ASSOCIATION OF LONG-TERM EXPOSURE TO AIR POLLUTION WITH ARTERIAL BLOOD PRESSURE AND HYPERTENSION

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ABBREVIATIONS AND UNITS

Abbreviations

ACE	angiotensin-converting enzyme
ANS	autonomic nervous system
AOD	automated oscillometric device
BMI	body mass index
BP	blood pressure
BPLM	blood pressure lowering medication
CHD	coronary heart disease
CI	confidence interval
CRP	C-reactive protein
CVD	cardiovascular disease
CYP1A1	cytochrome P450, family 1, member A1
DBP	diastolic blood pressure
DEE	diesel engine exhaust
DNA	deoxyribonucleic acid
eNOS	endothelial nitric oxide synthase enzyme
ET	endothelin
ETS	environmental tobacco smoke
EU	European Union
EURAD-CTM	the European Air pollution Dispersion Chemistry and Transport Model
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
HDL	high-density lipoprotein
HO	heme oxygenase
HR	hazard ratio
ICAM-1	intercellular cell adhesion molecule-1
IL	interleukin
iNOS	inducible nitric oxide synthase enzyme
IQR	interquartile range
JNC7	the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure
LDL	low-density lipoprotein

MONICA	monitoring trends and determinants in cardiovascular disease
NAD(P)H	nicotinamide adenine dinucleotide phosphate-oxidase
nNOS	neuronal nitric oxide synthase enzyme
NQO1	nicotinamide adenine dinucleotide dehydrogenase, qui-none 1
Nrf2	nuclear factor (erythroid-derived 2)-like 2
OR	odds ratio
PAH	polycyclic aromatic hydrocarbons
PM	particulate matter
PN	particle number
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species
RR	relative risk
RT-qPCR	real-time quantitative polymerase chain reaction
RZS	random-zero sphygmomanometer
SBP	systolic blood pressure
SD	standard deviation
SES	socio-economic status
T2DM	type 2 diabetes mellitus
TNFα	tumor necrosis factor alpha
UFP	ultrafine particles
VCAM-1	vascular cell adhesion molecule-1
VOC	volatile organic compound
WHO	the World Health Organization
WHR	waist-hip ratio

The abbreviations of chemical elements and compounds followed the International Union of Pure and Applied Chemistry nomenclature

Units

°C	degree(s) Celsius
g	gram(s)
kg	kilogram(s)
L	liter(s)
m	meter(s)
mm	millimeter
mmHg	millimeter of mercury
µ	micro (10^{-6})
Nm	nanometer
ppm	parts per million

ZUSAMMENFASSUNG

Bluthochdruck ist der wichtigste Risikofaktor für Mortalität und Morbidität weltweit. Daher besitzen Faktoren, die den Blutdruck erhöhen und auf große Teile der Bevölkerung einwirken, eine hohe Relevanz bei der Erforschung von kardiovaskulären Erkrankungen. Es gibt erste Hinweise darauf, dass Luftverschmutzung ein solcher Faktor ist.

Der Zusammenhang zwischen Luftverschmutzung und kardiovaskulärer Morbidität und Mortalität ist in den letzten zwei Jahrzehnten eingehend untersucht und nachgewiesen worden. Einige Studien haben gezeigt, dass eine kurzfristige Erhöhung der Konzentration der Luftschadstoffe zu einer akuten Steigerung des Blutdrucks führen kann. Es wird vermutet, dass Langzeitexpositionen gegenüber Luftverschmutzung stärkere Auswirkungen auf den Blutdruck haben könnten. Außerdem könnte chronisch erhöhter Blutdruck als Teil des pathologischen Wirkungspfads verstanden werden, wie Luftverschmutzung zu Herz-Kreislauf Erkrankungen und Mortalität führen könnte.

Bislang ist die Evidenz für den Zusammenhang zwischen langfristiger Belastung gegenüber Luftverschmutzung und arteriellem Blutdruck nicht ausreichend. Es ist außerdem nicht klar, welche Partikel, Gase und andere Komponenten der Luftverschmutzung für eine Erhöhung des Blutdrucks verantwortlich sein könnten. Auch der biologische Wirkungspfad der Luftverschmutzung auf den Blutdruck ist noch nicht etabliert beschrieben worden.

Ziel dieser Studie war es, sowohl die Assoziation zwischen der langfristigen Luftschadstoffexposition und dem arteriellen Blutdruck, als auch mögliche zugrundeliegende Wirkungspfade, zu untersuchen. Die Forschungsfragen wurden mithilfe zweier methodischer Ansätze beantwortet: (1) Auswertung von Daten einer großen bevölkerungsbasierten Kohortenstudie und (2) Messung und statistische Auswertung von Daten aus einem Tierexperiment.

Für den ersten methodischen Ansatz wurden Daten aus der Heinz Nixdorf Recall Studie, einer prospektiven Kohorte im Ruhrgebiet, ausgewertet. Langzeitkonzentrationen verschiedener Komponenten der Luftverschmutzung aus urbanem Hintergrund wurden mit einem Dispersions- und Chemie-Transport Modell geschätzt und den Wohnadressen der Studienteilnehmer

zugewiesen. Blutdruckmessungen, Laboruntersuchungen, anthropometrische Messungen sowie Befragungen zum Lebensstil, zur Bildung, zu Vorerkrankungen und anderen Risikofaktoren wurden von geschulten Mitarbeitern anhand standardisierter Vorgaben durchgeführt und erfasst. Außerdem wurden Informationen über Verkehrslärm und der sozio-ökonomische Status der Nachbarschaft erhoben. Der Zusammenhang zwischen Langzeitbelastung gegenüber Luftschadstoffen mit dem gemessenen Blutdruck und einer Hypertonie wurden mit multiplen Regressionsmodellen analysiert, welche für relevante Kovariablen und die Einnahme von antihypertensiven Medikationen adjustiert wurden. Multi-Pollutant Modelle (Einbeziehung mehrerer Luftverschmutzungskomponenten) wurden berechnet, um die verantwortlichen Komponenten für eine Blutdrucksteigerung zu identifizieren. Die Analysen wurden sowohl im Querschnitt (Blutdruck, Prävalenz der Hypertonie) als auch im Längsschnitt (Inzidenz einer Hypertonie) durchgeführt.

Die Analysen zeigten eine positive Assoziation zwischen der langfristigen Exposition gegenüber Feinstaub aus dem städtischen Hintergrund und einer Erhöhung des Blutdrucks. Dieser Zusammenhang war unabhängig von Expositionen gegenüber gasförmigen Luftschadstoffen (mit der Ausnahme von Ammoniak). Die Assoziation war linear, ohne Schwellenwert. Verkehrslärm, der sozio-ökonomische Status der Nachbarschaft und andere relevante Störfaktoren hatten auf diesen Befund keinen Einfluss. Zusätzlich weisen die Ergebnisse auf einen positiven Zusammenhang von langfristigen Ammoniak-Expositionen am Wohnort und Blutdruckwerten hin, welcher in weiteren Studien Bestätigung finden sollten. Assoziationen mit arterieller Hypertonie wurden nicht beobachtet.

Im experimentellen Tierversuch wurde die 13-wöchige Exposition gegenüber Dieselabgasen und Stickstoffoxiden untersucht. Die Expression von Genen, die auf dem Wirkungspfad zwischen Luftverschmutzung und höheren Blutdruckwerten beteiligt sein können, wurde mithilfe quantitativer Echtzeit-Polymerase-Kettenreaktion in Mäuselungen gemessen. Die Exposition gegenüber Dieselabgasen und Stickoxide wirkte sich auf die Aktivität von fünf Genen aus: CYP1A1, NQO1, iCAM, iNOS und TNF. Die Produkte dieser Gene sind in den Fremdstoffmetabolismus, in Entzündungsprozesse, oxidativen Stress und Gefäßtonus-Regelung eingebunden.

Zusammenfassend lässt sich festhalten, dass sich positive Assoziationen zwischen der langfristigen Exposition gegenüber Luftverschmutzung mit erhöhtem Blutdruck in einer bevölkerungsbasierten Kohorte zeigten. Erkenntnisse des experimentellen Teils dieser Studie deuten drauf hin, dass verkehrsbezogene Luftverschmutzungspartikel zu Entzündungen, oxidativem Stress und Gefäßreaktionen führen können, was wiederum den Bluthochdruck beeinflussen könnte.

Blutdruck ist einer der wichtigsten modifizierbaren Risikofaktoren für Morbidität und Mortalität weltweit. Die Zahl der Hypertoniker in der Bevölkerung steigt stetig an. Eine relativ geringe Erhöhung des Blutdrucks aufgrund der Einwirkung von Luftverschmutzung könnte, auf Bevölkerungsebene, zu einer wesentlich höheren Belastung der Bevölkerung führen. Die Ergebnisse dieser Studie liefern Hinweise dafür, dass weitere Maßnahmen zur Verringerung der Luftverschmutzung zu erheblichen gesundheitlichen Vorteilen für die Bevölkerung führen könnten.

ABSTRACT

High blood pressure is a major determinant for mortality and disability in the developed world. Therefore, factors which increase blood pressure and which affect large populations have a high priority in cardiovascular health research. One of such factors may be ambient air pollution.

The link between air pollution and cardiovascular morbidity and mortality has been established in the last two decades. There are studies showing that short-term elevations in air pollution can lead to increased blood pressure within hours or days. It has been suggested that long-term exposure to air pollution would have an even stronger effect on blood pressure. Moreover, chronically elevated blood pressure could be a part of the pathophysiologic mechanism on how exposure to air pollution can lead to cardiac events and mortality.

So far, only few studies have investigated the effect of long-term exposure to air pollution on blood pressure, with mixed results. Multiple questions remain unanswered yet. Which pollutant (or pollutants) in the air pollution mixture could be responsible for blood pressure elevation? Is the association of air pollution with blood pressure confounded by the influence of concurrent residential exposures, such as road traffic noise and neighborhood deprivation? Finally, it is not clear through which biologic pathways air pollution could chronically increase blood pressure and lead to hypertension. It is likely that inflammation and oxidative stress, triggered by inhaled pollutants, play a central role in this process. Oxidative damage, autonomic nervous system imbalance, and vascular endothelial dysfunction could be some of the mechanisms involved in chronic blood pressure elevation following long-term air pollution exposure.

The aim of this study was to investigate the effect of long-term air pollution and its specific components on blood pressure and hypertension and to investigate possible underlying pathophysiologic mechanisms. In the research part of this study, I applied two methodologies: (1) observational, including statistical analyses of the data from a prospective cohort study, and (2) experimental, using a controlled-exposure animal study.

I conducted the observational part of this study using a population-based cohort, the Heinz Nixdorf Recall Study in the highly urbanized Ruhr area of Germany. Long-term concentrations of urban background air pollution (particulate and gaseous pollutants) at participants' residences were modeled with dispersion and chemistry transport model. Related residential exposures (road traffic noise, area-level socio-economic status) were also assessed. Blood pressure was measured at baseline and after five years of follow-up with a standardized procedure. Information on cardiovascular risk factors, co-morbidities, lifestyle and socio-economic factors was collected. Cross-sectional and longitudinal multiple regression analyses were performed, taking into account relevant confounders, and the intake of blood pressure lowering medication. I also employed multi-pollutant models to identify the responsible compounds.

I found a positive association of long-term exposure to fine particulate matter with blood pressure. This association was independent of gaseous pollutants (except ammonia), traffic noise, other co-exposures, and relevant confounders. The association of air pollution with blood pressure was linear and without a threshold. I also found a positive association of long-term ammonia exposure at residence with blood pressure, which should be confirmed in further studies. I observed no associations with prevalent or incident hypertension.

In the experimental part of this study, I measured and compared gene expression in mice lungs after controlled 13-week exposure to diesel engine exhaust and nitrogen oxides. I found changes in the activity of the following genes: CYP1A1, NQO1, iCAM, iNOS, and TNFα. These genes are involved in xenobiotic metabolism, inflammation, oxidative stress and vascular tone regulation.

In summary, I found a positive association of long-term exposure to air pollution with elevated blood pressure in a population-based cohort. This association is likely attributable to fine particulate matter and independent from co-exposure to most gases in the air pollution mixture, to road traffic noise, to residential deprivation, or from relevant confounders. My findings from the experimental part of this study provide supportive evidence that traffic-related air pollution can induce inflammation, oxidative stress and vascular reactions, which might favor hypertension in the long run.

1. THEORETICAL BACKGROUND

1.1. Introduction

High blood pressure (BP) is a leading risk factor for global mortality and disability, accounting for at least 15% of all health loss in adults aged 50 and older and for more than 9 million deaths in 2010 worldwide (Ezzati et al. 2002; Lim et al. 2013; Lipfert et al. 2003). The risk for cardiovascular diseases (CVDs), such as heart failure, infarction, and stroke, doubles with every increase in either systolic BP (SBP) of 20 mmHg or diastolic BP (DBP) of 10 mmHg, even within a normal BP range (Carretero and Oparil 2000; Lewington et al. 2002). The association of BP with mortality is not linear, with a rapid increase in risk at values close to hypertension: 140 mmHg SBP and 90 mmHg diastolic DBP (Lipfert et al. 2003). The risk of developing hypertension for the population aged 55 and older is 90%, and the lifetime probability of receiving the BP lowering medication (BPLM) is 60% (Vasan 2002).

Even small absolute decreases in BP lead to a substantial reduction of CVD risk in the population (Cook et al. 1995; Erlinger et al. 2003). A reduction in SBP by 2 mmHg reduces stroke mortality by 5%, coronary heart disease (CHD) mortality by 4%, and total mortality by 3% (Whelton et al. 2002). A reduction in DBP by 2 mmHg has been linked to 6% decrease in the risk of CHD and 15% reduction in the risk of stroke and transient ischemic attack (Cook et al. 1995). Hence, even factors with relatively small impacts on BP, but affecting large proportions of the population, should have a high priority in cardiovascular health research.

Ambient particulate matter (PM) air pollution is one of such factors. It is a major risk factor for global mortality (Lim et al. 2013) and cardiovascular disease. According to estimation from the World Health Organization (WHO), urban outdoor air is responsible for 5% of all cardiopulmonary deaths worldwide (WHO 2014). In an updated Scientific Statement from the American Heart Association published in 2010, the authors concluded that exposure to fine outdoor PM can trigger CVD-related events and mortality within hours or weeks (Brook et al. 2010). This effect was even stronger with the long-term exposure (Brook et al. 2010).

Chronically elevated BP may be one of the underlying mechanisms of PM-induced cardiovascular morbidity and mortality (Brook 2007). Short-term increases in PM air pollution lead to

acute increases in arterial BP (Brook 2007; Brook et al. 2009; Chuang et al. 2010; Delfino et al. 2010; Dvonch et al. 2009; Mordukhovich et al. 2009; Zanobetti et al. 2004). It has been suggested that long-term exposure to outdoor PM can lead to a chronically elevated BP and possibly to hypertension (Brook et al. 2010). Indeed, like other cardiovascular outcomes, BP is more strongly associated with longer-term average air pollution than with short-term exposure to air pollution (Auchincloss et al. 2008). The associations between long-term air pollution with elevated BP and the elevated prevalence or incidence of hypertension have been reported in a few recent studies from Canada, China, Taiwan, and the USA (Chen et al. 2013; Chuang et al. 2011; Coogan et al. 2012; Dong et al. 2013; Johnson and Parker 2009; Schwartz et al. 2012). However, these studies differ with regard to the methodologies and the definitions of exposures and outcomes. The results are, in part, not consistent. For example, in one study long-term exposure to nitrogen dioxide, a marker of traffic-related air pollution, was associated with lower BP and a decreased prevalence of hypertension (Sørensen et al. 2011). Therefore, more research on the long-term effects of different pollutants on BP and hypertension is needed.

Mostly cross-sectional or repeated measures data have been used in studies of the association of air pollution with arterial BP. The cross-sectional analysis with BP as outcome may be complicated by participants' concurrent intake of BPLM, which amounts to 41.0–77.9% of the adult population in Europe and North America (Wolf-Maier et al. 2003). If the BP-lowering effect of medication is not properly accounted for, it may compromise the analysis with BP (Tobin et al. 2005).

Few biologic mechanisms have been suggested for the effect of air pollution on BP (Brook 2007). The inhalation of particles and their deposition in the lungs can cause local oxidative stress. First, the particles can stimulate lung autonomic receptors (either directly or through oxidative stress and inflammation) and can lead to systemic vagal withdrawal and autonomic imbalance, resulting in acute peripheral artery constriction (Brook 2007). Second, local oxidative stress may extend to systemic oxidative stress, together with elevated levels of circulating and vascular cytokines, which can instigate vascular endothelial dysfunction and lead to vasoconstriction (Brook 2007). The third hypothesized pathway is the translocation of nanoparti-

cles and soluble compounds to the systemic circulation, which can alter blood rheology and can result in oxidative stress in vascular tissue (Brook 2007). The imbalance of the autonomic nervous system (ANS) may be primarily responsible for acute BP elevation following air pollution, as shown by Brook et al. (2009). However, more research is needed on the exact mechanisms of air pollution-induced BP elevation.

1.2. Air pollution – the exposure of interest

1.2.1. Definition and characteristics

Atmospheric air pollution can be defined as the presence in the atmosphere of gases or particulate matter, which are not normal air constituents and can harm living organisms and the environment (Yu 2005). Air pollution consists of organic, inorganic, gaseous, particulate, and liquid compounds. There are natural components of air pollution, such as sea salt, dust, sand, pollen and spores. The major anthropogenic constituents of air pollution are combustion products: smoke, fumes, soot, and others (Dockery 2009). Traffic and industry emit particulate mixtures that transform further in the atmosphere. Interactions between different components can change the toxicity of the mixture. For example, sodium chloride (NaCl), frequent air pollution component, does not pose any hazard to health per se (Mills et al. 2009). However, it can form toxic compounds through synergy with other pollutants. For example, Last et al. demonstrated a synergistic interaction of NaCl with NO_2, which could potentially form a toxic compound, nitrosyl chloride, NOCl (Last and Warren 1987; Last et al. 1994). A synergistic effect of PM_{10} and NO_2 in emergency cardiac hospitalizations in Hong Kong has been recently reported by Yu et al. (2013). Air pollutants can be divided into two groups by their physical characteristics: particles, or PM, and gaseous pollutants, such as ozone, nitrogen oxides, and others.

PM is composed of solid and liquid aerosols that are dispersed in the air (Pope and Dockery 2006). PM is frequently used as an exposure in observational and experimental studies on air pollution effects, and the most consistent adverse health effects have been reported for this component of air pollution (Araujo and Nel 2009). The number of particles and their size, surface area, and chemical composition contribute to the toxicity of the air pollution mixture

(Mills et al. 2009).

Sources of air pollution in Germany

According to the German Federal Environment Agency (2013), energy production, such as the burning of fuels (industrial and private) and traffic (combustion, tire and brake wear), was a major source of $PM_{2.5}$ (70%), sulfur dioxide (81%), nitrogen oxides (84%), and carbon monoxide (77%). Industry (such as steel production) was the second major emitter of $PM_{2.5}$ (15%), PM_{10} (31%), sulfur dioxide (19%), and carbon monoxide (23%). The use of solvents and other products contributed mostly to volatile organic compounds (VOCs) emissions (68%) but also to $PM_{2.5}$ (9%) and PM_{10} (5%). Agriculture was a major source of atmospheric ammonia (94%), mostly emitted through the use of fertilizers.

1.2.2. ***Classification of PM***

Classification by size

Particle size is an important parameter, as it influences particle behavior in the atmosphere and within the human respiratory tract. PM is classified according to the aerodynamic diameter, defined as the diameter of a spherical particle with a density of 1000 kg/m^3 and the same settling velocity as an irregular particle. By definition, the aerodynamic diameter of 50% of the particles in the defined fraction should be less than or equal to the selected cut point (Chow 1995). The sizes of particles range from 0.5 mm to 10^{-7}mm (Yu 2005). In relation to health effects, the inhalable particles (also called "thoracic fraction") with diameters ≤ 10 µm are investigated. Thoracic fraction includes coarse, fine, and ultrafine PM.

Coarse PM consists of particles > 2.5 and ≤ 100 µm in diameter (Kelly and Fussell 2012). Size of coarse PM is comparable to some cells in the human body, e.g., red blood cells (Brook 2008). Particles with aerodynamic diameters ≤ 10 µm (PM_{10}) can be deposited in the nasopharynx, and smaller particles ($PM_{10-2.5}$) may be inhaled to the lungs (Kelly and Fussell 2012). PM_{10} is defined by the European Commission in Directive 2008/50/EC as *"particulate matter which passes through a size-selective inlet as defined in the reference method for the sampling and measurement of PM_{10}, EN 12341, with a 50% efficiency cut-off at 10-µm aero-*

dynamic diameter" (Directive 2008/50/EC). Sources of coarse PM include: black smoke, soil, agricultural and road dust, soil, crustal materials from roads, tire wear emissions, construction debris, farming, mining operations, volcano eruptions, sea salt, pollen, mold, spores, other plant parts, and secondary particles (Araujo and Nel 2009; Kelly and Fussell 2012; Pope and Dockery 2006). The lifetime of coarse PM is hours to days (Brook 2008). Coarse PM can disperse over large areas (10 to 100 km) and contributes to most of the total PM mass (Brook 2008).

