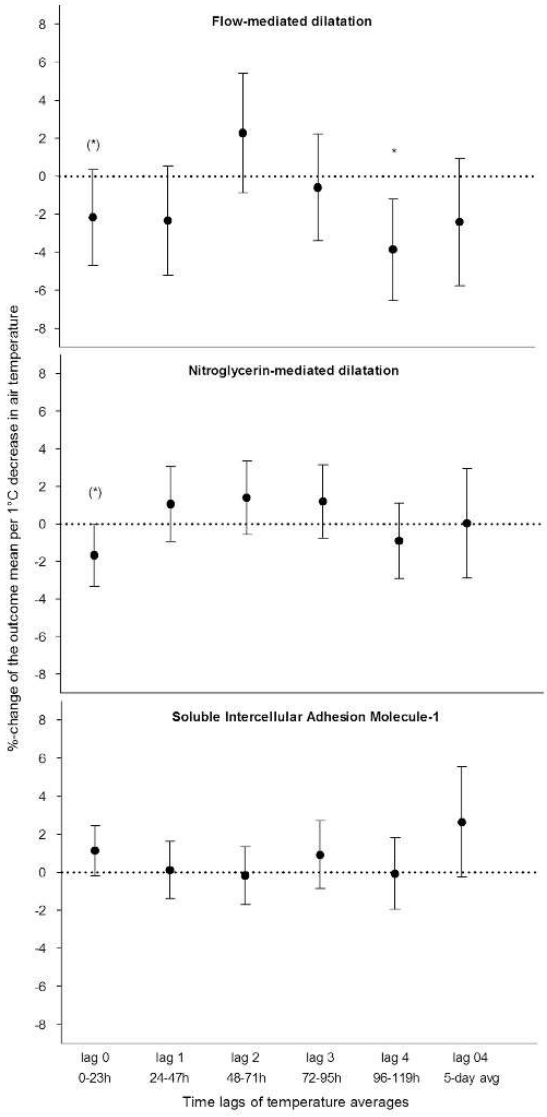

Fig. 1. Changes in flow-mediated dilatation, nitroglycerin-mediated dilatation and soluble intercellular adhesion molecule-1 in association with a 1 °C decrease in air temperature. $^{*}p$-value < 0.05, $(^{*})p$-value < 0.1.

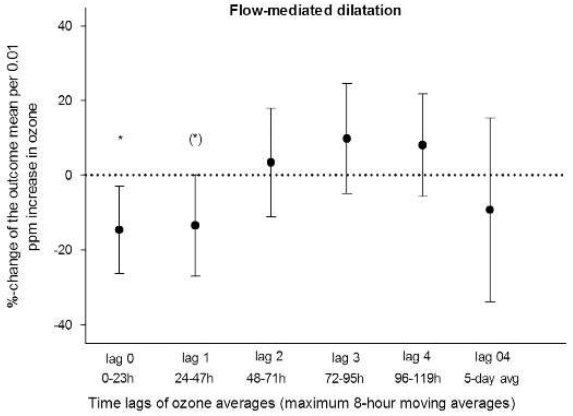

Fig. 2. Changes in flow-mediated dilatation in association with a 0.01 ppm increase in ozone. $^{*}p$-value < 0.05, $(^{*})p$-value < 0.1.

of the three mentioned participants had very high baseline levels of sICAM-1, with levels above the mean of 186.4 ng/ml and up to 986.5 ng/ml (data not shown). Subject-specific associations with a 0.01 ppm increase in ozone and FMD were also similar across all participants (Supplemental Fig. A.3B).

4. Discussion

4.1. Summary

A 1 °C decrease in air temperature was associated with an immediate as well as a delayed decrease in FMD. Moreover, NTGMD decreased immediately in association with a temperature decrement. With regard to sICAM-1, we found an increase on the same day as well as an increase in association with a 1 °C decrease in the 5-day temperature average. Furthermore, a 0.01 ppm increase in ozone led to a decrease in FMD on the same day and with a delay of one day. Effects of temperature decreases and ozone increases were observed during winter as well as during the summer. Temperature effects on FMD were more pronounced together with high $PM_{2.5}$ as well as high ozone concentration compared to medium or low air pollution concentrations. Immediate effects of temperature decreases on NTGMD were also stronger when $PM_{2.5}$ concentrations were high.