Fine particles have aerodynamic diameters > 0.1 and ≤ 2.5 μm, which is comparable to sizes of bacteria or viruses (Brook 2008). Together with ultrafine particles (UFPs) they are classified as $PM_{2.5}$. The European Commission defines $PM_{2.5}$ as *"particulate matter which passes through a size-selective inlet as defined in the reference method for the sampling and measurement of $PM_{2.5}$, EN 14907, with a 50% efficiency cut-off at 2.5-μm aerodynamic diameter"* (Directive 2008/50/EC). Fine particles consist of soot, organic compounds, endotoxin, sulfates, nitrates, metals, etc. (Brook 2008). Main sources of $PM_{2.5}$ include: combustion (fuel combustion and tailpipe and brake emissions from mobile sources, power plants, residential wood and coal burning, wildfires), industrial processes (smelters, cement plants, steel and paper mills), and secondary gas-to-particle conversion (Araujo and Nel 2009; Pope and Dockery 2006). $PM_{2.5}$ includes UFPs in the nucleation and Aitken modes, along with accumulation mode particles (0.1–1 μm; Araujo and Nel 2009). Fine particles have longer lifetimes than UFPs (up to weeks) and are distributed over large areas, forming the background air pollution (Brook 2008). Fine particles can reach alveoli and terminal bronchioles after inhalation (Dockery 2009; Kelly and Fussell 2012). Many adverse health effects, both acute and chronic, have been attributed to the fine particle fraction (European Environment Agency 2012). $PM_{2.5}$ can be distributed regionally (up to 1000 km; Brook 2008). Fine and ultrafine particles are major contributors to the number of particles and the surface area of PM, but not to its total mass.

UFPs have aerodynamic diameters ≤ 0.1 μm ($PM_{0.1}$), which is comparable to the size of molecules or smaller viruses (Brook 2008). UFPs are by far the most numerous particles in the PM mixture. They contribute mostly to surface area but not to the mass of PM (Kelly and

Fussell 2012). UFPs are very unstable, and they quickly (within minutes to hours) coagulate or condense into larger particles (Brook 2008). The major components of this fraction include sulfates, nitrates, elemental carbon, organic carbon, UFPs aggregates, endotoxin, and metals (Kelly and Fussell 2012). The major emission sources are combustion processes, such as automobile emissions (Araujo and Nel 2009; Pope and Dockery 2006). UFPs can also occur through the nucleation of gas-phase species to condensed-phase species in the newly emerging particles that did not grow (aerodynamic diameter < 0.01 μm, classified as nucleation mode) or through the coagulation of recently formed particles (0.01–0.1 μm, Aitken mode; Araujo and Nel 2009). Lifetime of UFPs is minutes to hours (Brook 2008). UFPs are mostly distributed within a 100-m distance from their source (Brook 2008); their concentration decreases exponentially with increasing distance from the road to approximately 300 m, where they cannot be further distinguished from background particles (Zhu et al. 2002). Combustion nanoparticles can carry on their surfaces many substances that can trigger adverse reactions in organisms: oxidized transition metals, polycyclic aromatic hydrocarbons, soluble organic compounds, and others. (Mills et al. 2009). The small size of the ultrafine particles allows them to penetrate the air-blood barrier in the lungs (Kelly and Fussell 2012; Mills et al. 2009).

Primary and secondary particles

Airborne particles are classified as primary, originating directly from various sources, and secondary**,** forming from the interactions of primary air pollutants (Dockery 2009). Primary PM includes larger particles, usually 1 to 20 μm in diameter (Yu 2005). The primary particles are emitted directly to the atmosphere by physical and chemical processes, such as combustion, erosion (Kelly and Fussell 2012). The secondary particles, such as sulfates (SO_4^{2-}) and nitrates (NO_3^-), are smaller than the primary particles. They are generated through chemical reactions in the atmosphere, e.g., the reactions of inorganic and organic gases with atmospheric oxygen, water vapor, free radicals, and reactive species (Araujo and Nel 2009). The primary particles contribute a minor amount to the total mass of PM, but can be more hazardous, than the secondary particles, because they act as condensation nuclei for the secondary aerosol mass and may carry various toxic trace species (Yu 2005).

1.2.3. Chemical composition of PM

The composition of PM depends on the source from which the particles originate. For example, sea salt aerosols consist mainly of sodium chloride, NaCl, but also include magnesium, sulfate, calcium, potassium, and some organic compounds (Perrino 2010). Sea salt is one of the dominant contributors to atmospheric aerosols worldwide: the yearly sea salt flux from the ocean to the atmosphere is estimated to be 3.3×10^{12} kg per year (Intergovernmental Panel on Climate Change 2007). The crustal matter originating from the Earth's crust consists of mineral oxides (Perrino 2010).

Organic matter is another major constituent of PM. It consists of various organic chemical compounds, primary (such as VOCs) and secondary (occurring through the oxidation of VOCs). VOCs can be of natural origin, e.g., isoprene, terpenoids, esters produced by plants, and anthropogenic, e.g., n-butane, ethylene, benzene from the motor vehicle exhaust (Blake and Blake 2003; Guenther 2003). Elemental carbon, also called black carbon, is another important compound of PM. Together, organic matter and elemental carbon form the carbonaceous fraction of PM.

Sulfates and nitrates are important compounds of PM (Kelly and Fussel 2012). Sulfates (SO_4^{2-}) are created through the oxidation of sulfur dioxide (SO_2). Similarly to sulfates, nitrates (NO_3^-) are formed through the oxidation of nitrogen dioxide (NO_2). Ammonium nitrate (NH_4NO_3) and bisulfate ($(NH_4)_2SO_4$) are common secondary particles and are formed from atmospheric ammonia, sulfate and nitrate (Perrino 2010). If ammonia is not present in the atmosphere, the following acids are formed instead: sulfuric acid (H_2SO_4) as liquid aerosol droplets, and nitric acid (HNO_3) as gas (Perrino 2010).

The relative contributions of different components to the total PM mass in Europe were investigated by Putaud et al. (2004). Approximately 70% of the total mass of the PM mixture was identified. The rest was assumed to be either water vapor or non-estimated matter. Organic matter was a major contributor to the annual average $PM_{2.5}$ and PM_{10} in the near-city and urban background (mass contribution 20% to PM_{10} and 22% to $PM_{2.5}$) and near the road (22% and 29%, respectively; Putaud et al. 2004). Sulfate was the second largest part of urban and

traffic PM and a major contributor to the masses of PM_{10} and $PM_{2.5}$ from the natural and rural background (Putaud et al. 2004). Nitrate was correlated with instances of high PM concentrations; the authors related this observation to specific meteorological conditions, such as cold periods, when atmospheric NH_4NO_3 was very stable (Putaud et al. 2004). The contribution of black carbon was relatively small in the urban background and rural sites and was higher near the road (Putaud et al. 2004).

Metals are an important part of the PM mixture: although not largely contributing to total mass or size, they define the chemical reactivity and toxicity of the mixture. The following transition metals were detected in $PM_{2.5}$ and UFP: iron (Fe), lead (Pb), mercury (Hg), cadmium (Cd), silver (Ag), nickel (Ni), vanadium (V), chromium (Cr), manganese (Mn), and copper (Cu; Lodovici and Bigagli 2011; Yu 2005). Transition metals can originate from the earth's crust, combustion sources, waste water discharges, and other sources (Lodovici and Bigagli 2011). Zink (Zn) is found in traffic-related PM, originating from waste oil (Lodovici and Bigagli 2011). Cu and Fe are components of brake wear; in addition, Fe is a soot suppressant emitted as UFP (Lodovici and Bigagli 2011). Particulate iron oxide (Fe_3O_4) can also be produced during combustion of coal which contains iron sulfide (FeS_2; Yu 2005):

$$3FeS_2 + 8O_2 \rightarrow Fe_3O_4 + 6SO_2 \qquad [1]$$

Lead halide particles can be produced during combustion of leaded gasoline, in a reaction of tetraethyl lead ($Pb(C_2H_5)_4$) with molecular oxygen and halogenated scavengers (dichlorethane and dibromoethane; Yu 2005):

$$Pb(C_2H_5)_4 + O_2 + halogenated\ scavengers \rightarrow CO_2 + H_2O + PbCl_2 + PbBrCl_2 + PbBr_2 \qquad [2]$$

Quass et al. (2004) analyzed the chemical composition of PM_1–PM_{10} from an urban background measurement station. They found that larger particles (PM_{10}) contained higher proportions of metal oxides (including aluminum and iron oxides), of calcium, potassium, magnesium, sodium and chloride. Smaller particles ($PM_{2.5}$ and PM_1) contained more ammonia, sulfates, nitrates, organic matter, and elemental carbon (Figure 1).

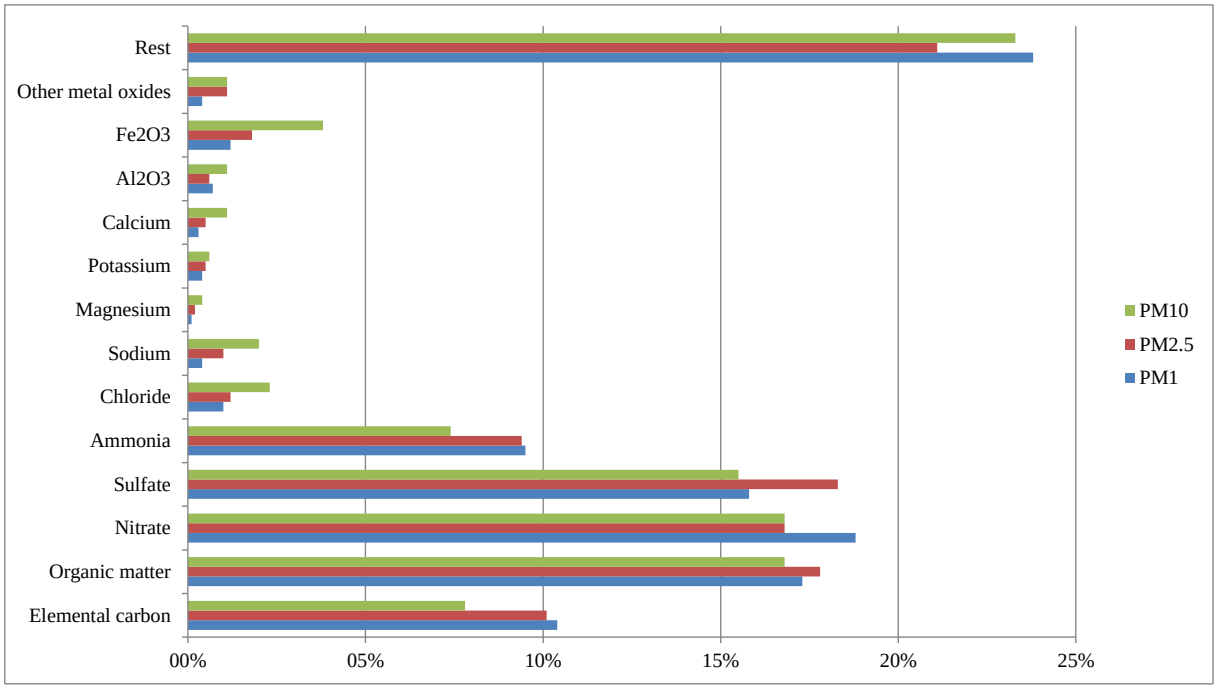


Figure 1. Chemical composition of PM_{10}, $PM_{2.5}$, and PM_1 from an urban background station in Germany.

Legend: Adapted from Quass et al. 2004, Figure 3. Mass concentration (%) of the respective components of PM_1, $PM_{2.5}$, and PM_{10} are presented.

1.2.4. Ozone

Ozone (O_3) is an important outdoor pollutant. As a secondary pollutant, it is formed in the troposphere through a complex series of reactions. These reactions involve nitrogen oxides (NO_x), VOCs, and the presence of sunlight (Brunekreef and Holgate 2002; Melkonyan and Wagner 2013). Brönnimann and Neu (1997) distinguish the following two types of chemical processes affecting ozone concentrations: (i) the photostatic equilibrium and (ii) the substitution of O_3 with peroxyl radicals. The photostationary equilibrium consists of three reactions:

$$NO_2 + h\upsilon^1(\lambda < 200\ nm) \rightarrow NO + O^* \quad [3]$$

$$O^* + O_3 \rightarrow O_3 \quad [4]$$

$$NO + O_3 \rightarrow NO_2 + O_2 \quad [5]$$

[1]hv – radiation energy with a frequency v at the wavelength λ < 400 nm (also < 420 nm); h – the Planck constant (Melkonyan and Kuttler 2012).

NO, emitted from traffic, is introduced to the equilibrium. Decrease in the concentration of NO leads to increases in the ozone concentration. Simultaneously, NO, originating from the traffic, scavenges O_3, which explains the lower concentrations of ozone observed in a city center compared to the suburbs (Brönnimann and Neu 1997; Brunekreef and Holgate 2002). The substitution of O_3 with a peroxyl radical occurs as follows:

$$NO + RO_2{}^{1} \rightarrow NO_2 + RO \quad [6]$$

$$NO + HO_2 \rightarrow NO_2 + HO^{*2} \quad [7]$$

[1]R is an organic part; RO_2 is peroxide radical. [2]Hydroxyl radical.

This process replaces reaction [5] in the photostationary equilibrium and decreases O_3 destruction, which results in a higher O_3 concentration. Peroxyl radicals are products of hydrocarbons (such as VOCs) and carbon monoxide in reactions with hydroxyl radicals (Brönnimann and Neu 1997).

Temperature and radiation influence chemical activity. The concentration of hydroxyl radicals increases in the sunlight (Melkonyan and Kuttler 2012). Decreasing the VOC emissions diminished the O_3 concentrations (Brönnimann and Neu 1997). On hot, sunny days, the synthesis of O_3 dominates over destruction, leading to elevated ozone levels. In contrast, the destruction of O_3 occurs more readily on cold, cloudy days (Brönnimann and Neu 1997). The reactive biogenic VOCs (isoprene, monoterpenes, etc.) are emitted in significant quantities on the hot days, resulting in increased amounts of ozone above green areas in the summer (Melkonyan and Kuttler 2012).

The measurement of atmospheric O_3 is conducted using continuous and discontinuous (such as passive sampler) methods (WHO 1999). The following methods to sample O_3 are commonly used: laminar flow, turbulent flow and sampling without a manifold (WHO 1999). The automated measurements most frequently employed to measure O_3 include chemiluminescence, ultraviolet photometry and differential optical absorption system spectrometry (WHO 1999). The reference method to measure O_3 in the European Union (EU) is continuous measurement by ultraviolet photometry according to the standard EN 14625:2005 (Directive 2008/50/EC).

1.2.5. Nitrogen oxides

The most common atmospheric nitrogen oxides are nitrogen monoxide (NO) and nitrogen dioxide (NO_2). Nitrogen oxides are produced from the reaction of nitrogen (N_2) and oxygen (O_2) gases during combustion, especially at high temperatures (Yu 2005):

$$N_2 + O_2 \rightarrow^1 2NO \quad [8]$$

$$2NO + O_2 \rightarrow 2NO_2 \quad [9]$$

[1]Reaction temperature 1210 °C (Yu 2005).

The primary sources of NO_2 include road transportation and power generation (Kelly and Fussell 2012). In the presence of oxidants (e.g., O_3) NO is transformed to NO_2, which is why the nitrogen oxides are usually referred to cumulatively as NO_x. The major sources of anthropogenic emissions of NO_x are transportation, power generation, and fossil fuel burning for heating (Brunekreef and Holgate 2002; Lodovici and Bigagli 2011). NO_x can also occur naturally during thunderstorms. NO_x contributes to the formation of tropospheric O_3 and fine PM. The reaction of NO_x with VOCs forms photochemical smog. The most substantial formation of photochemical smog occurs in summer. The major components of photochemical smog are ozone, nitrogen oxides, peroxyacetyl nitrate, radical oxygen forms, and various organic compounds (Yu et al. 2005). Nitrogen oxides are the precursors of nitric acid, which, when dissolved in atmospheric vapor, forms a component of acid rain.

The most commonly used methods to measure nitrogen oxides are passive and active sampling, automatic analysis and remote sensing (WHO 1999). In the EU, according to the standard EN14211:2005 (Directive 2008/50/EC), the reference method for the measurement of NO_x is chemiluminescence.

1.2.6. Sulfur dioxide

Sulfur dioxide (SO_2) is an important gaseous constituent of air pollution and constitutes a major part (95%) of anthropogenic emissions of sulfur (S) in the atmosphere (Yu 2005). SO_2 mainly originates from power generation and industrial processes including combustion of sulfur-containing fuels, such as coal or oil, petroleum refining, and nonferrous smelting

(Kelly and Fussell 2012; Yu 2005). In addition, domestic heating and diesel engines can also emit SO_2 (WHO 1999). Natural sources of SO_2 include volcanic eruptions and geothermal springs (WHO 1999). Concentrations of atmospheric SO_2 have been reduced substantially in the USA, Western Europe, and Japan in the past decades, whereas in other countries, such as China and South Korea, emissions are increasing (Environmental Protection Agency 2013; WHO Air Quality Guidelines for Europe 2000; Lu et al. 2000). SO_2 emissions worldwide have been largely reduced through flue-gas desulfurization, a technology to bind emitted SO_2 at power plants (Lu et al. 2000). Technologies that allow the removal of sulfur from fuels prior to combustion, such as the Claus and Stretford processes, are also used to reduce atmospheric SO_2 emissions. Similar to NO_x, SO_2 is a precursor for acid rain (forming sulfuric acid) and secondary particle formation. SO_2 is hazardous to both human health and the environment (European Environment Agency 2012; Lu et al. 2000). SO_2 is the most important precursor to $PM_{2.5}$ (European Environment Agency 2012).

The reference measurement method of SO_2 in the EU is automated measurement by ultraviolet fluorescence, as described in EN 14212:2005 (Directive 2008/50/EC). Other commonly used methods include active and passive sampling with spectrophotometry, chromatography, acidimetry, and others (WHO 1999).

NO_2 and SO_2 are the main sources of atmospheric acidity (Kumar et al. 2004). They can be oxidized by O_3, hydrogen peroxide (H_2O_2), or hydroxyl radical to sulfuric and nitric acids, respectively. The two acids can be further neutralized by atmospheric ammonia, forming ammonium bisulfate and ammonium nitrate and then remaining in the aerosol phase. Alternatively, sulfuric and nitric acids could be deposited on the Earth's surface (Kumar et al. 2004). NO_2 can also react with VOCs in direct sunlight, resulting in the production of ground-level O_3 (WHO 2003).

1.2.7. Ammonia

Anthropogenic ammonia (NH_3) is emitted during agricultural activities, animal feedlot operations, the decomposition of organic matter, and biomass burning, and, to a lesser extent, from industry, traffic, and volatilization from soils and oceans (Behera et al. 2013; Krupa 2003).

NH_3 is deposited within the first 4–5 km from its source (Krupa 2003) and is an important precursor of secondary particles in the atmosphere (Perrino 2010). Atmospheric NH_3 reacts with acids, such as sulfuric (H_2SO_4), nitric (HNO_3), nitrous (HNO_2), and hydrochloric (HCl) acids, and with oxides, e.g., SO_2. The emerging ammonium salts, such as ammonium nitrate (NH_4NO_3) or ammonium bisulfate ($(NH_4)_2SO_4$), form secondary particles, mostly in the $PM_{2.5}$ fraction (WBK and Associates Inc. 2004). Approximately 1% of total atmospheric ammonia is involved in the formation of NO (WBK and Associates Inc. 2004). It is estimated that the global emissions of NH_3 may increase due to increasing anthropogenic activity (Sutton et al. 2013).

1.2.8. Carbon monoxide

Carbon monoxide (CO) is present in small amounts in the atmosphere. CO is a product of (i) incomplete combustion of carbon-containing fuels, occurring when the supply of oxygen is not sufficient for complete oxidation, (ii) reactions between carbon dioxide (CO_2) and carbon-containing compounds at high temperature, and (iii) CO_2 dissociation at high temperature (European Environment Agency 2012; Yu 2005). The anthropogenic sources of CO include internal combustion engines, man-made fires and fuel combustion, and the natural source of CO is volcanic activity (WHO Air Quality Guidelines for Europe 2000). CO is involved in photochemical smog formation (Yu 2005). CO reacts with hydroxyl radicals and contributes to the synthesis of tropospheric O_3. CO is highly toxic because it reduces oxygen delivery to the body's organs and tissues (European Environment Agency 2012). The introduction of catalytic converters in road vehicles has reduced formerly significant amounts of CO at traffic sites (European Environment Agency 2012).

The most common routine measurement method of CO in the atmosphere is non-dispersive infrared spectrometry, an automated continuous method (WHO 1999). This method allows for the measurement of CO concentrations within the range of 0.5–115 mg/m³.

1.2.9. Measurement of air pollution

Commonly used measurement systems of suspended PM are high-, medium- and low-volume samplers (defined by the volume of air sampled per unit time; UNEP/WHO 1994). Every

sampler should conform to the CEN standard EN 12341 (WHO 1999). The samplers collect PM on a filter using high- or low-volume flow (WHO 1999). High- and medium-volume samplers can be equipped with PM_{10} inlets to allow fraction determination (UNEP/WHO 1994). The filter with collected particles can be analyzed using physical and chemical methods, the most important of which are the estimation of total sample mass, the characterization of PM size distribution, and the chemical analysis of particle fractions (UNEP/WHO 1994). The estimation of mass (gravimetry) is achieved using microbalance techniques, tapered element oscillating microbalance techniques, and radiometry (pseudogravimetry; UNEP/WHO 1994). Reflectometry (blackness of filter) is a measure of the total graphitic carbon content in the aerosol; thanks to technical simplicity and low cost, this method is widely used (UNEP/WHO 1994). Nephelometry (the light scattering capacity of the aerosol) is used as a measure of $PM_{2.5}$ (UNEP/WHO 1994). Chemical characterization of the PM allows for ion groups, metals and other functional entities in bulk specimens to be determined (UNEP/WHO 1994). Additionally, physical methods allow for the elementary molecular or crystalline compositions of the particles to be determined (UNEP/WHO 1994). The reference method EN 12341:1999 is used in the EU for the sampling and measurement of PM_{10}; for $PM_{2.5}$, EN 14907:2005 is used (Directive 2008/50/EC).

The methods to sample gases and vapors can be divided in two groups: (i) sampling in the original atmospheric state, without measuring concentrations and (ii) sampling of the atmosphere with an assessment of the concentrations of gases and vapors.

1.2.10. Current regulatory standards

In the EU, the standards for ambient air quality are set by Council Directive 2008/50/EC, which became active on 11 June 2008. This directive sets the maximum limits for 1-hour and 24-hour average SO_2 at 350 µg/m³ and 125 µg/m³, respectively. The 1-hour and 1-year limits for NO_2 are 200 µg/m³ and 40 µg/m³. The daily maximum for CO is set to 10 µg/m³. Daily PM_{10} has a limit of 50 µg/m³, which is not to be exceeded more than 35 times a calendar year, and a yearly limit of 40 µg/m³ (European Commission Air Quality Standards). The target value for $PM_{2.5}$ is 25 µg/m³ for the yearly average, which became active 1 January 2010; it will

be set as the limit beginning 1 January 2015. The additional objectives of Directive 2008/50/EC target the population exposure for $PM_{2.5}$. They include an average exposure indicator, a 3-year running annual mean concentration of $PM_{2.5}$ representing the urban background exposure. The EU member states are obligated to reach an average exposure indicator limit of 20 μg/m³ by 2015 (years 2013–2015). In addition, the exposure reduction target has been set; depending on its average exposure indicator value in 2010, each country is to reach a percentage reduction (0, 10, 15, or 20%) and to implement all measures to reach an AEI limit of 18 μg/m³ (European Commission Air Quality Standards).

According to the current WHO Air Quality Guidelines (2005), the recommended 24-hour mean for $PM_{2.5}$ is 25 μg/m³, and the recommended annual mean is 10 μg/m³; for $PM_{10,}$ these values are 50 and 20 μg/m³, respectively. It is estimated that reductions of PM concentrations to these levels will reduce air pollution-related mortality by 15% (WHO Air Quality Guidelines). In addition, three interim targets have been set for $PM_{2.5}$ and PM_{10}, suggested as helpful milestones in the process of reducing the exposure over time. For example, for annual mean concentrations of $PM_{2.5}$, the recommended interim targets were 35, 25 and 15 μg/m³. According to the report of the European Environment agency (2012), up to 30% of EU's urban population was exposed to $PM_{2.5}$ concentrations above the EU reference values in 2008–2010. In the same period, the vast majority (90–95%) of the EU population lived above the $PM_{2.5}$ threshold recommended by the WHO (European Environment Agency 2012).

Two countries outside the EU have already set limits for $PM_{2.5}$: USA, at 12 μg/m³ for the annual concentration (Environmental Protection Agency 2012), and Japan, at 15 μg/m³ (Ministry of the Environment 2009).

1.3. Arterial BP – the outcome of interest

1.3.1. The definition of BP

The cardiovascular system can be schematized as consisting of a pump (heart), a series of distributing and collecting tubes (arteries and veins), and a system of thin vessels that facilitate the rapid exchange between the tissues and circulation (Pappano and Wier 2013). Arterial BP, or simply BP, is one of the principal vital signs. It is defined as the lateral pressure exerted

by a column of blood upon the walls of blood vessels (Pal and Pal 2005). During a single heartbeat, BP changes from the maximum, SBP, to the minimum, DBP (Pappano and Wier 2013). Pulse pressure (PP) is defined as the difference between the SBP and the DBP. SBP depends mainly on the cardiac output and increases when cardiac output increases (Pal and Pal 2005). DBP depends on the peripheral resistance (Pal and Pal 2005). BP can be calculated using the following formula (Steffel and Lüscher 2011):

$$\mathrm{BP} = \text{Cardiac output} \times \text{Resistance} \qquad [10]$$

Cardiac output is defined as the total blood flow out of the left ventricle, usually per one minute (Pappano and Wier 2013). It is the product of stroke volume with heart rate. Stroke volume is the amount of blood pumped out from the ventricle with a heartbeat. Cardiac output is the blood flow perfusing all of the tissues of the body. It can change, adapting to the metabolic demands of the body: e.g., during severe exercise, it can increase four to five-fold (Pappano and Wier 2013). The following factors control cardiac output: heart rate, myocardial contractility, preload, and afterload (Pappano and Wier 2013). Cardiac output also depends on the vascular resistance (Pappano and Wier 2013).

Total peripheral resistance is the resistance of the peripheral vasculature to the blood flow (Pappano and Wier 2013). It increases during vasoconstriction and decreases during vasodilation. The main factors influencing the peripheral resistance are the tone of the small arterioles (also known as the resistance arterioles, diameters 100–450 μm), the tone of the pre-capillary arterioles (4–100 μm in diameter), and the blood viscosity. The regulatory factors influencing peripheral resistance include platelet-derived factors (such as serotonin), which dilate the vessel. Endothelium-derived NO, released following cholinergic stimulation, also works as a vasodilator (Pappano and Wier 2013). If the endothelium is damaged (e.g., removed), blood vessel dilation does not occur after these stimuli (Pappano and Wier 2013).

The factors controlling arterial BP may be divided in two large groups: “physical”, such as arterial blood volume and the elastic characteristics of the system, and “physiological”, such as cardiac output and peripheral resistance (Pappano and Wier 2013).

1.3.2. General mechanism of BP regulation

BP is regulated as follows: (i) sensors estimate the pressure and transfer a signal to an evaluator; (ii) evaluators translate the coded signal from the sensors, compare the BP with a set point (a value desirable under current conditions) and trigger, if needed, the compensatory mechanisms; and (iii) according to the signal from the evaluators, the effector mechanism can change the heart rate, the cardiac output or the total peripheral vascular resistance to stabilize the BP (Ackermann 2004). BP regulation can produce acute or long-term changes in pressure. The short-term responses are mainly produced by baroreceptors (the non-encapsulated nerve endings located in the arterial wall of the carotid sinus and the aortic arch) and by the stretch sensors in the cardiac atria (Ackermann 2004). The signal is conveyed to the midbrain (neurons in the nucleus tractus solitarius) and can trigger (i) the increased production of angiotensin II, which increases sodium and water retention in the kidneys, thereby increasing the blood volume; (ii) cardiac parasympathetic outflow, which results in lower heart rate, and, correspondingly, lower cardiac output and BP; and (iii) sympathetic nervous outflow, with the release of adrenalin, renin and noradrenaline, activating α- and β-adrenoreceptors in the cell membranes of the target tissues, resulting in increases in heart rate, cardiac contractility and vascular resistance – all of which can lead to a raise in BP (Ackermann 2004).

1.3.3. Regulation of BP through the control of blood flow

BP keeps blood flow at the level required for its metabolic activity, which is why maintaining a stable BP level is crucially important (Ackermann 2004; Sears and Casadei 2002). Control of blood flow and regulation of BP can be seen as two different aspects of the common process, each one affecting and being affected by the other. The auto-regulation of blood flow involves two processes: (i) metabolic, regulating the coupling between blood flow and tissue, and (ii) myogenic, modulating the blood vessel response to BP change (Sears and Casadei 2002). The metabolic response to BP change is affected by the concentrations of vasodilator metabolites and oxygen (Sears and Casadei 2002). Dilation of an arteriole can increase perfusion and oxygen supply. It has been suggested that the lack of oxygen also has a vasodilatory effect through a diminished adenosine triphosphate to adenosine diphosphate ratio and the

opening of $K_{adenosine\ triphosphate}$ channels, relaxing the smooth muscle (Sears and Casadei 2002). In addition, a vasodilatory effect was suggested for adenosine in coronary muscle and potassium in skeletal muscle (Sears and Casadei 2002). The myogenic response is a cascade of reactions in the vascular smooth muscle cells, occurring in response to changes in pressure (Sears and Casadei 2002). It has been suggested that stretching in the arteriolar wall leads to constriction of vessels: it activates calcium channels in the vascular muscle cells and that second messengers, such as arachidonic acid (an inhibitor of K_{Ca} channels), act as local vasoconstrictors (Sears and Casadei 2002).

1.3.4. Heart rate, stroke volume and peripheral resistance

BP regulation involves thee parameters: heart rate, stroke volume, and total peripheral vascular resistance (Ackermann 2004). Heart rate is set and controlled by cardiac pacemaker cells (located in the right atrium) which create the impulse (Ackermann 2004). The sympathetic nervous activity increases the heart rate, whereas the parasympathetic activity decreases it (Ackermann 2004). Stroke volume depends on cardiac performance: preload, afterload, heart rate and contractility. In a healthy heart, an increase in preload is associated with a higher ventricular performance, and the increase in afterload is associated with a higher ventricular contractility (Ackermann 2004). Short-term changes in the ventricular contractility occur due to neurohumoral factors, whereas long-term changes depend on the morphological changes in the contractile proteins actin and myosin (Ackermann 2004). Total peripheral vascular resistance is regulated mainly through changing the luminal arteriolar diameter (Ackermann 2004). The arteriolar diameter is determined by the degree of constriction or relaxation of smooth muscle cells located in the medial layer of the arteriole. This diameter results from the action of vasodilators or vasoconstrictors. Most of the factors that act as vasodilators (widening the arteriolar diameter) are byproducts of tissue metabolism, whereas vasoconstriction (narrowing of the arteriole) is achieved through sympathetic nervous activity and the actions of chemical compounds such as adrenaline, angiotensin II, or vasopressin (Ackermann 2004). Vasoconstriction, which is mediated sympathetically, controls peripheral vascular resistance, except for parts of vessels where vasodilatory products are produced by the tissue (Ackermann 2004).

1.3.5. Endocrine and paracrine regulation of BP

Endocrine regulation

The long-term control of BP involves the kidney and angiotensin II (Ackermann 2004). The kidney is the most important organ controlling blood volume and pressure (Sears and Casadei 2002). Kidneys regulate BP through two mechanisms: the modulation of sodium (and therefore liquid) balance and the production of vasoactive substances. These substances include angiotensin II, prostaglandin and NO, the endothelium-derived relaxation factor (Takenaka et al. 2004).

The endocrine regulation mechanisms include adrenal glands, renin-angiotensin system, and vasopressin release. The adrenal medulla of the adrenal gland produces two hormones influencing BP: adrenaline and noradrenaline. These hormones are produced in response to sympathetic stimulation, cause vasoconstriction and increased cardiac output (Sears and Casadei 2002). The receptors involved in vasoconstriction response are: (i) α_{1A} receptors in the vascular smooth muscle cells and (ii) α_{2C} receptors in the arteries and veins. The β_1 and β_2 adrenergic receptors, also located in the vasculature, mediate vasodilation (Sears and Casadei 2002).

The renin-angiotensin system is an important BP regulation system that involves the kidneys and controls the cardiac output by modulating urinary salt and water excretion (Suzuki and Saruta 2004). If BP is falling, a decrease in renal perfusion pressure, or sympathetic nervous activity, or prostacyclin production affects the activity of juxtaglomerular cells in the kidneys. This triggers the secretion of renin into circulation (Sears and Casadei 2002). Renin cleaves angiotensinogen to angiotensin I, and angiotensin-converting enzyme (ACE) subsequently cleaves it to angiotensin II; ACE catalyzes the breakdown of the vasodilator bradykinin to inactive peptides (Sears and Casadei 2002). Angiotensin II mediates vasoconstriction and sodium and liquid retention (through the release of aldosterone from the adrenal cortex), increasing blood volume and elevating BP (Fujita 2001; Sears and Casadei 2002). Angiotensin II also stimulates smooth muscle proliferation (Sears and Casadei 2002). In addition to the kidney renin-angiotensin system, there are local systems within the vasculature (Ching and Beevers 1991). A local renin system may be activated by local vascular damage, independent

of the systemic BP (Ching and Beevers 1991). Angiotensin also plays a role in vascular remodeling through the stimulation of angiotensin type 2 receptors (Sears and Casadei 2002).

Vasopressin, or antidiuretic hormone, acts to retain water in the body and to constrict blood vessels. It is secreted in nervous fibers of the supraoptic and paraventricular nuclei of the hypothalamus in response to reductions in plasma volume (activated by baroreceptors; a > 10% reduction in blood volume activates the secretion) and increases in plasma osmolality (osmoreceptors in the hypothalamus) and in response to cholecystokinin, a substance secreted by the small intestine (Ashton 2007). Angiotensin II activates and atrial natriuretic peptide inhibits vasopressin secretion (Ashton 2007).

Paracrine regulation

The auto-regulation of BP (paracrine regulation) produces immediate responses to local changes and alters the perfusion of tissues in the area. Different effector mechanisms operate at the different sites of the vascular system (Storkebaum and Carmeliet 2011). The release of endothelial NO, a decreased supply of O_2, decreased pH, an increased supply of CO_2, increased temperature, and histamine release (local inflammation) have local vasodilatory effects (Storkebaum and Carmeliet 2011). Prostaglandins and ETs released by damaged endothelial cells are local vasoconstrictors (Silldorff et al. 1995).

1.3.6. The neural regulation of blood pressure

The neural regulation of BP results in acute adjustments to systemic changes and body-wide responses, controlled by the medulla oblongata (Schmidt et al. 2011). Neural regulation of BP responds to baroreceptors and chemoreceptors. Baroreceptors are located in the high- and low-pressure receptor zones in the left and right carotid sinuses, in the aortic arch, and in arteries (Sears and Casadei 2002). The baroreceptors detect changes in arterial pressure and send signals to a region in the medulla part of the brainstem (the rostral ventrolateral medulla). The rostral ventrolateral medulla adjusts the arterial pressure by altering heart rate (force and speed of contractions) and systemic vascular resistance. Baroreceptors in the low-pressure receptor zones are located in pulmonary veins, the venae cavae, the atria, and the kidneys (renal baroreceptors; Ashton 2007). They regulate BP by affecting the secretion of vasopressin,

renin and aldosterone, which results in increases in blood volume and cardiac output and, as a result, increased BP (Ashton 2007). Chemoreceptors are located in the aorta and carotid arteries (Ashton 2007). They react to changes in the concentrations of O_2, CO_2, and pH (Ashton 2007).

1.3.7. The role of vascular endothelium

The vascular endothelium plays a major role in maintaining local homeostasis through its sensory and effector capacity (Wilson and Lerman 2001). The vasoregulatory role of the endothelium is achieved through the emission of relaxing or constricting factors. NO, the endothelium-derived relaxing factor, is synthesized by endothelial nitric oxide synthase enzyme (eNOS) as a response to shear stress (Sears and Casadei 2002). NO diffuses from the inner vascular layer (endothelium) to the medial layer (smooth muscle cells) and triggers vasodilation through a decrease in Ca^{2+} release and activation of K channels; this process involves the enzymes guanylate cyclase and protein kinase G (Ching and Beevers 1991; Sears and Casadei 2002). NO has a half-life of a few seconds and acts rapidly (Wilson and Lerman 2001). The circulating agents, such as bradykinin, adenosine, serotonin, histamine, acetylcholine, may also activate NO synthesis (Sears and Casadei 2002; Wilson and Lerman 2001). In addition to NO, the vascular endothelium can produce other vasodilatory substances, such as prostacyclin (Sears and Casadei 2002). In response to angiotensin, vasopressin, thrombin, and adrenaline, endothelial cells can release ET-1, a vasoconstrictor (Ching and Beevers 1991; Sears and Casadei 2002).

Endothelium plays the role of an anti-inflammatory barrier: it prevents infiltration of the circulating inflammatory cells under normal conditions (Wilson and Lerman 2001). Endothelium-derived NO has an additional, “antiatherogenic”, function: it interacts with adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule-1, also known as e-selectin, to prevent the endothelial adhesion of leukocytes and their further migration to the arterial wall; in addition, NO inhibits platelet aggregation (Wilson and Lerman 2001). The same antiatherogenic role was observed for the other endothelium-derived relaxing factor, prostacyclin

(Sears and Casadei 2002). Endothelium-derived vasoconstrictor ET-1 is a proatherogenic factor (Wilson and Lerman 2001).

The imbalance between NO and endothelin may be associated with CVD (Sears and Casadei 2002). Endothelial dysfunction is characterized by abnormal endothelium-dependent relaxation, impaired vasoreactivity, decreased NO bioavailability, and increased expression of adhesion molecules and transendothelial migration of circulating monocytes (Wilson and Lerman 2001). Endothelial dysfunction is associated with early stages of atherosclerosis and may play an important role in atherogenesis (Wilson and Lerman 2001). However, the results of a recent analysis in the Multi-Ethnic Study of Atherosclerosis suggest that impaired endothelial function is not an independent predictor of hypertension incidence and may not play a substantial role in hypertension development (Shimbo et al. 2010).

There are two other nitric oxide synthase enzymes, which produce NO in mammals: inducible (iNOS) and neuronal (nNOS). INOS plays an important role in the immune defense (because of the free radical properties of NO). ENOS and nNOS are constitutively expressed calcium-dependent isoforms (Lee and Yen 2008). INOS is either not expressed or expressed at a minimal level under normal, non-pathological conditions (Lee and Yen 2008). INOS expression is induced in vascular cells by proinflammatory cytokines (Lee and Yen 2008). NO plays a dual role: on the one hand, the constitutive production of NO is a mechanism to protect the cells of the vascular endothelium, on the other hand, disproportionate (i.e., too much or too little) NO production in pathological conditions can result in cytotoxic effects (Lee and Yen 2008). In addition to NO production, iNOS simultaneously catalyzes the production of peroxynitrite, a potent oxidant that can trigger oxidative damage of the vascular endothelium and can mediate protein nitration, guanidine nitration, and deoxyribonucleic acid (DNA) single-strand breakage, processes that are toxic and mutagenic (Lee and Yen 2008). It has been suggested that the increase of iNOS expression results from vascular injury (Lee and Yen 2008).

1.3.8. The interplay of CO and NO in BP regulation

NO and CO are important gas transmitters involved in the compensatory regulation of blood pressure during the genesis of hypertension (Lee and Yen 2008). Under pathological stress

conditions, NO is produced by iNOS (Lee and Yen 2008). CO is produced by heme oxygenase (HO), an enzyme catalyzing the oxidative degradation of heme to biliverdin, CO and iron (Lee and Yen 2008). There are three isoforms of this enzyme: HO-1, HO-2, and HO-3 (Lee and Yen 2008). Isoforms 2 and 3 are active constitutively, whereas isoform 1, similar to iNOS, is normally not active (Lee and Yen 2008). HO-1 expression is induced by oxidative stress, cytokines, NO, and other stimuli (Lee and Yen 2008). HO-1 is expressed in the endothelial and foam cells of atherosclerotic lesions (Lee and Yen 2008).

Though CO was originally considered a toxic metabolic waste product, its cytoprotective function was discovered recently (Lee and Yen 2008). CO can act as a vasodilator similarly to NO (Lee and Yen 2008). Depending on the experimental conditions, CO can act as a vasoconstrictor or vasodilator (Lamon et al. 2009). The vasoconstricting function of CO is activated by the oxidative stress response (Lamon et al. 2009). Alternatively, it has been suggested that CO promotes vasoconstriction by inhibiting the formation of NO, the endothelium-derived relaxation factor (Johnson and Johnson 2003). It has been suggested that NO and CO dynamically affect each other and that under certain pathological conditions, such as oxidative stress, iNOS and HO-1 cooperate and compensate for each other (Lee and Yen 2008).