4.2. Effects of air temperature and ozone on endothelial function

Nawrot et al. 2005 and Widlansky et al. 2007 reported an association between temperature and FMD (Nawrot et al., 2005;

Table 4
Changes in flow-mediated dilatation (lag 0, lag 4) and nitroglycerin-mediated dilatation (lag 0) in association with A) a 1 °C decrease in air temperature, B) a 10 μg/m³ increase in $PM_{2.5}$, C) interactive effects between air temperature and $PM_{2.5}$ (temperature effect is shown).

	A) Air temperature		B) $PM_{2.5}$[e]	C) Interactive effects		
	Main model[d]	add. adjusted for $PM_{2.5}$[e]	Main model[f]	Temperature and $PM_{2.5}$[e] $PM_{2.5}$[e] concentrations:		
	%-Change (95%-CI[c])	%-Change (95%-CI[c])	%-Change (95%-CI[c])	<25%-quartile %-Change (95%-CI[c])	25%-74% %-Change (95%-CI[c])	≥75%-quartile %-Change (95%-CI[c])
FMD[a] lag 0	−2.2 (−4.7;0.3)	−3.9 (−6.7;−1.2)	−27.4 (−47.2;−7.7)	−3.1 (−6.9;0.8)	−4.7 (−7.9;−1.6)	0.2 (−5.5;5.8)
FMD[a] lag 4	−3.9 (−6.5;−1.2)	−2.9 (−5.8;0.0)	19.0 (−5.3;43.3)	−1.8 (−5.5;1.8)	−1.8 (−4.8;1.2)	−8.7 (−13.0;−4.4)
NTGMD[b] lag 0	−1.7 (−3.3;−0.04)	−1.4 (−3.2;0.4)	4.6 (−6.7;15.9)	−0.9 (−3.0;1.3)	−1.4 (−3.3;0.6)	−4.2 (−7.5;−0.9)

[a] Flow-mediated dilatation.
[b] Nitroglycerin-mediated dilatation.
[c] Confidence interval.
[d] See Supplemental Table A.3.
[e] Particulate matter with a size range of <2.5 μm in aerodynamic diameter.
[f] See Supplemental Table A.3+adjusted for air temperature using the same lag as the analyzed $PM_{2.5}$ lag.

Table 5
Changes in flow-mediated dilatation (lag 0, lag 1) in association with A) a 1 °C decrease in air temperature, B) a 0.01 ppm increase in ozone concentration, C) interactive effects between temperature and ozone (temperature effect is shown).

	A) Air temperature		B) Ozone	C) Interactive effects		
	Main model[c]	add. adjusted for Ozone	Main model[d]	temperature and ozone Ozone concentrations:		
	%-Change (95%-CI[b])	%-Change (95%-CI[b])	%-Change (95%-CI[b])	<25%-quartile %-Change (95%-CI[b])	25%-74% %-Change (95%-CI[b])	≥75%-quartile %-Change (95%-CI[b])
FMD[a] lag 0	−2.2 (−4.7;0.3)	−3.0 (−5.5;−0.4)	−14.6 (−26.3;−2.9)	−1.6 (−4.9;1.8)	−1.5 (−4.8;1.9)	−5.3 (−10.3;−0.2)
FMD[a] lag 1	−2.3 (−5.2;0.5)	−2.6 (−5.4;0.3)	−13.5 (−27.0;0.04)	−1.0 (−4.5;2.6)	−2.6 (−6.3;1.2)	−7.3 (−13.2;−1.4)

[a] Flow-mediated dilatation.
[b] Confidence interval.
[c] See Supplemental Table A.3.
[d] Adjusted for time trend, day of the week, air temperature (lag 4), relative humidity (lag 0) and barometric pressure (lag 4).