1.3.9. Individual factors affecting BP

Among the factors influencing the average BP level, are age, sex, race, family history, socioeconomic status, early life experiences, body mass index (BMI), nutrition (in particular, sodium, potassium, calcium and magnesium intake, fish oil intake, licorice, tyramine-containing foods, coffee consumption, alcohol intake), physical activity, and environmental factors (Chobanian et al. 2003; Drøyvold et al. 2005; Geleijnse et al. 2005; Steffel and Lüscher 2011; Whelton 1994).

BP at birth is measured at approximately 70 mmHg SBP to 50 mmHg DBP (Whelton 1994). SBP rises gradually over childhood, adolescence, and adulthood, reaching approximately 140 mmHg by 70-80 years of age (Whelton 1994). The estimated annual increase of SBP in adults is approximately 0.9 mmHg (Wills et al. 2012). DBP also increases over a substantial period of life, although the rate of increase is smaller than that for SBP (Whelton 1994). After the age

of 50, the average DBP stops increasing or even declines due to the growing rigidness of the arteries (Whelton 1994).

The prescription drugs that affect BP levels include cortisone and other steroids, estrogens, non-steroidal anti-inflammatory drugs, phenylpropanolamines, cyclosporine and tacrolimus, erythropoietin, sibutramine, antidepressants (especially venlafaxine), clozapine, and others (Chobanian et al. 2003). Environmental chemicals, such as lead, mercury, heavy metals, and lithium salts, are also associated with arterial hypertension (Chobanian et al. 2003).

During the measurement, the BP reading may be influenced by many factors. For example, using different recording devices in one study produces a systematic difference in measurement. In the analysis comparing a random-zero sphygmomanometer (RZS) with an automated oscillometric measurement device (AOD), the differences (AOD-RZS) constituted 3.9 mmHg for SBP and 2.6 mmHg for DBP (Stang et al. 2006). The measurement procedure can influence BP levels, particularly, the resting time before the measurement, the consumption of food or beverages before the measurement, rigorous exercise, smoking, the use of medication, appropriateness of cuff size for measurement, body position, and the arm with which the measurement is performed (Kuulasmaa et al. 1998). Therefore, it is important to use a standardized measurement procedure.

1.3.10. Arterial hypertension

Hypertension can be defined as a condition when the vasodilatory response is inefficient or when the arteries are injured in the inflammatory process and are therefore less compliant and constricted (Lee and Yen 2008). The clinical definition of hypertension given in the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) is SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, or medication with antihypertensive agents (Chobanian et al. 2003). Hypertension can be further divided to stage 1, characterized with SBP 140–159 mmHg or DBP 90–99 mmHg, and stage 2, with SBP ≥ 160 mmHg or DBP ≥ 100 mmHg (Chobanian et al. 2003). Normal blood pressure is defined as SBP ≤ 120 mmHg and DBP ≤ 80 mmHg. Higher BP values indicating prehypertension are defined as SBP 120–139 mmHg or DBP 80–89 mmHg. Prehypertension is independently as-

sociated with cardiovascular risk (Chobanian et al. 2003; Erbel et al. 2012).

Essential (primary) hypertension is arterial hypertension without an underlying primary disease; it constitutes 90–95% of all hypertension cases (Carretero and Oparil 2000; Steffel and Lüscher 2011). Secondary hypertension occurs as a symptom of another primary disease, such as kidney disease, endocrine disruptions, obstructive sleep disturbance, and others (Steffel and Lüscher 2011).

Three hemodynamic subtypes of essential hypertension are defined: isolated systolic hypertension (DBP is normal), isolated diastolic hypertension (SBP is normal), or combined hypertension (both SBP and DBP are high; Victor and Kaplan 2008). Isolated diastolic hypertension is more frequent in men than in women; it is also attributed to middle-age weight gain (Victor and Kaplan 2008). If not treated, isolated hypertension can progress to combined hypertension (Victor and Kaplan 2008).

The global burden of hypertension is rather high. In 2002, up to 20% of the population in the developed countries had high BP, and it was estimated that at least 500 million people worldwide had or would eventually have hypertension (Mulvany 2002). Hypertension was deemed the major risk factor for global mortality in the Global Burden of Disease Study 2010 (Lim et al. 2013).

1.3.11. Pathogenesis of hypertension

The pathogenesis of hypertension, despite the large volume of research and attention given to this topic, is not yet completely understood (Hayashi 2001; Mulvany 2002). Hypertension is associated with endothelial dysfunction, altered vascular contractility, and arterial remodeling (Paravicini and Touyz 2006). The underlying pathology of arterial hypertension involves elevated systemic vascular resistance, increased cardiac output, or both (Steffel and Lüscher 2011). The early stages of hypertension are characterized by increased cardiac output, likely due to left ventricular hyperactivity (Ching and Beevers 1991). In advanced hypertension, the cardiac output is normal, whereas the systemic vascular resistance is increased (Ching and Beevers 1991). In response to increased cardiac output in hypertension, the peripheral arteries contract, thereby increasing the systemic vascular resistance and further increasing the BP

(Ching and Beevers 1991). The regulation of BP is a complex process, and an increase in the BP value arises from an imbalance between the vasoconstrictive (such as angiotensin II, endothelin, thromboxane) and vasodilatory (NO, prostacyclin) substances (Steffel and Lüscher 2011). At the cellular level, hypertension is associated with changes in vascular muscle cell growth, apoptosis, cell migration, inflammation, and fibrosis (Paravicini and Touyz 2006).

It has been suggested that the kidney plays a role in hypertension development (Hayashi 2001). For example, sodium retention, a result of inadequate renal sodium excretion, is a well-known determinant of hypertension (Hayashi 2001). It has been suggested that salt sensitivity plays a role in hypertension development (Hayashi 2001). The kidney is also responsible for the production of various vasoactive substances, such as the renin-angiotensin system, prostaglandins, and NO (Hayashi 2001).

It has been suggested that the endothelial damage occurring in hypertension increases free radical production and thus destroys endothelium-derived NO; this process can be reversed by antioxidants such as superoxide dismutase, which scavenges free radicals (Ching and Beevers 1991). In addition, a damaged endothelium produces lower amounts of endothelium-derived NO compared to a healthy endothelium (Ching and Beevers 1991). Tissue damage related to reactive oxygen species (ROS) activity potentiates vasoconstriction by destroying endothelium-derived NO (Ching and Beevers 1991).

1.3.12. Small artery remodeling

The wall of a small artery consists of three layers: (i) the intima, the inner layer, which consists of longitudinally arranged endothelial cells; (ii) the media, the middle layer, which consists of circumferentially aligned smooth cells; and (iii) the adventitia, the outer layer, which consists of connective tissue and sympathetic nerves (Mulvany 2002). In hypertensive individuals, the structure of a small artery is altered: the lumen is reduced, and the ratio of media to lumen is increased (Mulvany 2002). This process is called remodeling. It has been suggested that the small artery diameter decreases and causes thickening of the arterial wall, ensuring that the wall tension remains normal (Mulvany 2002).

The only parameter that is consistently above normal values in hypertensive individuals is the

peripheral vascular resistance; most of the other factors, such as sympathetic activity and plasma renin activity, remain within normal range (Mulvany 2002). Increased peripheral vascular resistance results in higher BP, according to formula [10]. It has been suggested that vascular remodeling is the largest contributor to the increased peripheral resistance, possibly through narrowing of the resistance arterioles (Mulvany 2002). Narrowed arteriole diameter was a predictor of hypertension development in a rat model (Mulvany 2002).

The following factors could initiate remodeling: the activation of alpha receptors of the ANS by adrenaline; triggering noradrenaline release, which causes vasoconstriction; and circulating vasoconstriction hormones, such as angiotensin, vasopressin, prostaglandins, and serotonin (Ching and Beevers 1991). The cellular mechanisms of vascular remodeling are not yet completely understood. It has been shown that the phosphorylation of extracellular signal-regulated kinases 1 and 2, the expression of the proto-oncogenes c-fos and c-myc, and the activities of the platelet-derived growth factor beta receptor and matrix metalloproteinases 2 and 9 are involved in remodeling (Mulvany 2002). BPLM intake can somewhat reverse vascular remodeling by increasing the lumen diameter, but this process is impaired and does not suffice to fully normalize the small artery structure (Heagerty et al. 2010).

1.3.13. Medication against high blood pressure

The control of BP, especially of SBP, achieved either with lifestyle modification or medication, reduces total mortality, cardiovascular mortality, stroke and heart failure (Chobanian et al. 2003). The first step in BP control is lifestyle modification. If the aimed BP value is not achieved, then medication is prescribed (Chobanian et al. 2003). According to the Anatomical Therapeutic Chemical Classification System of the WHO Collaborating Centre for Drug Statistics Methodology, BPLM includes the following classes of drugs or any combination of them: diuretics, β-blockers, ACE inhibitors, angiotensin-receptor antagonists, calcium-channel blockers, α-blockers, centrally active antihypertensive drugs, and hydralazine. In the initial treatment phase, thiazide-type diuretics are used, sometimes in combination with other classes of drugs (e.g., an angiotensin converting enzyme inhibitor). A drug from another class can be chosen if the initial drug is tolerated. Most often, hypertension is treated with two or

more drugs, especially if the initial BP values are very high (Chobanian et al. 2003).

BP values can diminish substantially after effective therapy with BPLM. Clinical trials have reported reductions in SBP of approximately 5.4–8.4 mmHg and in DBP of 2.3–4.2 mmHg in response to medication (Turnbull 2003). In diabetic patients, antihypertensive treatment with nebivolol yielded an approximately 20 mmHg decrease in SBP (Schmidt et al. 2007). Similar results were reported for combination therapy in other studies (Everett et al. 2008; Mourad et al. 2007).

1.4. Association of air pollution with CVD

1.4.1. Air pollution and cardiovascular mortality and events

Positive associations of short-term elevations in PM_{10}, $PM_{2.5}$, UFP, elemental and organic carbon with cardiovascular mortality were identified in several studies, as reviewed by Rückerl et al. (2011). As estimated in a few time-series U.S. studies, an increase in the 24-hour mean $PM_{2.5}$ by 10 µg/m³ is associated with an increase in the relative risk for daily cardiovascular mortality by 0.4–1.0% (Brook et al. 2010). Brook et al. (2010) also noted that the risk is not equally distributed in the population: the susceptible groups, such as the elderly and subjects with coronary artery or structural heart disease, may be at higher risk of cardiovascular mortality due to PM. In Europe, the analysis of two large population-based studies showed a positive association with cardiovascular mortality: assuming the association was linear, a 10 µg/m³ increase in PM_{10} was associated with a 0.7% increase in cardiovascular mortality (Samoli et al. 2004). In a later re-analysis with North American and European studies together, Samoli et al. (2008) found that results for short-term mortality were quite similar.

Similar to acute effects, the long-term exposure to PM was also associated with cardiovascular deaths (Brook et al. 2010; Brunekreef et al. 2009; Miller and Siscovick 2007; Pope et al. 2004). For example, in the study with approximately 500,000 adults from the USA, a 10 µg/m³ increase in PM_{10} was associated with a 6% elevation in cardiopulmonary mortality risk (Brook et al. 2010). Additionally, reduced exposures to air pollution are associated with lower mortality (Laden et al. 2006). In a Dutch cohort, an increase in the yearly mean $PM_{2.5}$ by 10 µg/m³ was related to a 4% higher risk or cardiovascular mortality (Brunekreef et al. 2009). In

a recent meta-analysis of studies from the USA, Canada, China, Japan, Germany, the Netherlands, Switzerland, Italy, and New Zealand, an increase in the long-term $PM_{2.5}$ by 10 µg/m³ was associated with an increase in cardiovascular mortality by 11% (Hoek et al. 2013).

Short-term elevations in PM concentrations have shown associations with CVD events, such as the triggering of an acute myocardial infarction, heart failure and ischemic stroke hospitalization, and discharge of implanted automatic cardioverter defibrillators (Araujo and Nel 2009). Short- and long-term exposure to PM is associated with hospital admissions for ischemic heart disease, heart failure, cerebrovascular disease, cardiac arrhythmias and arrest (Brook et al. 2010; Domínguez-Rodríguez et al. 2011) In a meta-analysis of 35 studies on air pollution and heart failure, daily increases in CO, SO_2, NO_2, $PM_{2.5}$, PM_{10} were positively associated with heart failure hospitalization or death (Shah et al. 2013). In a recent meta-analysis of 11 European cohorts, long-term exposure to traffic-related air pollution was associated with an elevated risk of new coronary events, even at air pollution levels below the current European limits (Cesaroni et al. 2014).

1.4.2. Air pollution and atherosclerosis

The association of different air pollutants with cardiovascular events may share a common underlying pathology – atherosclerosis. This disorder is characterized by atherogenesis (the development of atheromatous plaques) and, in more advanced stages, smooth muscle cell proliferation, fibrous cap formation, necrotic cores, calcification, rupture, hemorrhage and thrombosis (Araujo and Nel 2009). Atherogenesis is a lifelong process (Künzli et al. 2011). The early central features of the artery wall pathology are oxidative stress, followed by systemic and vascular inflammation, endothelial dysfunction, and lipid deposition in the arterial wall (Künzli et al. 2011). These pathologic changes result in reduced vascular reactivity, arterial stiffening, thickening of the arterial wall, arterial stenosis, and plaque formation (Künzli et al. 2011). The atheromatic plaque formation (lipid deposition and oxidation in the artery wall) is regarded as an intermediate between risk factor exposure and clinical events (Erbel et al. 2010). Coronary plaque rupture leads to acute coronary manifestations, such as myocardial infarction; carotid plaque rupture causes cerebral stroke and vascular dementia (Künzli et al.

2011).

According to a lifetime model of atherosclerosis development, progression of atherosclerosis can be divided into 3 phases: normal, preclinical and clinical disease manifestation (Künzli et al. 2011). Acute clinical events may manifest at approximately 40 years of age or later, depending on the degree of individual risk (Künzli et al. 2011). The degree of atherosclerosis at any age reflects a combination of genetic predisposition and cumulative exposure to internal and external factors that have either protective or deteriorating effects on vascular health (Künzli et al. 2011).

An external factor possibly affecting vascular health is outdoor air pollution. It has been suggested that air pollution plays roles in pulmonary and systemic inflammation, cardiac autonomic function impairment, and accelerated atherosclerosis (Mills et al. 2009; Pope et al. 2004). Some studies indicated associations between air pollution and systemic inflammation and endothelial dysfunction (Brook et al. 2010; Hertel et al. 2010; Hoffmann et al. 2009; Krishnan et al. 2012). Exposure to air pollution induced vascular inflammation and oxidative stress and promoted atherosclerotic plaque expansion or rupture (Brook et al. 2010; Mills et al. 2009). Künzli et al. (2004, 2010) have identified a cross-sectional and a prospective association of air pollution with subclinical atherosclerosis, measured as carotid intima-media thickness. The studies with a population-based German cohort showed that living close to a major road and being exposed to elevated concentrations of $PM_{2.5}$ were positively associated with the markers of subclinical atherosclerosis (Bauer et al. 2010; Hoffmann et al. 2007). A positive, though much weaker, association of long-term PM with intima-media thickness was also identified in an American population-based cohort (Diez Roux et al. 2008). Two hypotheses of the association of air pollution with atherosclerosis have been suggested by Künzli et al. (2011): (i) long-term exposure to outdoor air pollution causes atherogenesis, therefore, exposed individuals would have a faster progression of vascular pathologies and would reach the clinical manifestations of atherosclerosis at earlier ages than non-exposed individuals; and (ii) short-term exposure to air pollution can trigger cardiovascular events in susceptible individuals (i.e., those with pre-existing subclinical atherosclerosis). Considering the longitudinal nature of atherogenesis, the chronic effect of air pollution on atherosclerosis, described by

hypothesis (i), is relevant at any age. The acute effect on cardiovascular events, described by hypothesis (ii), is more relevant at an older age, when atherogenesis reaches the pre-clinical stage.

1.4.3. Association of air pollution with BP and hypertension

The causal association between air pollution and atherosclerosis and cardiovascular events may involve elevated BP and hypertension (Brook et al. 2009). In a few recent studies, a positive association between short-term increases in PM air pollution and acute elevations in arterial BP has been reported (Brook et al. 2010, 2011; Chuang et al. 2010; Delfino et al. 2010; Dvonch et al. 2009; Hoffmann et al. 2012; Mordukhovich et al. 2009; Zanobetti et al. 2004). The association was reported in both the general population (Auchincloss et al. 2008; Brook et al. 2011) and among more susceptible individuals, such as diabetics (Hoffmann et al. 2012), the elderly (Mordukhovich et al. 2009; Wilker et al. 2010), and subjects with chronic obstructive pulmonary disease (Linn et al. 1999). In a Dutch cohort of pregnant women, the residential outdoor PM_{10} concentration was associated with increases in BP in the second and third trimesters (van den Hooven et al. 2011). Some studies report inverse associations of short-term air pollution exposure with BP. For example, ambient PM_{10} concentration was associated with higher BP in the first trimester of pregnancy and lower BP in the later trimesters in a French cohort; higher NO_2 concentrations were associated with decreased BP throughout the entire pregnancy period (Hampel et al. 2011). In a Taiwanese cohort, short-term exposure to various air pollutants was negatively associated with SBP and positively associated with DBP (Chen et al. 2012).

Based on the evidence from short-term studies with air pollution and BP, a positive association between long-term exposure to air pollution and elevated BP, independent from and possibly also stronger than short-term fluctuations, has been proposed (Rückerl et al. 2011). Stronger associations with long-term than with short-term exposure levels have already been shown for other health outcomes (Brook et al. 2010; Diez Roux et al. 2006). There is also some supporting evidence for a long-term association with BP. For example, Auchincloss et al. (2008) reported that the association of longer-term averages (30- or 60-day means) of

$PM_{2.5}$ with BP was stronger than the results observed for daily or weekly averages.

Thus far, the evidence for long-term effects is rather scarce. In two American studies with selected populations (elderly men and black women, respectively), traffic-related air pollution was linked to higher BP or hypertension (Coogan et al. 2012; Schwartz et al. 2012). Long-term exposures to PM and gaseous air pollutants were associated with high BP and hypertension in two large Asian cohorts (Chuang et al. 2011; Dong et al. 2013). Long-term PM concentrations were positively related to self-reported hypertension among white American adults (Johnson and Parker 2009). In a large cohort of non-hypertensive adults from Canada, long-term exposure to $PM_{2.5}$ was positively associated with incident physician-diagnosed hypertension (Chen et al. 2013). One study reported a negative long-term association of air pollution with BP: in a large population-based Danish cohort of older adults, long-term exposure to nitrogen oxides, indicators of traffic-related air pollution, was associated with decreased BP and a lower prevalence of self-reported hypertension (Sørensen et al. 2012).

1.5. Pathophysiology of the adverse cardiovascular effects of air pollution

Oxidative stress, a condition involving chronically elevated levels of ROS, plays a central role in the development of adverse effects of air pollution on the cardiovascular system (Brook et al. 2010; Paravicini and Touyz 2006). Three general pathways have been suggested based on the current evidence: (i) pulmonary inflammation and oxidative stress, (ii) ANS imbalance and (iii) direct translocation of PM constituents to the systemic blood flow (Brook et al. 2010).

1.5.1. Initiation of pulmonary inflammation and oxidative stress

Inhalation of particles into the lung can trigger a localized inflammatory response in the lung (Mills et al. 2009). Exposure to PM has been correlated with the concentrations of redox-active compounds and macrophage damage in bronchial epithelium (Lodovici and Bigagli 2011). Transition metals, such as Fe, Pb, Ni, V, Cr, Mn, Cu, can be adsorbed on the surfaces of fine and ultrafine particles and can generate ROS (e.g., as hydroxyl radical) with Fenton's reaction (Lodovici and Bigagli 2011):

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + HO^* + H_2O \quad [11]$$

For example, when polycyclic aromatic hydrocarbons (PAH) are metabolized with cytochrome P450, redox-active quinones are formed, which can trigger ROS production (Lodovici and Bigagli 2011). PM can inhibit the oxidative stress responses; activate the proinflammatory genes, such as tumor necrosis factor alpha (TNFα); and stimulate the production of proinflammatory cytokines and chemokines (Lodovici and Bigagli 2011). Diesel exhaust particles can cause lipid peroxidation and ROS production (Lodovici and Bigagli 2011). Gaseous pollutants, such as ozone and nitrogen oxides, can induce oxidative stress and inflammation. For example, O_3 induces oxidative stress through the formation of ozonide O_3^- and hydrogen peroxide (Lodovici and Bigagli 2011).

1.5.2. Transformation from local to systemic inflammation and oxidative stress

The cytokines, activated immune cells, and platelets, which are synthesized or released due to pulmonary inflammation and oxidative stress, can reach the systemic circulation. The plasma levels of cytokines, such as interleukin (IL) 1β, IL-6, and granulocyte-macrophage colony-stimulating factor, are elevated after exposure to PM (Mills et al. 2009). PM is associated with the increased release of neutrophils and monocytes into the systemic circulation (Mills et al. 2009). Finally, the acute-phase proteins and coagulation markers, such as C-reactive protein (CRP), ICAM-1, VCAM-1, fibrinogen, and platelets are associated with short- and long-term exposure to PM and gaseous pollutants, such as NO_2 and CO, in the general population (Bind et al. 2012; Delfino et al. 2008; Hennig 2014; Hertel et al. 2010; Lucking et al. 2008; Pekkanen et al. 2000).