Widlansky et al., 2007). The findings of Widlansky et al. (2007) are consistent with our results showing FMD to be lowest in the coldest temperature quartile and to be highest in the warmest quartile of outdoor temperature. In contrast to our findings Nawrot et al. showed a linear decrease in FMD with increasing temperature on the day of the visit (Nawrot et al., 2005). The inconsistent findings may be in part due to differences in study cohorts; we investigated diabetic individuals, whereas, Nawrot and co-authors investigated a randomly selected sample of the general population (Nawrot et al., 2005). For example, the individuals examined by Nawrot et al. were younger (mean age: women 40.1 years, men 41.1 years) and were not obese, with a mean BMI of 25.0 kg/m² in women and 26.1 kg/m² in men, respectively. The mean temperature (11.2 °C (SD 6.6)) during the study period was lower compared to our study (Nawrot et al., 2005). Zanobetti et al. 2014 reported a linear relationship of temperature and brachial artery diameter, but observed no association between temperature and FMD or NTGMD (Zanobetti et al., 2014). However, all the results show that even if people are exposed to temperature changes for a short period of time, since people generally spent most of their time indoors; their body reacts to that environmental stimuli.

Halonen et al. 2010 reported a linear increase in sICAM-1 in association with a temperature decrease (Halonen et al., 2010). We also found a trend for increased sICAM-1 associated with decreased temperature, though it did not quite reach statistically significance. Halonen et al. (2010) also only observed a statistically significant increase in sICAM-1 associated with temperature decreases over cumulative lags of three and four weeks whereas, we observed the strongest association for a 5-day average immediately preceding the participant's visits.

Several studies have reported a decrease in FMD associated with an increase in air pollution (Briet et al., 2007; Hashemi et al., 2012; O'Neill et al., 2005; Schneider et al., 2008). We observed an immediate 14.6%[−26.3;−2.9%] decrease in FMD and a 13.5% [−27.0;0.04%] decrease with a one day lag associated with a 0.01 ppm increase in ozone. This corresponded to a 36.5% [−65.7;−7.3%] immediate decrease in FMD in association with an interquartile range (IQR) increase of 0.025 ppm in ozone. These findings are consistent but stronger than previous results based on the same data showing an immediate 16.1%[−32.2; 0.0%] decrease in FMD on the same day in association with an IQR increase of 9.3 μg/m³ in $PM_{2.5}$ (Schneider et al., 2008). We observed no association between ozone and sICAM-1, sVCAM-1, E-Selectin and vWF. Schneider et al. 2010 also reported no associations of $PM_{2.5}$ with sICAM-1, sVCAM-1, E-Selectin and vWF (Schneider et al., 2010).

For FMD and NTGMD, subject-specific responses to temperature and ozone were similar across all participants. Moreover, the effects were similar in winter and summer, suggesting that our findings do not present a pure "cold effect" but also an effect due to mild temperature decreases in summer. Comparable effects of temperature decreases during winter and summer have also been reported in a study that examined the association between air temperature and the occurrence of myocardial infarctions (Wolf et al., 2009). Therefore, it is likely that temperature decreases might

have an influence on endothelial function independently of the season.

Random slopes for sICAM-1 were slightly heterogeneous and, thus, might explain the non-significant results for sICAM-1. Three individuals differed from the others showing a decrease instead of an increase in sICAM-1 in association with a temperature decrement. In particular, one of the three mentioned individuals had very high sICAM-1 levels with values between 627.8 ng/ml and 986.5 ng/ml. Therefore, we assume that the difference in the temperature effects is due to the high baseline levels of sICAM-1, given that an increase in sICAM-1 was more likely when levels were around the mean value of 186.4 ng/ml.

4.3. Interactive effects between air temperature and air pollution

Our results suggest that temperature and $PM_{2.5}$ as well as temperature and ozone might have additive effects on endothelial function. Interactive effects between air temperature and air pollution have also been shown for mortality, suggesting that interactive effects between temperature and other environmental exposures should be taken into account (Burkart et al., 2013; Roberts, 2004; Stafoggia et al., 2008). However, to the best of our knowledge this is the first study to show interactive effects between air temperature and air pollution on endothelial function.

4.4. Plausible biological mechanisms

Regulation of vascular tone is impaired in individuals with T2D (Caballero et al., 1999), contributing to impaired adaptation to temperature decreases or air pollution increases that may not be clinically significant in healthy individuals. We suggest that endothelial dysfunction might be a possible mechanism explaining cardiovascular events in diabetic individuals associated with temperature and air pollution. It has been shown that a decrease in air temperature leads to an activation of the sympathetic nervous system that results in peripheral vasoconstriction and impaired vasodilatation in response to hyperemia (Hanna, 1999; Widlansky et al., 2007). This may be due in part to decreased bioavailability of NO, reflecting endothelial dysfunction. Since nitroglycerin serves as an exogenous NO donor, the association of NTGMD with temperature suggests that altered smooth muscle cell function may also play a role (Deanfield et al., 2007). Since we observed an immediate temperature effect on FMD as well as on NTGMD, we assume that temperature decreases have an immediate impact on the function of the smooth muscle cells rather than on the bioavailability of NO.

The delayed temperature effect on FMD (but not NTGMD) for lag 4 suggests an endothelium-dependent mechanism, possibly through changes in the synthesis and bioavailability of NO. Previous studies have also suggested an influence of air temperature on the bioavailability of NO, but did not examine NTGMD (Nawrot et al., 2005; Widlansky et al., 2007). However, the delayed effect on FMD was an unexpected finding which certainly needs further investigation. Increased ozone concentration was only associated with decreased FMD. Similarly, Schneider et al., 2008 also observed an immediate decrease in FMD associated with an increase in $PM_{2.5}$, but no association between NTGMD and $PM_{2.5}$ (Schneider et al., 2008). Hence, we conclude that decreases in FMD in association with changes in air pollution are best explained by a reduced synthesis and bioavailability of NO.

4.5. Strength and limitations

One of the strengths of the study is that all participants adhered to the protocol and completed most of the four visits. However, autocorrelation should be considered as the visits took place on consecutive days. We checked for autocorrelation as a sensitivity analysis and the results were similar when using an autoregressive covariance structure. A limitation of the study is the small sample size, which may account for the non-significant results for several outcomes. A further strength of the study is that in contrast to previous studies, FMD as well as NTGMD was analyzed. Hence, it was possible to determine whether FMD was mediated by endothelial dysfunction (lack of NO bioavailability) or via an endothelium-independent mechanism such as changes in smooth muscle cell function. Air pollution was measured at different geographical sites within the airshed encompassing the residences of the study participants, and the correlation between the sites was very strong, suggesting that we were accurately assessing ambient exposure of the study participants. Further, all the participants lived within a 30-mile radius of Chapel Hill, and no one had any unusual exposure to air pollution because of residence or occupation (Schneider et al., 2008). However, our study shows the general limitation of accurately measuring exposure of participants as all panel studies with a similar design.

Moreover, our findings were quite robust to several sensitivity analyses. Because we investigated a small cohort having the clinical diagnosis T2D the results are not generalizable to the general population. However, as persons having diabetes are regarded as an especially susceptible group this might give better insight into possible biological mechanism explaining cardiovascular events in association with environmental changes. Since we investigated a 1 °C decrease in temperature and a 0.01 ppm increase in ozone rather than extreme events, the results might also be generalizable to other regions with similar climate to the Raleigh/Durham/Chapel Hill metropolitan area.

5. Conclusion

Our findings suggest a linear association between temperature decreases as well as ozone increases on endothelial function in diabetic individuals. Moreover, we hypothesize that there are interactive effects between air temperature and air pollution on cardiovascular health. We observed similar associations during winter and the summer. Hence, we conclude that endothelial dysfunction might be a possible mechanism explaining cardiovascular events in association with environmental changes that have been observed during winter as well as during summer.

Ethical standards

All participants gave written informed consent. The University of North Carolina Human Studies Biomedical Institutional Review Board and the U.S. EPA approved the study protocol.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this paper can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.08.003.

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SUPPLEMENTAL MATERIAL

Dew point temperature (dp) was calculated as follows:

$$dp = \frac{1}{\frac{1}{temp + 241.413} - \frac{\log_{10}(\frac{rh}{100})}{1838.675}} - 241.413 \quad (1)$$

with temp= air temperature and rh=relative humidity.