1.5.3. Inflammation, oxidative stress and arterial BP

There are many links from the immune system and inflammatory processes CVD, and to hypertension in particular. For example, the Toll-like receptors, which are part of the innate immune system and can "sense" pathogens, toxins and other substances, play a role in CVD. These receptors can be activated by oxidized lipoproteins, are involved in atherosclerosis development, and can send inflammatory response signals that can affect the cardiovascular system (e.g., ROS, reactive nitrogen species [RNS], etc.; Harrison et al. 2011).

Oxidative stress is an imbalance between ROS formation and antioxidant defense mechanisms (Touyz and Schiffrin). If ROS production in the cell predominates the antioxidant defense reactions, cellular oxidative stress may develop, triggering signaling pathways as a result of redox disequilibrium that result in the activation of cytokine and adhesion molecule expression (Araujo and Nel 2009). It has been suggested that oxidative stress mediates the adverse cardiovascular effects of air pollution with PM (Araujo and Nel 2009).

The vasculature is a rich source of ROS and RNS. Under normal conditions, these molecules function to maintain vascular integrity, which is achieved through the regulation of smooth cell contraction, relaxation, and growth (Touyz and Schiffrin). Common endogenous ROS include superoxide anion $^{*}O_2^{-}$, hydrogen peroxide H_2O_2, hydroxyl radical HO^{*}, and hypochlorous acid HOCl; common RNS include NO and peroxynitrite ($ONOO^{-}$; Touyz and Schiffrin). Under pathological conditions, the same compounds act detrimentally: they may trigger endothelial dysfunction, smooth muscle cell apoptosis, inflammation, and other vascular damage (Touyz and Schiffrin).

Though the clear cause-and-effect relationships are not yet completely understood, it is known that oxidative stress occurs in hypertension (Khullar et al. 2003). Patients with hypertension have elevated concentrations of oxidative stress byproducts and demonstrate decreased activity levels of endogenous antioxidant enzymes in whole blood and mononuclear cells compared to healthy individuals (Redón et al. 2003). Oxidative stress in hypertension can promote vascular smooth muscle cell proliferation and hypertrophy and collagen deposition; can stimulate the expression of proinflammatory molecules, such as adhesion molecules and chemotactic proteins; and can mediate the oxidation of lipids and cell migration(Touyz and Schiffrin). All of these processes contribute to arterial remodeling and hypertension development (Touyz and Schiffrin).

Oxidative stress and hypertension are closely related. On the one hand, the production of ROS and other markers of oxidative stress is increased during hypertension; on the other hand, the deactivation of free radicals with antioxidants improves vascular function and leads to reduced BP (Touyz and Schiffrin). Furthermore, animal models that are unable to produce ROS

tend to have lower BPs compared to controls (Touyz and Schiffrin). ROS have multiple effects on the organism that are relevant to hypertension; they promote sympathetic outflow in the central nervous system, and they trigger blood vessel constriction and sodium and volume retention in the kidneys (Harrison et al. 2011). In addition to this prohypertensive action, ROS activate the inflammatory response, which might also be relevant for hypertension. Harrison et al. (2011), analyzing the findings of their and other researchers' studies, hypothesize that hypertensive stimuli, such as angiotensin II, may activate T-cells and, in turn, the entry of other inflammatory cells into the vasculature; these events lead to the release of cytokines causing vasoconstriction, sodium retention, and other effects that further increase BP and lead to more severe hypertension.

Paravicini and Touyz (2006) delineate the three most relevant sources of ROS in vascular pathology: xanthine oxidase, eNOS and nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase. Of them, NAD(P)H oxidase is the main ROS source (Paravicini and Touyz 2006). The NAD(P)H oxidase enzyme is a multi-component, NADPH-dependent enzyme that has been characterized in three types of vascular wall cells. The enzyme generates superoxide anion in the presence of molecular oxygen (Yu et al. 2008). NAD(P)H oxidase plays a significant role in atherosclerosis progression, and its inhibition can slow down adverse cardiovascular processes (Yu et al. 2008). The increased activity of vascular NAD(P)H oxidase is associated with hypertension pathology (Touyz and Schiffrin). NAD(P)H oxidase is activated by vasoactive hormones (such as angiotensin II), growth factors and mechanical stimuli (Paravicini and Touyz 2006). Therefore, angiotensin II may be one of the factors linking hypertension with oxidative stress (Khullar et al. 2003). Its effects are mediated by ROS, which are produced with the help of NAD(P)H oxidase (Paravicini and Touyz 2006). The antioxidant defense enzymes, such as superoxide dismutase, play an important role in inflammation-mediated BP regulation (Gongora et al. 2006; Lob et al. 2010).

1.5.4. The role of endothelial dysfunction

An important function of the endothelium is to maintain vascular homeostasis (Widlansky et al. 2003). This function is achieved via the interactions of the endothelium with the cells in

the vessel wall and lumen (Widlansky et al. 2003). Endothelial dysfunction is characterized by the impaired vasomotion and vascular tone, the pro-thrombotic and pro-inflammatory state, and proliferation in the arterial wall (Widlansky et al. 2003). Vascular oxidative stress decreases the bioavailability of NO, a potent vasodilator; together with an inflammatory response, this effect may promote endothelial dysfunction (Touyz and Schiffrin). Inflammation mediates oxidative stress in CVD and leads to vascular injury and endothelial dysfunction (Lin et al. 2013). Oxidative stress and endothelial dysfunction are often observed in hypertensive subjects, and these conditions have been suggested to play causal roles in hypertension development (Lin et al. 2013). Endothelial dysfunction is also considered an early step in atherosclerosis development (Sitia et al. 2010). It has been hypothesized that vascular damage due to oxidative stress is a link from hypertension to atherosclerosis (Alexander 1995).

The inflammatory response induced by air pollution exposure can impair the function of the vascular endothelium. For example, in a cohort study with 704 elderly American men, short-term exposure to traffic-related pollutants ($PM_{2.5}$, NO_2, CO) was associated with vascular cell adhesion molecules related to both inflammation and vascular function (Bind et al. 2012). Vascular tone regulation was affected by short-term exposure to diesel exhaust in a panel study with healthy volunteers (Mills et al. 2005). In an animal model of PM_{10} exposure, anti-inflammatory drugs reduced atherosclerosis and improved endothelial function, protecting against the adverse effects of PM_{10} (Miyata et al. 2013). Anti-inflammatory and antioxidant medications (such as statins) have beneficial effects on endothelial progenitor cells and therefore protect against endothelial injury and aging (Tousoulis et al. 2008).

1.5.5. Air pollution, ANS imbalance and hypertension

Short-term exposure to PM can affect the autonomic control of the heart rate, resulting mostly in decreased heart rate variability (Brook et al. 2009, 2010; Chuang et al. 2007; Elder et al. 2007). Air pollution can activate the sympathetic nervous system and trigger parasympathetic withdrawal, resulting in an acute increase in BP (Brook et al. 2010; Miller et al. 2012). It is hypothesized that deposited particles could alter central nervous system activity either directly or by interacting with lung receptors and by altering ANS reflex arcs (Brook et al. 2004).

Sympathetic nervous system activation is the most probable mechanism of BP elevation shortly after exposure; it has been suggested that decreased heart rate variability leads to elevated DBP (Brook et al. 2009). PM inhalation increases baroreceptor reflex sensitivity, which corresponds to the upregulation of vagal reflexes (Shannahan et al. 2012). Additionally, it was suggested that the contradictory results of animal experimental studies, where PM increased BP in some studies and decreased in others, were due to the stimulation of either the sympathetic or the parasympathetic nervous system (Shannahan et al. 2012).

Typically, in the state of ANS imbalance, the sympathetic nervous system is hyperactivated whereas the parasympathetic nervous system is hypoactive (Thayer et al. 2010). This autonomic imbalance is related to a number of pathologic cardiovascular conditions (Thayer et al. 2010). In particular, vagal tone is lower in persons with hypertension, and it has been suggested that decreased heart rate variability may precede hypertension (Thayer et al. 2010). In a recent case-control study, the ANS imbalance contributed to prehypertension status and cardiovascular risks caused by insulin resistance, dyslipidemia, inflammation, and oxidative stress in diabetic subjects (Pal et al. 2013).

1.5.6. Translocation of particles into the circulation

It has been suggested that ultrafine and nanoparticles could rapidly translocate to the circulation (Mills et al. 2009). This translocation has been demonstrated in some recent animal studies (Mills et al. 2009; Shannahan et al. 2012). Moreover, the capacity of UFPs to translocate to secondary organs, such as the liver, the kidney, and the heart, was demonstrated (Shannahan et al. 2012). Both particle size and charge play roles in the translocation (Shannahan et al. 2012). It is also possible that particles do not cross the lung-blood barrier directly, but are ingested and transported by alveolar macrophages (Mills et al. 2009).

Translocated UFPs or soluble compounds can induce arrhythmias, reduce myocyte contractility, and reduce the coronary blood flow (Lodovici and Bigagli 2011). UFP exposure stimulates the expression of genes related to inflammation and coagulation, and diesel exhaust may trigger inflammation and oxidative stress (Mills et al. 2009; Shannahan et al. 2012). Translocated particles affect vascular reactivity, inhibiting vascular NO production and stimulating vaso-

constriction through oxidative stress (Shannahan et al. 2012). Apart from the particles' effects, additional toxicity was attributed to transition metals and organic components from UFPs, which could also translocate through the lung-blood barrier and had cardiotoxic effects (Shannahan et al. 2012). The process of translocation can be compared to the accumulation of LDL particles (which are approximately the size of a nanoparticle) in the arterial wall, which constitutes the pathophysiology of atherosclerosis (Mills et al. 2009).

1.6. Methodological challenges in the investigation of air pollution associations with BP and hypertension

1.6.1. The responsible components

Many constituents of air pollution are correlated. Therefore, the adverse health effects, observed with specific components of the mixture, may actually reflect the toxicity of other components correlated with them. For example, NO_2 is an indicator of traffic-related air pollution but also contributes to the generation of O_3 and oxidant pollutants and is a precursor of nitrates in PM (WHO 2003). It is necessary to disentangle the effects of individual compounds, which can be achieved with multipollutant models, such as models including both particulate and gaseous components. The results with multipollutant models may differ substantially from single-pollutant models. For example, though it is generally considered that the PM component of air pollution contributes to elevated BP, Coogan et al. found that NO_x but not $PM_{2.5}$ was associated with the incidence of hypertension and diabetes mellitus in a two-pollutant model to which both these exposures were included (Coogan et al. 2012).

1.6.2. Related exposures at the residence

In studies of air pollution effects, it is important to take into account other residential exposures that may confound the results. One of such exposures is road traffic noise. Daytime noise at a level of 65 dB is harmful for health (Babisch 2008). It is assumed that the adverse effects of noise occur through the stress response system (Babisch 2008). Road traffic noise was associated with the risk of myocardial infarction in a dose-response manner (Babisch 2008). The association of road traffic noise with BP and hypertension has been extensively studied. Laboratory studies demonstrated that acute noise exposure affects the sympathetic

ANS and endocrine system, triggering a number of nonspecific physiological responses, including altered heart rate variability, vasoconstriction and elevated BP, stress hormones, and others. (Babisch and Kamp 2009). Although it is likely that road traffic noise does not confound the results with air pollution and cardiovascular outcomes, as shown in recent studies (Beelen et al. 2009; Viehmann et al. 2010), it is important to investigate the role of road traffic noise in the effects observed with air pollutants.

Residential characteristics, such as neighborhood-level income, population density, and other socio-economic factors, are associated with CVD (Diez Roux 2003; Dragano et al. 2009b; Gerber et al. 2011). Similarly to road traffic noise, residential deprivation may confound the association with air pollution and needs to be taken into account.

1.6.3. Correction for BPLM intake in BP analysis

The lifetime risk of developing hypertension for normotensive individuals aged 55–65 is more than 90% (Chobanian et al. 2003). Therefore, a substantial proportion of the general population is on BPLM. In medicated subjects, the measured value will be greatly influenced by medication and will not reflect the actual ("underlying") value. This disparity could lead to bias in an analysis with BP as the outcome (Tobin et al. 2005).

Thus far, there is no universally used strategy to correct for BPLM intake in the analyses with BP. Many researchers still use the "naïve" methods of adjustment, which are not recommended because they can yield biased results (McClelland et al. 2008; Tobin et al. 2005). "Naïve" adjustment strategies include (i) ignoring the problem, (ii) adjusting for BPLM intake with an indicator variable, (iii) excluding medicated participants from the analysis (McClelland et al. 2008), or (iv) adjusting for baseline BP, which all lead to biased results in genetic association studies (McArdle and Whitcomb 2009). The only conditions under which the model ignoring treatment will provide an unbiased estimate of the actual effect are if (i) no participants are taking any medications and if (ii) the medication has no effect (McClelland et al. 2008). These adjustments can barely be fulfilled in a real-life cohort, which is why it is very important to use more sophisticated techniques to correct for medication intake.

In a study by Tobin et al. (2005), various techniques to correct for BPLM were estimated us-

ing simulated and real data. Among the recommended methods that had already been used in a few other studies were fixed addition (adding a fixed increment to measured BP values in medicated individuals; Newton-Cheh et al. 2009; Timpson et al. 2009); censored normal regression (censoring BP values in medicated individuals with the assumption that the distribution of BP in medicated and non-medicated subpopulations does not differ); and a semi-parametric method to substitute BP values in medicated individuals, based on averaging the ordered residuals (Tobin et al. 2008). These and other methods of correction for BPLM imply some assumptions for the dataset and should be carefully considered before the analysis strategy is chosen.

1.7. Summary: rationale for this project

The long-term effects of air pollution on BP are not completely understood. In 2010, high BP was deemed one of three major risk factors for mortality worldwide (Lim et al. 2013). It is hypothesized that long-term exposure to air pollution could raise BP chronically and lead to hypertension (Brook 2007), but the evidence is scarce and not conclusive.

The components of the air pollution mixture that are responsible for the positive association with BP should be identified. Thus far, the most consistent association with CVD has been reported for $PM_{2.5}$. However, it is possible that other components, such as nitrogen oxides or carbon monoxide, can affect cardiovascular health. Due to the correlations of individual mixture components, the results with one exposure may actually reflect the toxic effects of the other component. It is also possible that PM interacts with gaseous pollutants, which may alter the composition and toxicity of the mixture (WHO 2003).

Detailed information is needed regarding the exact mechanistic pathways through which air pollution can lead to hypertension. It is hypothesized that air pollution could elevate BP through oxidative stress and inflammation, ANS imbalance, and vascular dysfunction (Brook et al. 2010). Experimental animal studies or controlled-exposure human studies are scarce and do not provide conclusive information.

It is important to disentangle air pollution effects from other related exposures. Exposure to traffic particles co-occurs with exposure to road traffic noise. Traffic noise acts through stress-

response mechanisms, which may overlap with those involved in particle effects or may interact with them (Babisch 2008). Additionally, air pollution may correlate with the neighborhood-level deprivation. The effects of air pollution may thus reflect the differences in health caused by numerous factors related to socio-economic status.

The choice of an appropriate strategy to correct for the effect of BPLM is important. Outcome-affecting medication is an acknowledged constraint in analyses (Tobin et al. 2005). The literature offers recommendations regarding methods to correct for this effect.

2. STUDY AIMS AND HYPOTHESES

Aim of the study

The aim of this study is to investigate the effect of long-term air pollution and its specific components on BP and hypertension in humans and to investigate possible pathophysiologic mechanisms in an animal model. $PM_{2.5}$ is the primary exposure of interest in this study. In addition, coarse (PM_{coarse}, PM_{10}) and ultrafine ($PM_{1,}$ particle number (PN)) particles will be analyzed as exposures. Common gases of the air pollution mixture will be investigated as co-exposures.

Specific objectives

1. Study the long-term association of $PM_{2.5}$, PM_{10}, PN, PM_1, nitrogen oxides (NO_2, NO), SO_2, CO, NH_3, and O3 with arterial BP and hypertension in a population-based cohort.
    a. Perform cross-sectional and longitudinal analysis, controlling for the relevant confounders.
    b. Study the robustness of the results using different covariate specifications.
    c. Test the independence of results with PM from co-exposure to the gasous compounds, using multipollutant models and other methods.
    d. Test the independence of results for PM from other residential exposures: i.e., road traffic noise and neighborhood characteristics.
    e. Identify the susceptible population groups using effect modification analysis.
    f. Select and apply different strategies to correct for BPLM intake in the analysis of the effect of air pollution on BP.
2. Investigate the expression profiles of genes related to air pollution and hypertension in murine lung tissue after experimental exposure to diesel exhaust.
    a. Prepare the biologic materials.
    b. Measure the expression levels of selected genes involved in inflammation, oxidative stress, and vascular response and compare them to housekeeping genes.
    c. Investigate quantitative data on gene expression in exposed animals compared to non-exposed controls.

3. Integrate the results, discuss them and make conclusions.

Hypotheses

1. Long-term residential exposure to fine PM is linearly associated with an increase in arterial BP and risk of hypertension in the general population. This association is independent from the following factors: short-term air pollution fluctuations, co-exposure to common gases in the air pollution mixture, road traffic noise, neighborhood social factors, and personal risk factors.

2. Traffic-related air pollution can affect the expression of genes related to elevated BP and hypertension in an animal model setting.

3. MATERIALS AND METHODS

3.1. Observational part

3.1.1. Study population

I used data from the prospective ongoing Heinz Nixdorf Recall (Risk Factors, Evaluation of Coronary Calcium and Lifestyle; HNR) study. The HNR study aims to evaluate the prognostic value of noninvasive visualization and quantification of atherosclerosis (e.g., electron-beam computed tomography for the prediction of cardiac events), in a population-based cohort, and investigate whether electron-beam computed tomography, as a novel method of risk assessment, provides independent information in addition to the traditional analysis of risk factors (Erbel et al. 2012; Schmermund et al. 2002). Parallel to the main aim of the study, a large amount of individual data, such as health characteristics, biomarkers, socio-economic indicators, genetic polymorphisms, environmental exposures, residential context factors, etc., is being collected and used in various investigations. The study population was randomly selected from the mandatory registries of three adjacent cities: Bochum, Essen, and Mülheim an der Ruhr. These three cities are located in the densely populated Ruhr area, with a tight traffic and industrial network. Study participants were aged 45 to 75 years at baseline. If a participant had overt CHD, defined as diagnosed myocardial infarction or revascularization of coronary arteries, including balloon dilatation and coronary bypass surgery, he (or she) was excluded from the primary HNR analysis as a prevalent case, but not from the HNR cohort or the analysis cohort, included in the current study.

Baseline examination

In total, 4,814 participants were invited to the university hospital of Essen to undergo the first (baseline) physical examination and medical history assessment. The first baseline examination of a participant was performed on December 11, 2000 and the last on August 13, 2003. All participants signed informed consent forms. Examinations in the cohort were conducted in accordance to the recommendations for research on human subjects, adopted by the 18th World Medical Assembly, Declaration of Helsinki (World Medical Association 1964) and later revisions, and were approved by the ethics committee at the University of Essen, Ger-

many (Schmermund et al. 2002). An external review board evaluated the study. Study personnel underwent certification (the German Institute for Standardization, Euronorm ISO 9001:2000), and regular quality checks.

During the baseline visit, the following measurements were performed: anthropometric measurements, laboratory blood tests (blood sugar, lipids, inflammatory factors and others), BP measurement, resting and exercise electrocardiograms, ankle-brachial index measurement, coronary calcification assessment with non-contrast-enhanced electron-beam computed tomography, and carotid artery intima-media thickness measurement with B-mode sonography (Bauer et al. 2010; Schmermund et al. 2002). Information on current medication, lifestyle (diet, physical activity, smoking, and alcohol consumption), and individual socio-economic status (SES, defined as education, economic activity, household income) was collected in a computer-assisted personal interview. Except for electron-beam computed tomography results and experimental findings (novel risk factors, genetic polymorphisms), the results were reported to a participant and, upon each participant's agreement, to his primary physician (Schmermund et al. 2002).

Follow-up period and second examination

During the follow-up period, self-report questionnaires were sent to participants annually by post. In these questionnaires, the study end points were assessed. The primary endpoints included nonfatal myocardial infarction and cardiac death. The secondary endpoints included all-cause mortality, cerebrovascular events, coronary revascularization, angiographically defined incident peripheral vascular disease, hospitalization for cardiac disease, and initiation of medical therapy for cardiac disease (Schmermund et al. 2002). Endpoints were affirmed by the endpoint committee, including cardiologists and at least one epidemiologist. Some additional information, e.g., lifestyle, nutrition, traffic noise annoyance, etc., was also gathered in annual questionnaires. Five years after the baseline interview, the participants were re-invited to a follow-up assessment of their medical history, physical and health risk factor status, blood tests, blood pressure measurement, non-contrast electron-beam computed tomography imaging, and electrocardiograms (Lehmann et al. 2014). Follow-up assessment was completed for

4,359 participants. Loss to follow-up constituted 9.5% of the study population, which was lower than the estimation of 15% (Schmermund et al. 2002). The first follow-up investigation was performed on May 15, 2006, and the last one on September 18, 2008.

Participants included in the current analysis

In the analysis with baseline data I included all participants with no missing information on: (i) exposure (modeled air pollution concentrations); (ii) outcome (measured BP and BPLM intake); and (iii) residential co-exposures and covariates in the main model. In total 4,584 participants, comprising 95.2% of the baseline sample, were included to the current analysis with baseline data (Figure 2).

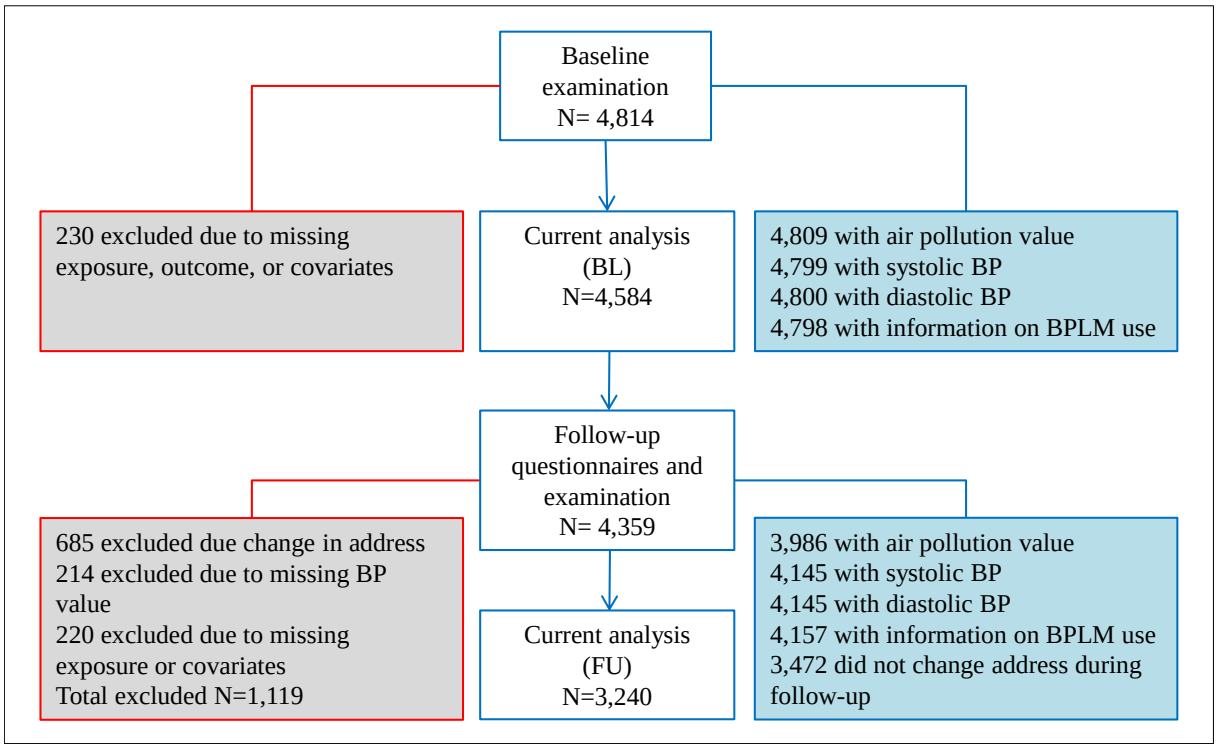


Figure 2. Baseline and follow-up examination in the HNR study and the inclusion of participants in this analysis.

Legend: BL = baseline examination, FU = follow-up examination.

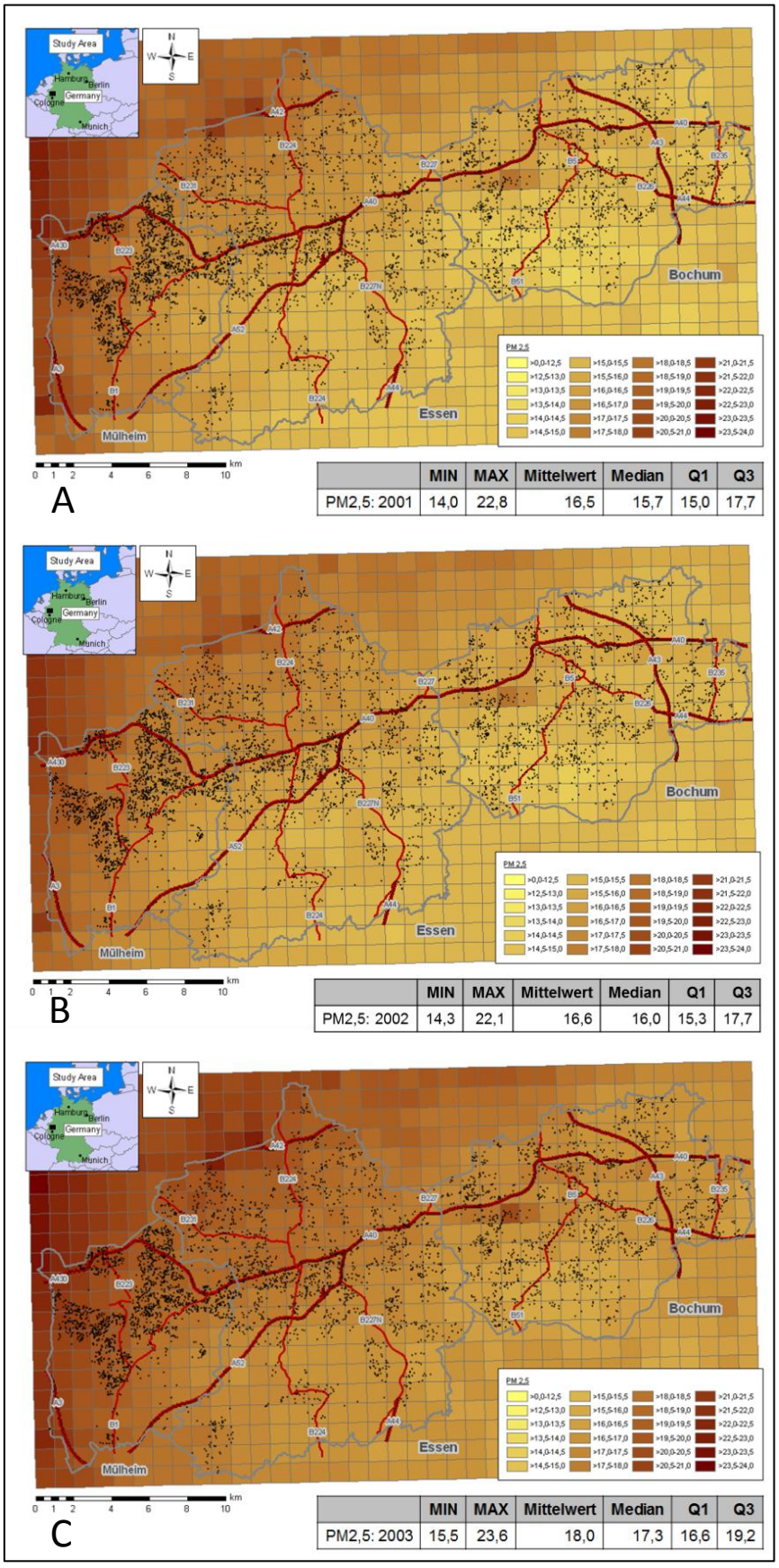
Out of 4,359 participants who filled out the follow-up questionnaires, arterial BP was measured in 4,157 participants. I excluded 685 participants who changed their residences during follow-up (to avoid exposure misclassification) and 220 with missing information on expo-

sures or covariates. The remaining sample of 3,240 participants was included in the analysis of the follow-up data.

3.1.2. Exposure assessment

Air pollution

The European Air pollution Dispersion Chemistry and Transport Model (EURAD-CTM) was employed to assess individual air pollution exposure at the home addresses of the HNR participants (Ebel et al. 2007; Memmesheimer et al. 2004). The EURAD-CTM is a validated, time-dependent, three-dimensional model. With the help of this model, the daily mass concentrations of PM_1, $PM_{2.5}$, PM_{10}, PN, NO_2, NO, SO_2, O_3, CO, and NH_3 were modeled for the study area (approximately 600 km²) with a resolution of 1 km², as shown in Figure 3. This resolution is the smallest for the anthropogenic emissions, such as industrial sources, household heating, traffic, agriculture, etc., provided by the North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection. The EURAD-CTM *"simulates the physical, chemical and dynamical processes that control emission, production, transport and chemical transformation and deposition of atmospheric trace species"* (Rhenish Institute for Environmental Research); it is based on the fundamental physical principles of conservation of mass, momentum and energy (Hennig et al. 2014). The model combines the fluid dynamic equations with information on chemical and physical transformations that air pollution constituents undergo in the atmosphere (Hennig et al. 2014). The upper vertical boundary of the model is 16 km; the lowest layer is approximately 40 m high (Hennig et al. 2014). The EURAD-CTM uses the sequential nesting method, starting from the large Europe-wide scale and narrowing down to the Ruhr area (in total, there were 4 nests with grid sizes of 125 km, 25 km, 5 km, and 1 km), which allowed to include the long-range transport and formation of secondary particles in the atmosphere (Hennig et al. 2014; Memmesheimer et al. 2004).



	MIN	MAX	Mittelwert	Median	Q1	Q3
PM2,5: 2001	14,0	22,8	16,5	15,7	15,0	17,7

	MIN	MAX	Mittelwert	Median	Q1	Q3
PM2,5: 2002	14,3	22,1	16,6	16,0	15,3	17,7

	MIN	MAX	Mittelwert	Median	Q1	Q3
PM2,5: 2003	15,5	23,6	18,0	17,3	16,6	19,2

Figure 3. Modeled yearly concentrations of urban background $PM_{2.5}$ in the study area.

Legend: A = year2001, B = year 2002, C = year 2003. Source: I. Vanberg, unpublished (personal communication).

With the help of the EURAD-CTM model, the mean daily concentration of each respective pollutant was modeled in 1 km^2 grid cells. The primary output concentrations were then calibrated with measured PM_{10} concentrations from 6 routine monitoring sites throughout the study area, taking into account the nature of the monitoring station (traffic, industrial site, urban background, regional background). Data from measurement stations were supplied by the North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection. The calibrated values were assigned to the participants' addresses using the geographic information system (ArcView 9.2, ESRI, Redlands, CA, USA). I calculated the mean of the 365 daily PM values prior to the participant's examination date to assess the individual long-term residential exposure. To discriminate the spatial contrast in exposure from the temporal contrast, I calculated a long-term grid-specific mean concentration as the average of the baseline period (2000–2003) and the follow-up period (2006–2008) for each grid cell 1×1 km. The long-term grid-specific mean concentration was assigned to each participant's residence.

Short-term air pollution and meteorology

Short-term exposure was included as a covariate in the analysis. I calculated the respective short-term mean (the choice of averaging time was based on model fit), subtracting the yearly mean to avoid over-adjustment. In addition to air pollutant concentrations, the EURAD-CTM model predicts meteorological parameters (Hennig et al. 2014). I used mean of lags 0–6 with all PM metrics, PN, NO_x, SO_2, and CO, mean lags 0–1 with NH_3, and mean of lags 0–2 with O_3. Daily mean temperature (t°), precipitation, and wind speed were assigned to each participant's address, similarly to air pollution. Two-day mean temperature and East-West wind were selected as the short-term meteorology variables based on the best model fit and were included as covariates in some adjustment models.

Traffic indicators

Distance from participant's residence to the nearest major road was estimated using digitalized maps (ArcView 9.2, ESRI, Redlands, CA, USA) and daily traffic counts provided by the North Rhine–Westphalia State Agency for Nature, Environment and Consumer Protection. A major road was defined as a road section in the upper quartile of the daily traffic count (>

22,980 vehicles per day for general traffic, > 756 vehicles per day for heavy-duty traffic and > 4,341 for diesel traffic). I categorized distance to major road as follows: ≤ 50 m, 51-100 m, 101-200 m, and > 200 m (reference category). When entered as a covariate in the analysis with air pollution or road traffic noise as main exposures, linear distance to road in upper quartile of diesel vehicle density was used as a covariate, based on the better model fit.

Road traffic noise

Long-term road noise was modeled according to the Directive 2002/49/EC of the European Parliament and of the Council as weighted 24-hour mean (L_{den}) and night-time mean (L_{night}). The following formula was used:

$$L_{den} = 10lg\frac{1}{24}\left(12 \times 10^{\frac{L_{day}}{10}} + 4 \times 10^{\frac{L_{evening}+5}{10}} + 8 \times 10^{\frac{L_{night}+10}{10}}\right) \quad [12]$$

where L_{day} was the A-weighted long-term average sound level, as defined in ISO 1996-2: 1987, determined over all of the day periods of the year; $L_{evening}$ was the A-weighted long-term average sound level, as defined in ISO 1996-2: 1987, determined over all of the evening periods of the year; and L_{night} was the A-weighted long-term average sound level, as defined in ISO 1996-2: 1987, determined over all of the night periods of a year (Directive 2002/49/EC). The small-scale topography of the area, dimensions of buildings, noise barriers, street axis, vehicle type-specific traffic density, speed limit, and type of street surface were included in the noise model (Directive 2002/49/EC). The calculation method VBUS/RLS-90 (VBUS 2006; RLS 90) and the software CadnaA (DataKustik GmbH 2014) were used for the noise modeling at façade points. The highest façade point noise level within a buffer of 10 m from the residence was used as the individual noise level. The reference level was set to 45 dB for L_{den} and to 40 dB for L_{night}. I additionally categorized continuous values of road traffic noise in 5-dB categories. Merging of the continuous noise variable into 5-dB categories was performed to correct for pseudo-precision – a very precise modeling of noise combined with no information on actual exposure (i.e., location of bedroom, ventilation patterns, etc.). This definition (continuous noise variable in 5-dB categories) was used when road traffic noise was entered as a covariate in the analysis with air pollution or traffic indicators as main exposure. According to a better model fit, L_{night} was used as a covariate in these analyses. Tram noise,

obtained from isophone maps, was included to the analyses as well. It was defined with 5-dB categories, ranging from ≤5 to >65 dB. In the analysis with noise as a main exposure, I additionally included noise as categorical variable to account for a possible threshold of a biological effect.

Neighborhood-level socio-economic status

Table 1. The neighborhood-level social characteristics obtained for the HNR study area and their definitions.

Variable	Definition
Population density	$N_{population}$[1]/$Area_{Neighb.}(km^2)$
Percentage of elderly residents	$(N_{aged \geq 65}/N_{population})\times 100$
Unemployment rate	$N_{unemployed}/(N_{Economicly\ active})\times 100$
Social welfare rate	$(N_{receiving\ welfare\ benefits}/N_{population})\times 100$
Residential turnover	$((N_{moved\ in} + N_{moved\ out})/N_{population})\times 100$

Source: Jöckel et al 2010.
[1]Number of residents in the neighborhood.

The study area was divided into 106 neighborhoods, each corresponding to a statistical unit with a median size of 11,263 inhabitants (interquartile range 7,875–16,022) (Dragano et al. 2009a). The socio-economic characteristics for each neighborhood in the study area were retrieved from the city departments in charge of statistics and monitoring in Essen, Bochum, and Mülheim an der Ruhr (Jöckel et al. 2010). The neighborhood-level data were assigned to the HNR participants by address linkage (Jöckel et al. 2010; Dragano et al. 2009a). The following data were retrieved: population density, age structure, unemployment rate, social welfare rate, and residential turnover (Table 1).

3.1.3. Assessment of study outcomes

Blood pressure measurement

BP measurements at baseline and follow-up were conducted according to the WHO monitoring trends and determinants in cardiovascular disease (MONICA) measurement protocol (Hense et al. 1995). BP was recorded using the AOD (Omron HEM-705CP; OMRON Corporation, Hoofddorp, the Netherlands), and the RZS (Mark II; Hawksley, Lancing, United King-

dom; Stang et al. 2006). The rationale of using two BP recording devices on one subject was to investigate differences between obtained values, to compare the values, and to develop a conversion algorithm that could be used in other studies in which comparisons between populations or population groups are limited because of the different devices used (Stang et al. 2006). AOD is currently used in the vast majority of epidemiologic studies with BP (Stang et al. 2006). In the current study, the values obtained with an AOD were used (unless the AOD values were missing; see details below). The AOD displayed BP values to the nearest 1 mmHg, and the RZS displayed the values to the nearest 2 mmHg (Stang et al. 2006). The measurement devices were regularly calibrated by the Bureau of Standards, the Board of Weights and Measures (Stang et al. 2006).

According to the standards of the WHO MONICA BP recording protocol, study personnel conducting BP measurements were certified and regularly trained in measuring BP (Hense et al. 1995; Stang et al. 2006). Arm circumference was measured before BP recording, and the appropriate cuff size was chosen according to the protocol (Stang et al. 2006). The HNR participants were asked not to drink coffee or any other beverages containing caffeine before the measurement. BP measurements were performed on the right arm, in a seated position with at least 5 minutes of rest before measurement and with a 3-minute interval between the BP readings. To ensure that these standards were followed, the BP measurement was conducted in the middle of the computer-assisted personal interview; the participants were asked at least 53 questions before their BP was measured (Stang et al. 2006).

At baseline, participants were randomly assigned to the order of measurement devices, depending on whether their personal identification number was odd or even (Stang et al. 2006). At baseline, BP was measured with both devices in all participants, whereas at follow-up, BP was measured with AOD in all participants and RZS in a subsample (n = 885, 21% of the follow-up sample). At follow-up, for those 885 participants whose BPs were measured with both devices, the order of devices was opposite to baseline. On average, the intervals between the two devices were 22 minutes at baseline (Stang et al. 2006) and 23.5 minutes at follow-up. BP was measured three times with both types of measurement device. Due to the time constraints and personnel shortage, a reduced program of BP recordings (two measurements with AOD

and one with RZS) was applied for approximately 13% of the study participants at baseline (Stang et al. 2006). At the follow-up measurement, the reduced program was not applied.

During the measurement, some information related to BP value and measurement conditions was recorded: room temperature, participant's intake of coffee and other beverages before the measurement, whether a participant was diagnosed with hypertension and whether he or she received antihypertensive treatment.

A standard quality assurance protocol was applied to check the BP values for plausibility at baseline (Author: A. Stang, personal communication). Briefly, the following parameters were checked:

- The availability of each measurement (1^{st}, 2^{nd}, 3^{rd}) of SBP and DBP with both recording devices;
- With RZS measurements only: the availability of the correction factor;
- Whether the correct sleeve size was used during measurement;
- Temperature in the measurement room (allowed range: 18–28 °C);
- Whether the order of devices was the same as assigned to the participant;
- Plausibility of the measured BP values and their variability within one participant.

The final SBP and DBP values at baseline and follow-up ("best-off" BP) were calculated as shown at Figure 4. BP was calculated as the mean of the 2^{nd} and 3^{rd} measurement values with AOD. The 1^{st} measurement was generally disregarded, as it was assumed to be higher than the consequent measurements (Stang et al. 2006; Pickering et al. 2005).

A strategy to diminish the number of missing values was used (Author: A. Stang, personal communication). If the readings 2 and 3 with AOD were missing, the first available of the following options was selected: (i) mean of the readings 1&3 or 1&2 with AOD; (ii) the single available BP with AOD; (iii) mean of readings 2&3 with RZS; (iv) mean of the readings 1&3 or 1&2 with RZS; (v) the single available BP with RZS; or (vi) the BP value from the pre-stress phase of the ergometric stress testing. PP was calculated as the difference (SBP minus DBP) of the final BP values.