Supplemental Table 1. Spearman's rank correlation coefficients for clinical measurements

	FMD[a]	NTMGD[b]	sICAM-1[c]	sVCAM-1[d]	E-Selectin[e]	vWF[f]
FMD[a]	1	0.52	-0.05	-8	-0.28	-0.38
NTGMD[b]		1	0.13	0.22	0	-0.27
sICAM-1[c]			1	0.46	0.44	0.20
sVCAM-1[d]				1	0.26	0.55
E-Selectin[e]					1	0.04
vWF[f]						1

[a]Flow-mediated dilatation

[b]Nitroglycerin-mediated dilatation

[c]Soluble intercellular adhesion molecule-1

[d]Soluble vascular cell adhesion molecule-1

[e]Soluble endothelial-leukocyte adhesion molecule

[f]Von Willebrand factor

Supplemental Table 2. Spearman's rank correlation coefficients for meteorological and air pollution parameters.

	Air temperature (°C)	Relative humidity (%)	Barometric pressure (hPa)	$PM_{2.5}$[a] ($\mu g/m^3$)	Ozone (ppm)
Air temperature (°C)	1	0.42	-0.18	0.40	0.59
Relative humidity (%)		1	-0.23	0.07	0.07
Barometric pressure (hPa)			1	0.12	-0.12
$PM_{2.5}$[a] ($\mu g/m^3$)				1	0.33
Ozone (ppm)					1

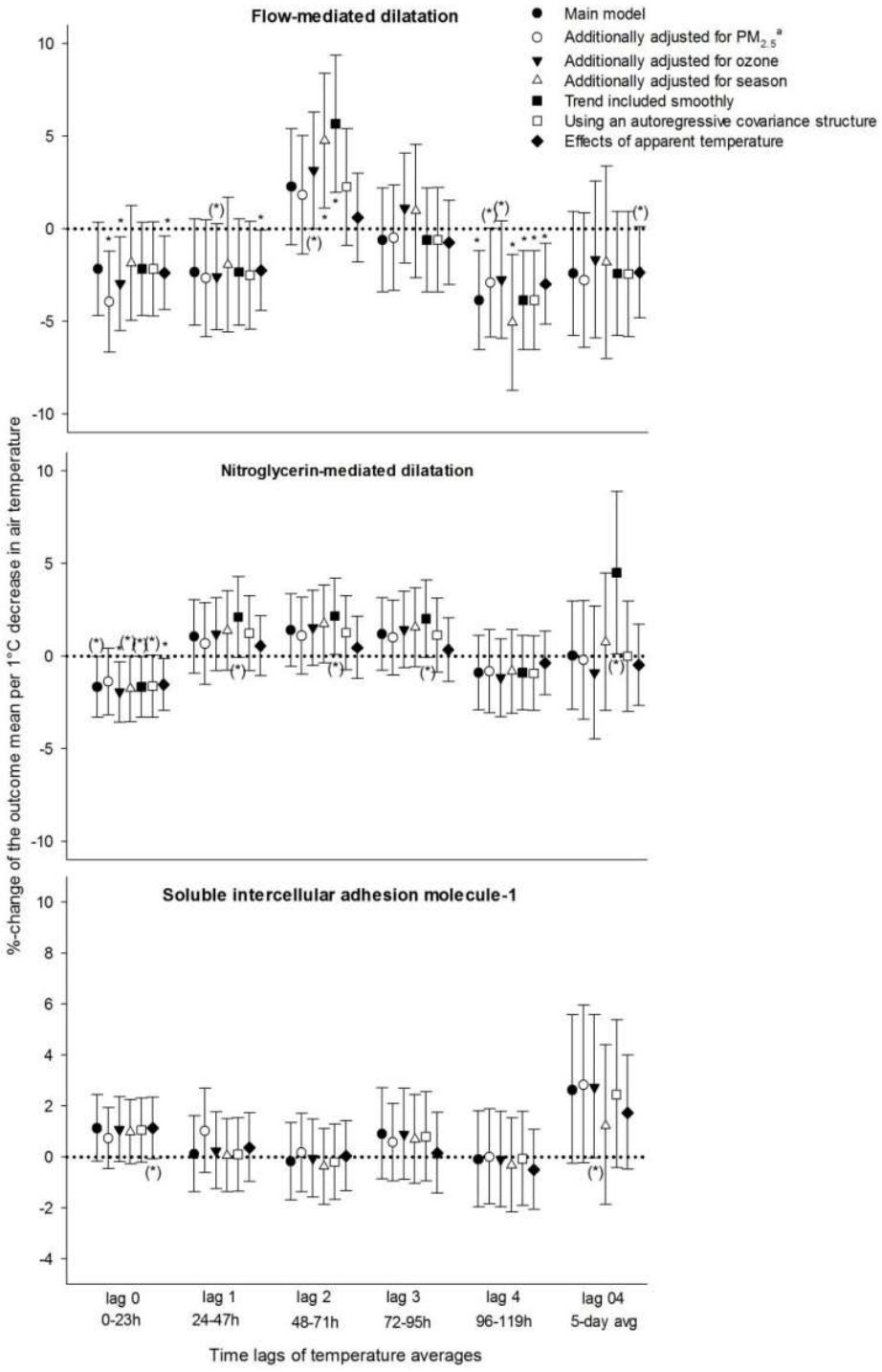
[a]particulate matter with a size range of <2.5 μm in aerodynamic diameter