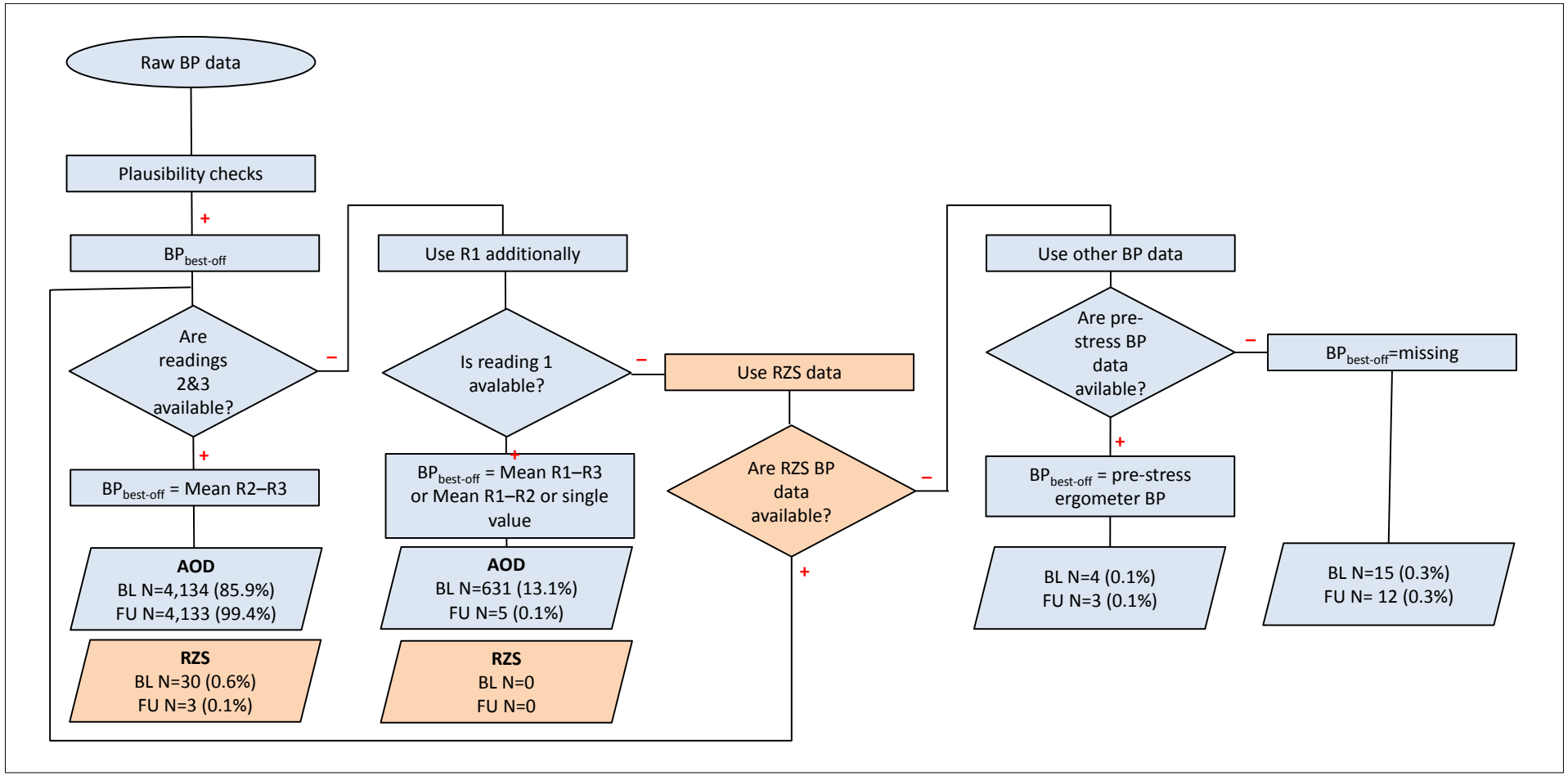


Figure 4. The "best off" BP value calculation strategy.

Legend: R = reading; BL = baseline measurement; FU = follow-up measurement.

Assessment of BPLM intake

Current BPLM intake was assessed at the baseline visit in a physician-conducted interview. Participants were additionally asked to bring to the examination all packages of medication, which they had been taking during the past 7 days. The list of drugs included as BPLM was defined by HNR study cardiologists using the WHO Anatomical Therapeutic Chemical Classification System; in 294 participants (6% of the total HNR sample) missing information on ATC codes was replaced with self-reported intake of BPLM, recorded during BP measurement or ergometric stress testing was used instead.

Table 2. Blood pressure-lowering medications in the HNR study.

Class	**ATC codes included**
Diuretics	Thiazides: C03EA14, C03BA04, C03BA03, C03AA03, C03BA11, C03BA08, C03BA02, C03BA10. Loop diuretics: C03CA02, C03CX01, C03CA01, C03CA03, C03CA04.
Beta-blocking agents	Selective: C07AB04, C07AB03, C07AB05, C07AB07, C07AB08, C07AB02, C07AB12, C07AB13. Non-selective: C07AA01, C07AA19, C07AA30, C07AA15, C07AA14, C07AA18, C07AA12, C07AA02, C07AA23, C07AA03, C07AA05. Alpha and beta blocking agents: C07AG02.
ACE inhibitors	C09AA07, C09AA01, C09AA08, C09AA02, C09AA09, C09AA03, C09AA13, C09AA04, C09AA06, C09AA05, C09AA11, C09AA10.
Angiotensin II antagonists	C09CA06, C09CA02, C09CA04, C09CA01, C09CA07, C09CA03.
Selective calcium channel blockers	Dihydropyridine derivatives: C08CA01, C08CA02, C08CA03, C08CA09, C08CA13, C08CA04, C08CA05, C08CA10, C08CA07, C08CA08. Benzothiazepine derivatives: C08DB01. Phenylalkylamine derivatives: C08DA02, C08DA01.
Alpha-adrenoreceptor antagonists	C02CA07, C02CA04, C02CA01, C02CA08.
Anti-sympathetic nervous system agents	C02AC01, C02CC07, C02AC02, C02AB01, C02AC05.
Arteriolar vasodilators	C04AX37, C02DB01, C02DC01.
Other BP-lowering agents	C03BX03.
Combination drugs	Diuretics and potassium-sparing agents: C03EA01, C03EA06, C03EA12, C03EA14, C03EA15, C03EA21, C03EA41, C03EB01, C03EB02, C03EB21. Beta blocking agents and diuretics: C07BA01,

Class	ATC codes included
	C07BA02, C07BA05, C07BA12, C07BA14, C07BA18, C07BB02, C07BB03, C07BB04, C07BB07, C07BG02, C07CA02, C07CA03, C07CA05, C07CA23, C07CB02, C07CB03, C07CB04, C07CB08, C07DA05, C07DB01. Loop diuretics and ACE inhibitors: C09BA01, C09BA02, C09BA03, C09BA04, C09BA05, C09BA06, C09BA07, C09BA08, C09BA09, C09BA13. Thiazides and angiotensin II antagonists: C09DA01, C09DA03, C09DA04, C09DA06, C09DA07. ACE inhibitors and calcium channel blockers: C09BB05, C09BB10. Calcium channel blockers and diuretics: C08GA01, C08GA02.

Definition of hypertension

Prevalent hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or as the current use of BPLM (Chobanian et al. 2003). In the analysis of incident hypertension, the prevalent cases (hypertensive at baseline) were excluded.

Self-reported hypertension and BPLM intake

At baseline and follow-up examinations (during a computer-assisted interview), the HNR participants were asked whether they had physician-diagnosed hypertension and, if yes, whether they were treated for high BP with medications. These variables were used as additional outcomes: self-reported physician diagnosed hypertension and self-reported BPLM intake.

3.1.4. Individual-level covariates

Age, sex, lifestyle, and co-morbidities

Age at the examination date was calculated as the difference in years with the date of birth. Lifestyle was represented with smoking, alcohol consumption, and physical activity. BMI was calculated as weight divided by squared height in meters. I assessed current and previous smoking, the lifetime cumulative exposure in pack-years, and any environmental tobacco smoke (ETS) exposure (at home, at work, at other places). Amount of alcohol intake was given as a number of drinks per week (one drink defined as 0.25 L beer, 0.1 L wine, or 0.02 L spirits) and was categorized as 0, 1–3, 4–6, and > 6 drinks per week. I categorized each participant's sport and physical activity as self-reported times of physical exercise per week: < 1, 1, 2–3, and > 3 times/week. CVD, representing chronic disease or markers of deprived health, was included as CHD (self-

reported history of myocardial infarction or coronary intervention) and type 2 diabetes mellitus (T2DM; prior physician diagnosis of T2DM or use of antidiabetic drugs or random blood glucose ≥ 11.1 mmol/L or fasting blood glucose ≥ 7 mmol/L).

Individual socio-economic status

Individual level SES was assessed as years of formal education (United Nations Educational, Scientific, and Cultural Organization 1997) and was categorized as low (≤ 10 years), medium (11–17 years), and high educational level (≥ 18 years). Economic activity was categorized as employed, retired, unemployed, or economically inactive.

Other covariates

As surrogates for the study surface area characteristics (influencing the distribution, accumulation and transport of the pollutants), I used indicator variables for city (Mülheim, Essen, Bochum) and geographic area (North, Center, South). To account for temporal variations in exposure and outcome, I calculated the time trend variable as the count of days starting from the first examination date at baseline or follow-up, correspondingly, to the examination date of each participant.

3.1.5. Statistical analysis

Causal graphs to define the adjustment sets

The adjustment sets were identified using causal graphs (Glymour and Greenland 2008). The most likely causal relations between variables were based on prior biological and epidemiological knowledge and derived adjustment sets (Figure 5). The minimal sufficient sets were obtained using DAGitty 2.0 (Textor et al. 2011). Two minimal sufficient adjustment sets were identified to estimate the direct (causal) effect of air pollution on BP. They included the following concepts: (i) short-term air pollution, age, sex, lifestyle, CVD, and road traffic noise; and (ii) short-term air pollution, age, sex, BPLM intake, lifestyle, and road traffic noise. The adjustment set (i) was selected as the main model because the relationship between air pollution BPLM intake and the resulting BP was investigated separately (see the subsection “Correction for BPLM intake”).

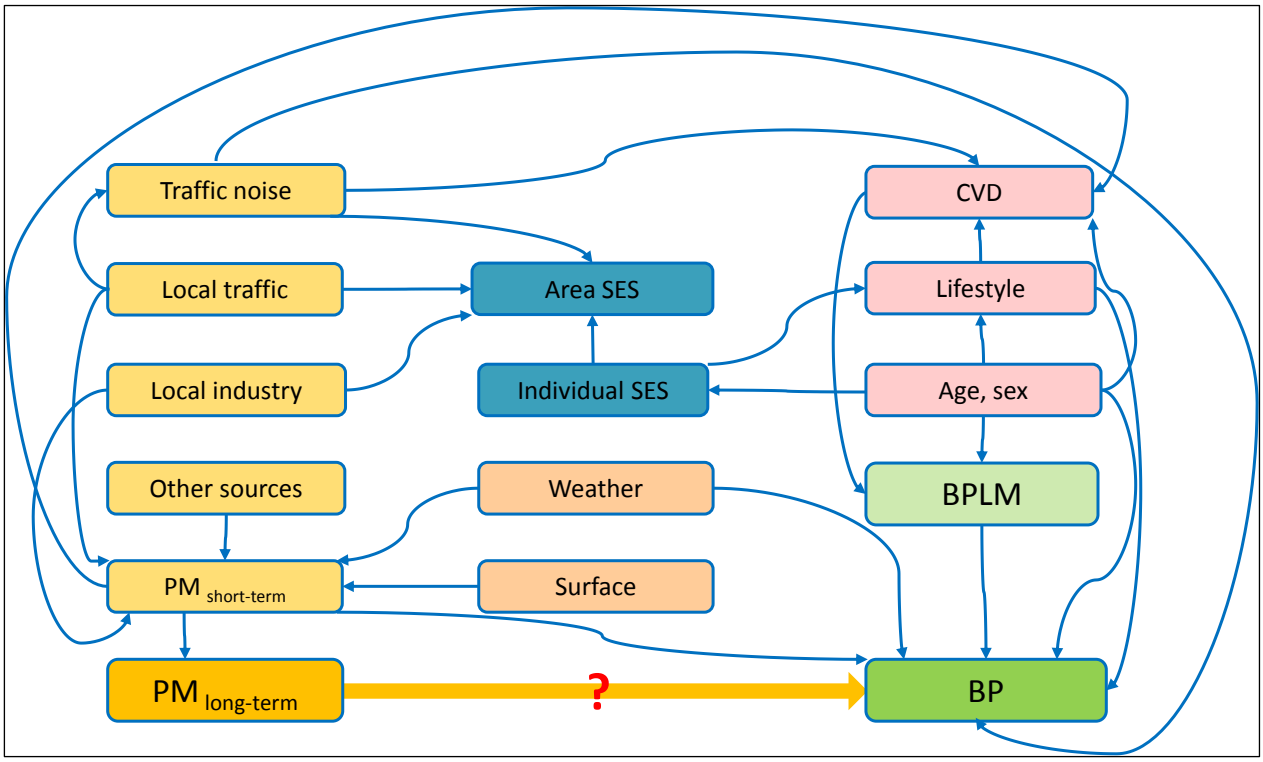


Figure 5. Hypothesized associations between air pollution, individual and neighborhood-level risk factors, and arterial BP.

Legend: question mark (?) = the study question; weather = short-term meteorology; surface = study area characteristics.

Selection of variables for the statistical model

I checked how many missing values each of the variables, suggested for the main or extended adjustment sets, had and selected the variables with fewer missing values. For example, intima-media thickness, which is a measure of the progression of atherosclerosis, had 1,000 missing values and was therefore omitted from the analysis. I checked all of the variables for the plausibility of values. Pack-years of smoking at baseline had one extremely high value (400 pack-years, which was twice as high as the second highest value). After consulting with data managers and the HNR study team, I assigned this participant the second highest value (204 pack-years), which resulted in the improved model fit. If more than one variable was available for controlling for possible confounding, for example, different blood lipids as a surrogate for the nutrition component of lifestyle, the decision on the variable was made in the model checks step.

Statistical analysis

Statistical analyses were performed using the following software: SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), STATA version 12.0 (StataCorp, College Station, TX, 12 USA; www.stata.com) and R version 2.13.1 (R Core Team 2013).

Univariate analyses of data, such as descriptive statistics, correlations, cross-tables and subgroup comparisons were performed prior to the regression analysis. The distribution of longitudinal variables was checked for normality. The parametric statistical procedures (e.g., Pearson's correlation coefficient calculation and the T-test in two groups) were employed regardless of the variables' distribution based on the central limit theorem, because the number of participants in the analysis was substantial.

Exposure was entered as a linear term in all analyses based on the reported linearity of the association with cardiovascular mortality (Brook et al. 2010). Regression estimates were presented per interquartile range (IQR) of respective pollutant concentration. In addition, the dose-response relationship of exposure with outcome was tested using categorical exposure (by quartiles) and penalized splines (Hastie and Tibishirani 2007).

Outcomes were not transformed for the analysis. BP was analyzed with a linear regression model, and BPLM and hypertension with a logistic regression. To correct for the BPLM intake in a sensitivity analysis, I also used a right-censored regression (see the section "Correction for BPLM intake"). In the analysis with the incidence of hypertension as an outcome, a Poisson regression with robust variance estimation was used.

I conducted cross-sectional and longitudinal analysis. For the cross-sectional analysis, exposure and outcome values from the same time period were used (baseline or follow-up, respectively). Longitudinal analysis was conducted in few different ways: (i) the association of baseline air pollution concentrations with BP, the incidence of hypertension, and BPLM intake at follow-up; (ii) the association of baseline air pollution concentration with change in BP from baseline to follow-up, the incidence of hypertension, and BPLM intake at follow-up; and (iii) the association of change in air pollution from baseline to follow-up with change in BP from baseline to follow-up, the incidence of hypertension, and BPLM intake at follow-up. The longitudinal analysis with change in BP as the outcome was conducted in a subsample of participants not taking BPLM.

The non-linearity of covariate-outcome relationships was investigated using squared terms cen-

tered on the mean. Non-linearity was tested for the following covariates: age, BMI, lipid ratio (LDL/HDL), HDL, triglycerides, and time trend. Of them, a significant non-linear relationship was observed for age, but only with DBP; this observation is consistent with the evidence that DBP increases only until a certain age, after which, with the progression of arterial stiffness, DBP starts to decrease (Chobanian et al. 2003; Whelton 1994). Non-linear terms were also significant for BMI, triglycerides (only with DBP), and time trend. The variables which showed a deviation from a linear relation with BP were included to the main adjustment model as polynomials and linear terms, centered on the mean.

Regression model checks

I checked the following assumptions of the linear regression model: collinearity of predictor variables, distribution of residuals, and presence of influential observations. These checks were performed with the main adjustment set, but without the exposure in the model (the "empty" model). The collinearity of predictor variables was checked using correlations. Variables with Pearson's correlation coefficients > 0.7 were not included in the model simultaneously. For example, of the two highly correlated meteorological variables humidity and temperature, only humidity was left in the model. In addition, I considered the variance inflation factor (VIF) as another measure of collinearity: a variable with VIF > 10 was excluded from the analysis. The distribution of residuals was close to normal, although the Kolmogorov-Smirnov test revealed a deviation from normality. I checked the leverage of individual observations plotting the leverage against the squared residual plot. One observation had a substantially high leverage. This participant was deleted from the analysis, which resulted in a better model fit. Using the main adjustment set with SBP, DBP and PP as outcomes, I computed the plot of residuals versus fitted values, and found no evidence that the model was making unusually large or small predictions.

The main adjustment model

The main adjustment set with SBP, PP, hypertension and BPLM as outcomes included time trend (linear and squared terms, centered on mean), short-term exposure (mean of lags 0–1 with NH_3, mean of lags 0–2 with O_3, and mean of lags 0–6 with all PM metrics, PN, NO_x, SO_2, and CO; for all short-term exposures, yearly mean was subtracted), L_{night} traffic (linear, in 5-dB categories), L_{den} tram (linear, in 5-dB categories), distance to major road (upper quartile of diesel vehicle density), age, sex, BMI (linear and squared terms, centered on the mean), waist-hip ratio (WHR), blood lipids (lipid ratio, HDL, triglycerides), smoking status, ETS, physical activity (in catego-

ries), education (in categories), and economic activity (in categories). The main adjustment set with DBP as the outcome additionally included linear and squared terms for age and the concentration of blood triglycerides.

The following covariates in the main model were significant predictors of SBP: sex, age, BMI, current smoking, alcohol consumption > 6 drinks/week, sport < 1 time/month, educational status, road traffic noise, and the city of residence (the model estimates with all outcomes are presented in Tables 38–42 in the Appendix).

Subjects excluded from the analysis at baseline

I preformed complete case analysis, excluding participants with missing values. At baseline, 230 subjects were excluded due to missing values (Appendix Table 43). There were slightly more men, smokers, subjects with CHD, participants with lower educational levels, those practicing no sport, and retired people; the distributions of exposure, outcome and the remaining personal characteristics were quite similar to the main analysis subset. At follow-up, 685 participants were not included because they changed their addresses during the follow-up period. This group was not compared to the main group. The examination of personal characteristics of the 232 subjects excluded from the follow-up sample (those who did not change their addresses) due to missing values did not reveal any substantial differences to the follow-up analysis subset with regard to exposure, though some characteristics differed slightly (Appendix Table 44); the excluded subjects had lower BP values, higher rates of medication use and hypertension, more women, more subjects with CHD, fewer smokers, more exposure to CHD, lower educational levels and no sport activity.

Sensitivity analyses

In addition to the analysis with the main outcomes (BP, hypertension based on BP and BPLM intake, and BPLM intake), I conducted a sensitivity analysis with self-reported hypertension and BPLM intake as outcomes.

In the extended adjustment sets, those variables that were not included in the main model but were nevertheless important predictors of BP were added, including (i) short-term meteorology (humidity and wind speed), (ii) alcohol and pack-years of smoking (available for a subset of 4,368 participants), (iii) co-morbidities (CHD, T2DM), (iv) city of residence and geographic area (North, Center, South), and (v) area-level SES (in the mixed-effects regression model). In the

additional analysis, I deleted 3 influential observations (with high residual/leverage).

I investigated the spatial component of exposure definition. The individual 365-day mean reflects both spatial and temporal differences in the study area during the baseline period: for example, two participants living on the same street, but coming for baseline measurements of BP on different dates would be assigned different 365-day mean concentrations of residential air pollution. Therefore, in a sensitivity analysis, the grid-specific mean of the baseline period (2000–2003) representing only spatial differences was used as a main exposure. I did not adjust for time trend in this model because the grid-specific mean had only spatial variation.

I calculated the two-exposure models combining particulate and gaseous components: in one model, both of the exposures were entered as independent predictors, in the other interactions with high levels of one of the pollutants were tested. For the interaction analysis, the pollutant was dichotomized at the 75th percentile, and analyses with product terms $Exposure_1 \times Exposure_2$ (dichotomous) were conducted.

Analysis of effect modification.

I tested for effect modification of results with BP as the outcome using the product term exposure×effect modifier. The Z-test was used to assess the significance of effect modification. The following factors were tested: sex, age ≥ 60 years, BMI ≥ 30 kg/m², CHD, T2DM, current smoking, passive smoking, acute inflammation (CRP > 3 μg/L), high alcohol consumption, no sport activity, low education level, T2DM, CHD, city of residence, meteorological season (winter vs. other), road traffic noise ≥ 65 dB vs. lower, and living < 100 m to the major road (upper quartile of the diesel vehicles density) vs. living farther away.

Correction for BPLM intake

As a first attempt to correct for medication effect, I included medication as a covariate. An air pollution-related increase in BP may lead to BPLM intake in the most susceptible individuals. Therefore, BPLM intake may lay in the causal pathway from air pollution to BP. Adjustment for the variable that mediates the effect of exposure on the outcome may lead to a biased estimate of the main association, which is why other methods of correction were applied as well.