Supplemental Table 3. Confounder models for the association between air temperature and endothelial function.

	Potential confounders			
Outcomes	**Time trend**	**Day of the week**	**Relative humidity**	**Barometric pressure**
Flow-mediated dilatation	✓ linearly	✓	✓ linearly (time lag 0)	✓ linearly (time lag 4)
Nitroglycerin-mediated dilatation	✓ linearly	✓	✓ linearly (time lag 4)	✓ linearly (time lag 04)
Soluble intercellular adhesion molecule-1[a]	✓ smoothly	✓	✓ smoothly (time lag 3)	✓ linearly (time lag 3)
Soluble vascular cell adhesion molecule-1	✓ linearly	✓	✓ linearly (time lag 1)	✓ linearly (time lag 3)
Soluble endothelial-leukocyte adhesion molecule[a]	✓ linearly	✓	✓ smoothly (time lag 3)	✓ linearly (time lag 3)
Von Willebrand factor	✓ linearly	✓	✓ linearly (time lag 4)	✓ linearly (time lag 04)

Confounders were included linearly and smoothly as penalized splines depending on the value of Akaike's Information Criterion.

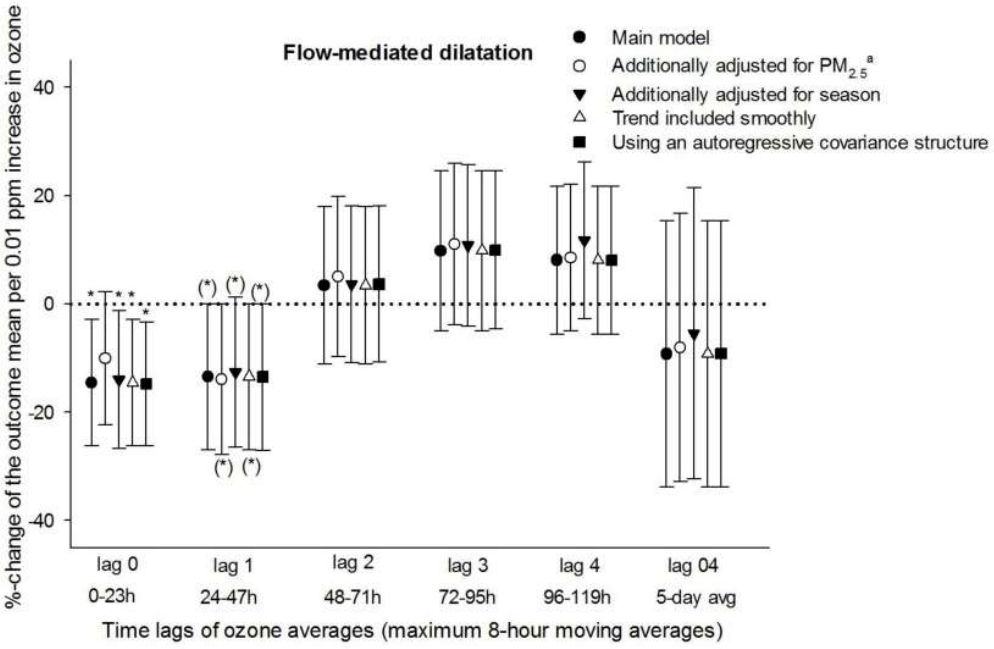
[a]Outcome log transformed



Supplemental Figure 1. Changes in flow-mediated dilatation, nitroglycerin-mediated dilatation and soluble intercellular adhesion molecule-1 in association with a 1°C decrease in air temperature / apparent temperature.

*p-value<0.05, (*)p-value<0.1

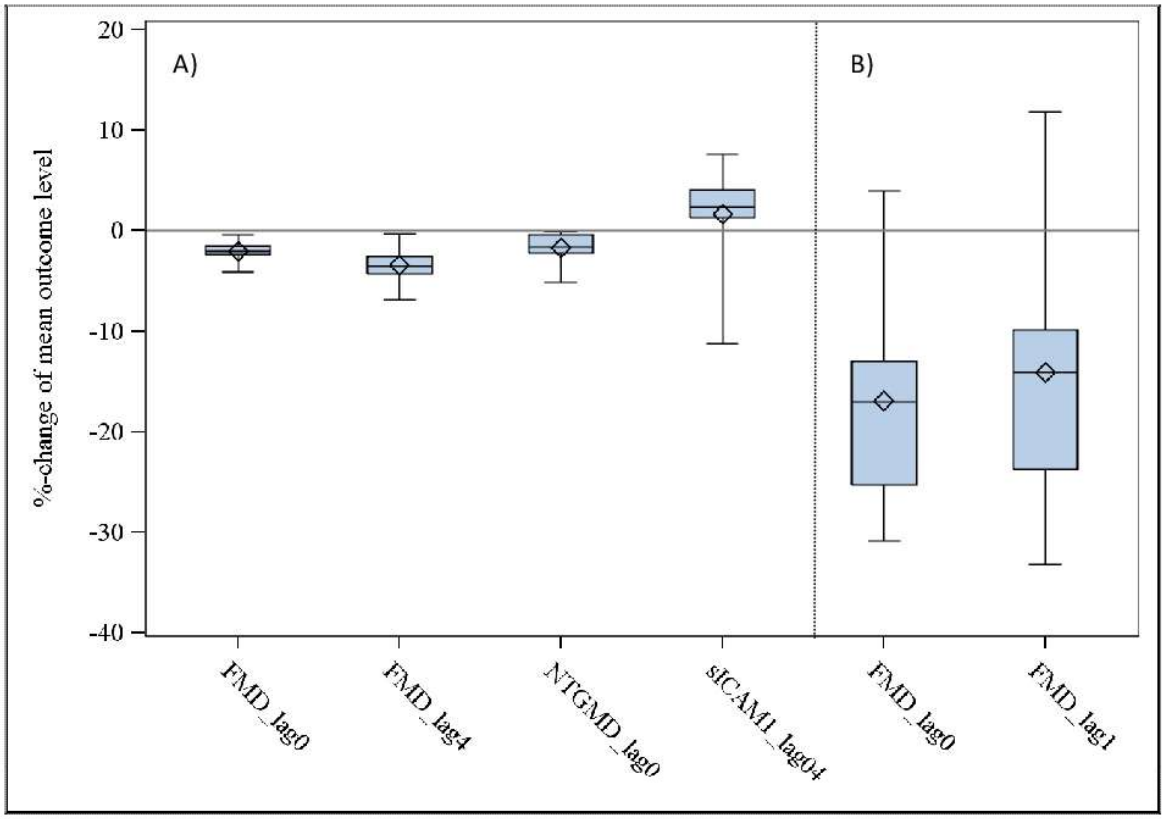
[a]particulate matter with a size range of <2.5 µm in aerodynamic diameter



Supplemental Figure 2. Changes in flow-mediated dilatation in association with a 0.01 ppm increase in ozone.

*p-value<0.05, (*)p-value<0.1

[a]particulate matter with a size range of <2.5 μm in aerodynamic diameter



Supplemental Figure 3. A) Subject-specific responses to a 1°C decrease in air temperature on flow-mediated dilatation (lag 0, lag 4), nitroglycerin-mediated dilatation (lag 0) and soluble intercellular adhesion molecule-1 (5-day average (lag 04)). B) Subject-specific responses to a 0.01 ppm increase in ozone on flow-mediated dilatation (lag 0, lag 1).

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