My second approach was to use binary outcomes: BPLM intake and hypertension. The latter outcome combines BPLM and high BP values. Using these outcomes would help to diminish the bias in the analysis. However, dichotomizing the variable implies power loss. Additionally, it is

possible that in the less susceptible participants, a change in BP related to air pollution would likely not result in hypertension. Thus, it is possible that a null association of air pollution with BPLM intake and hypertension will be observed.

The third approach was to implement some of the correction techniques recommended in the literature (Tobin et al. 2005). I chose to use the fixed addition method. With this method, a fixed increment (e.g., 5 or 10 mmHg) is added to BP values of medicated participants. The advantages of this method are that it is simple and straightforward. The limitation of this method is that an assumption regarding treatment effect is made. In a population-based cohort, the participants will vary greatly by the treatment scheme, achieved control of hypertension, and other related factors.

Along with the fixed addition method, I implemented another recommended strategy: the normal censored regression. With this method, BP values in the medicated participants are right-censored: it is assumed that the "real", underlying BP (without the treatment effect) in subjects taking BPLM is at least as high as the measured value (Tobin et al. 2005). The model is fit into a linear regression equation as follows:

$$BP^* = \beta_0 + \beta_1 * PM + \beta_2 * Covariate_2 + \cdots + \beta_x * Covariate_x + \varepsilon_i \quad [13],$$

where BP^* = censored BP (BP^* = BP in participants not taking BPLM and $BP^* \geq$ BP in participants taking BPLM); β_0 = intercept; $\beta_1 \ldots \beta_x$ regression coefficients; and ε_i = error term (Tobin et al. 2005). Model parameters were estimated by maximum likelihood using a Newton-Raphson algorithm (Jennrich and Robinson 1969). Standard errors were estimated from the inverse of the observed information matrix.

In addition, I estimated the association of air pollution with blood pressure in participants taking BPLM ("medicated") and not taking BPLM ("non-medicated") separately. The interaction term exposure×BPLM intake was used to increase power. This method avoids making assumptions about treatment effect and BP distribution. BPLM intake was entered as a separate outcome in the study.

3.2. Experimental part

3.2.1. Experimental setting

Study animals

Eight-week-old C57BL/6J female mice obtained from the Charles River Company (Sulzfeld,

Germany) were used in the experiment. The Animal Ethics Committee of the Dutch National Vaccine Institute approved the experiment. Animals were housed in inhalation chambers during the entire experiment. The temperature was maintained at 22 ± 2 ^{0}C and the relative humidity at 30–70% with a 12-hour light/dark cycle. The animals were allowed ad libitum access to a commercially available rodent diet (CRM) and tap water via an automatic drinking water system (Gerlofs-Nijland 2012).

Exposure

Diesel engine exhaust (DEE) was generated using a 100 KVA common-rail diesel generator (Stammis, Heerhugowaard, Netherlands) under load (38 KW) and idling conditions fueled with EN590 diesel. Exposure level was controlled with a proportional-integral-derivative controller. NO_2 was generated by heating liquid nitrogen and mixing it with diluted diesel in two different concentrations to obtain low and high NO_2 conditions. To remove the particulate fraction (filtered DEE), the flow was split after the addition of NO_2 and partially passed through a HEPA filter (Gerlofs-Nijland 2012).

Table 3. Group codes, exposure test atmospheres, and concentrations of NO_2.

Group	Abbreviation	Exposure	NO_2 concentration
1	Control	Clean air	–
2	DEE	DEE (~ 1 mg/m³)	2 ppm[1]
3	DEE + NO_2 (low)	DEE + low NO_2	4 ppm
4	DEE + NO_2 (high)	DEE + high NO_2	15 ppm
5	DEE (filtered) + NO_2 (low)	Filtered DEE + low NO_2	4 ppm
6	DEE (filtered) + NO_2 (high)	Filtered DEE + high NO_2	15 ppm
7	NO_2 (high)	High NO_2	15 ppm

[1]ppm = parts per million

The following test atmospheres were generated: (i) control with clean air (HEPA filtered, chemically purified with activated charcoal and purafill and conditioned to a temperature of 21 ^{0}C and a relative humidity of 55%); (ii) DEE diluted to 1 mg/m^3 with clean and conditioned air; (iii) DEE combined with low-concentration NO_2 (volume concentration NO_2/NO_x approximately 20%); (iv) DEE combined with high-concentration NO_2 (NO_2/NO_x ~ 50% v/v of NOx); (v) filtered DEE (only the gaseous fraction) combined with low-concentration NO_2; (vi) filtered DEE

combined with high-concentration NO_2; and (vii) high-concentration NO_2 alone (Table 3). The animals (10 per exposure group) were exposed over 13 weeks (5 days per week, 6 h per day) to different test atmospheres in whole-body exposure units. On the day after the last exposure day, the animals were anesthetized with a mixture of Ketamine (100 mg/ml) and Xylazine (20 mg/ml) in a 10:8 ratio and sacrificed via exsanguination. Necropsy was performed on the day after the last exposure day. Lung tissue samples were snap-frozen in liquid nitrogen and stored at -80 °C (Gerlofs-Nijland 2012).

3.2.2. Measurement of gene expression in the lung tissue

Lung tissues were ground and homogenized in Trizol®. To extract ribonucleic acid (RNA), the RNeasy® mini kit was used in combination and the DNAse treatment with the QIAGEN ® RNAse-free DNAse set. The extraction was performed according to the manufacturer's instructions (Qiagen 2012; 5 PRIME 2007). Next, coding DNA was synthesized from 0.5 µg of RNA with the iScript Advanced coding DNA Synthesis Kit for real time quantitative polymerase chain reaction (RT-qPCR) (BioRad, CA, USA). Coding DNA was consequently diluted 1:15 in DNA-free water (Van Berlo et al. 2010).

Five genes involved in oxidative, inflammatory, or vascular responses, were investigated: CYP1A1, iNOS, TNFα, NAD(P)H dehydrogenase quinone 1 (NQO1), and ICAM-1. Three housekeeping genes were also selected for this study: glyceraldehyde 3-phosphate dehydrogenase (GAPDH), hypoxanthine-guanine phosphoribosyltransferase (HPRT), and β-actin. The sequences of the RT-qPCR primers are presented in Table 4. The primers were designed using Primer Express software Version 3.0 (Applied Biosystems). The PCR efficiencies of the primers were 90% or higher (data not shown). I used the SYBR© Green Supermix (BioRad), diluted coding DNA, and 0.3 µM primers in a total volume of 25 µL. The RT-qPCR reactions were run with the iQ5™ real-time PCR detection system (BioRad, CA, USA). Cycling conditions were as follows: 3 min denaturation at 95 °C, and then 40 cycles of 15 s at 95 °C and 45 s 60 °C (Van Berlo et al. 2010). Melt curves (60–95°C) were produced for product identification and purity. The threshold cycle (C_t), the PCR cycle at which fluorescence rises above threshold background fluorescence, indicating that the amount of amplified material has reached the threshold (Livak and Schmittgen 2001), was calculated using iQ5™ Optical System Software (BioRad, CA, USA).

Table 4. Primer sequences used in the current study

Gene	Sense (forward) primer	Antisense (reverse) primer
CYP1A1	5′-CCTCATGTACCTGGTAACCA-3	5′-AAGGATGAATGCCGGAAGGT-3
iNOS	5′-AACATCAGGTCGGCCATCA-3	5′-CGTACCGGATGAGCTGTGAA-3
TNFα	5′-AGGCTGCCCCGACTACGT-3	5′-ACTTTCTCCTGGTATGAGATAGCAAAT-3
NQO1	5′-CCATGGCGGCGAGAAG-3	5′-CATGGCGTAGTTGAATGATGTCTT-3
ICAM-1	5′-GTCCGCTGTGCTTTGAGAACT-3	5′-CGGAAACGAATACACGGTGAT-3
GAPDH	5′-AACCTGCCAAGTATGATGACATCA-3	5′-GGTCCTCAGTGTTAGCCCAAGAT-3
HPRT	5′-AAGACTTGCTCGAGATGTCATGAA-3	5′-AAAGAACTTATAGCCCCCCTTGA-3
β-actin	5′-CGTGAAAAGATGACCCAGATCA-3	5′-CACAGCCTGGATGGCTACGT-3

3.2.3. Relative gene expression calculations ($2^{-\Delta\Delta Ct}$ method)

I calculated difference in gene expression in each exposed group compared to the control group using a housekeeping gene as an endogenous reference. For that, I used the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). I ran a linear regression model as follows:

$$\Delta C_t = \beta_0 + \beta_1 \times Group2 + \beta_3 \times Group3 + \beta_4 \times Group4 + \beta_5 \times Group5 + \beta_6 \times Group6 + \beta_7 \times Group7 + \varepsilon \quad [14]$$

The dependent variable ΔC_t was calculated as:

$$\Delta C_t = C_t(Gene\ of\ interest) - C_t(Housekeeping\ gene) \quad [15]$$

In the formula [14], $ß_0$ is the intercept; $ß_1$ to $ß_7$ are linear regression coefficients for each of the exposure groups (independent predictors in the linear regression model); and ε is residual error. The fold change of gene expression for the exposed group X compared to the control group was calculated as $2^{-\beta_X}$. The 95% confidence interval was calculated as $2^{-(\beta_X \pm 1.96 \times Standard\ Error_{\beta_X})}$. As a sensitivity analysis, I calculated the fold change in expression without normalizing for the housekeeping gene. In addition, I tested for an exposure-response relationship with NO_2 concentration, which was entered as a continuous predictor in the linear regression model. The data were prepared using Microsoft Office Excel 2010. Analyses were performed using SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). Graphs were produced with R 2.13.1 (R Core Team 2013).

4. OBSERVATIONAL STUDY RESULTS

4.1. Cross-sectional analysis with BP and hypertension at baseline

4.1.1. Description of study population

At baseline, 4,584 participants with no missing values for exposure, outcome, and covariates were included. The distribution of outcomes in the study population at baseline is summarized in Table 5. On average, the study participants at baseline had slightly elevated SBP: 133.2 mmHg (standard deviation (SD) = 20.8 mmHg), which, according to the JNC7 classification, is in the pre-hypertensive range (Chobanian et al. 2003). DBP was very close to normal range, with a mean value of 81.4 mmHg (standard deviation, SD, 10.9 mmHg). The average PP was 51.7 mmHg (SD 14.7 mmHg). At the time of the baseline measurement, 1,628 (35.5%) participants took BPLM, and 2,611 (57.0%) had prevalent hypertension according to the JNC7 definition (Chobanian et al. 2003). During baseline examination, participants also indicated whether they had hypertension diagnosed by a physician (42.7%) and whether it was treated with medications (31.2%).

Table 5. Average BP values and rates of hypertension and BPLM intake in the analysis sample.

Variable (unit)	Statistics	Description	N missing
SBP (mmHg)	Mean ± SD	133.2 ± 20.8	–
DBP (mmHg)	Mean ± SD	81.4 ± 10.9	–
PP (mmHg)	Mean ± SD	51.7 ± 14.7	–
Hypertension	n (%)	2,611 (57.0%)	–
BPLM intake	n (%)	1,628 (35.5%)	–
Self-reported hypertension[1]	n (%)	1,955 (42.7%)	10
Self-reported BPLM intake[1]	n (%)	1,432 (31.2%)	1

N = 4,584.
[1]*For the complete case analysis, participants with missing values of BP or BPLM intake were excluded; the outcomes in the sensitivity analyses contained few additional missing values.*

The distribution of BP was quite symmetric but was slightly skewed either to the left (PP, SBP) or to the right (DBP) of the mean value (Figure 6). The distribution deviated from normal ($p < 0.01$ with Shapiro-Wilk test of normality for SBP, DBP and PP).

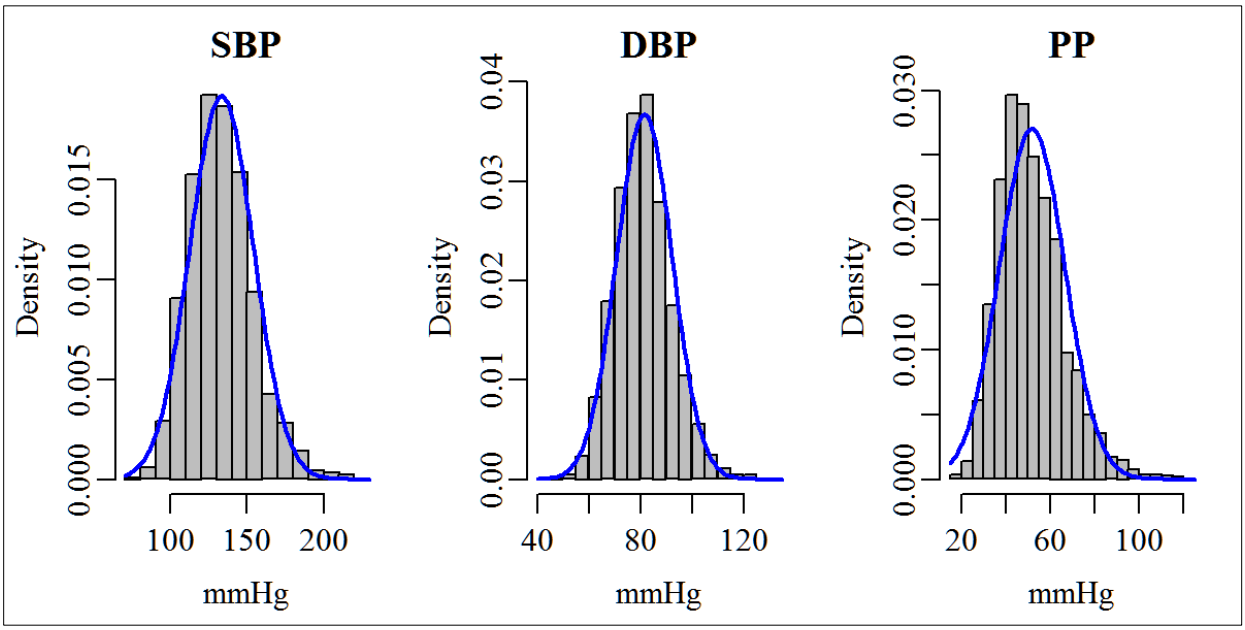


Figure 6. Distribution of BP in the study population.

Legend: blue line indicates normal distribution.

I compared the distribution of hypertension and BPLM intake according to the definition used in this study: the JNC7 definition for hypertension (Chobanian et al. 2003) and BPLM intake according to the ATC codes (see Methods, "Assessment of BPLM intake") in Table 6.

Table 6. Difference between hypertension and BPLM intake, included as main outcomes according to the study definition, and self-reported hypertension and BPLM intake.

Hypertension (JNC7 definition)	**Self-reported hypertension = No**	**Self-reported hypertension = Yes**	**Self-reported hypertension = Missing**
No	1,747 (88.5%)	220 (11.2%)	6 (0.3%)
Yes	872 (33.4%)	1,735 (66.4%)	4 (0.2%)
BPLM intake (ATC codes)	**Self-reported BPLM intake = No**	**Self-reported BPLM intake = Yes**	**Self-reported BPLM intake = Missing**
No	2,870 (97.1%)	85 (2.9%)	1 (0.0%)
Yes	281 (17.3%)	1,347 (82.7%)	0 (0.0%)
N = 4,584.			

Of 2,611 participants with hypertension according to the study definition, 66.4% reported physician-diagnosed hypertension. Of 1,973 non-hypertensive (study definition), 11.2% reported having physician-diagnosed hypertension. The overall agreement for main definition of hypertension and for self-reported hypertension was 76.1%, Cohen's kappa was 0.53, which corresponds to

fair to good agreement. Among 1,628 participants taking BPLM according to the study definition, 82.7% also reported medication use. The overall agreement for the study definition of BPLM and self-reported BPLM intake was 92.0%. Cohen's kappa was 0.82, which corresponds to excellent agreement.

Table 7. Distribution of personal characteristics in the baseline analysis sample.

Variable (unit), statistics	**Description**	**N missing**
Age (years), Mean ± SD	59.6 ± 7.8	–
Sex (male), n (%)	2,274 (49.6%)	–
CHD, n (%)	296 (6.5%)	–
T2DM, n (%)	623 (13.6%)	–
BMI (kg/m²), Mean ± SD	27.9 ± 4.6	–
LDL:HDL ratio, Mean ± SD	2.7 ± 1.1	–
Smoking, n (%)		–
Current	1,064 (23.2%)	
Former	1,585 (34.6%)	
Never	1,935 (42.2%)	
Smoking pack-years[1], Mean ± SD	16.1 ± 24.6	112
ETS exposure, n (%)	1,658 (36.2%)	–
Alcohol (drinks/week)[1], Mean ± SD	5.3 ± 10.2	107
No sport, n (%)	2,100 (45.8%)	–
Education, n (%)		–
< 10 years (low)	517 (11.3%)	
11–17 years (middle)	2,558 (55.8%)	
≥ 18 years (high)	1,509 (32.9%)	
Economic activity		–
Employed	1,847 (40.3%)	
Unemployed	290 (6.3%)	
Housewife/homemaker	642 (14.0%)	
Retired	1,805 (39.4%)	

N = 4,584.

[1]*For the complete case analysis, missing values in all variables in the main adjustment model were excluded; some variables, used only in the sensitivity analyses, may contain missing values: pack-years of smoking, alcohol consumption.*

Other characteristics of the study population are presented in Table 7. On average, the study participants were 59.6 (SD 7.8) years old. Gender distribution was equal (49.6% men). CHD was reported by 6.5%. T2DM was found in 13.6%. The average BMI of the participants was 27.9 kg/m² (SD 4.6 kg/m²), which corresponds to the WHO definition of overweight, indicating the presence of overweight subjects in the population. Twenty-three percent (N = 1,064) were smokers, and thirty-five percent (n = 1,585) were former smokers, leaving forty-two percent of the analysis sample (n = 1,935) with no history of smoking. This percentage is lower than the one estimated by the World Health Organization for the year 2000 for the European region (29.9%) and for Germany (34.8%; European health for all database 2014). On average, participants reported 16.1 pack-years of smoking (SD 24.6 pack-years). Approximately one-third of the study population (36.2%) reported exposure to ETS. Forty-five percent of study participants did not practice sport regularly. Approximately half of all of the study population at baseline had secondary education (11-17 years; n = 2,558, 55.8%), thirty-three percent reported having tertiary education (≥ 18 years; n = 1,509), and the remaining 517 (11.3%) had primary education only (≤ 10 years). Equal proportions of the population (approximately 40%) reported being employed or retired at the time of the baseline measurement.

4.1.2. Description of exposure

Particulate matter

The distributions of modeled long-term concentrations of PM in the analysis sample at baseline are given in Table 8. The mean concentration of background $PM_{2.5}$ 365 days prior to BP measurement at baseline was 16.7 µg/m³ $PM_{2.5}$ (SD 1.6 µg/m³). The grid-specific mean of the baseline period (2000–2003) was slightly higher than the individual 365-day mean: 17.1 µg/m³ (SD 1.4 µg/m³). The average concentration of the individual 365-day mean PM_{10} was 20.7 µg/m³ PM_{10} (SD 2.6 µg/m³). Equivalently to $PM_{2.5}$, the grid-specific mean value of PM_{10} was slightly higher than the individual 365-day mean: 21.1 µg/m³, SD 2.6 µg/m³. For comparison, the yearly mean concentration of PM_{10} for the Rhein–Ruhr area in 2000 was 24 µg/m³, as reported by the North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection.

On average, 365-day mean PM_{coarse} reached 4.0 µg/m³ (SD 1.5 µg/m³). The mean concentration of PM_1 was 11.6 µg/m³ (SD 1.3 µg/m³) one year before the baseline measurement. The mean 365-day concentration of particle number, also representing the ultrafine fraction of particles, was 8.8×10^4/L (SD 1.9×10^4/L). The grid-specific mean values for the entire baseline period (2000–

2003) for PM_{coarse}, PM_1, and PN did not differ from the individual 365-day mean values.

Table 8. Concentrations of particulate matter in the study population at baseline.

Exposure (unit)		Individual 365-day mean			Mean of 2000–2003			Correlation
		Mean ± SD	***Min –Max***	***IQR***	***Mean ± SD***	***Min –Max***	***IQR***	***Pearson's ρ***
$PM_{2.5}$	(µg/m³)	16.7 ± 1.6	13.3 – 22.4	2.4	17.1 ± 1.4	14.5 – 21.6	2.3	0.875*[1]
PM_{10}	(µg/m³)	20.7 ± 2.6	15.8 – 29.3	4.0	21.1 ± 2.6	17.2 – 29.1	4.1	0.933*
PM_{coarse}	(µg/m³)	4.0 ± 1.6	2.1 – 11.8	1.7	4.0 ± 1.5	2.2 – 9.2	1.9	0.895*
PM_1	(µg/m³)	11.6 ± 1.3	8.7 – 16.1	1.9	12.0 ± 1.1	9.9 – 15.7	1.7	0.863*
PN	($\times 10^4$/L)	8.8 ± 1.9	4.9 – 18.4	2.7	8.8 ± 1.9	5.0 – 18.1	2.7	0.987*

N = 4,584.
[1] *Significance: * = p < 0.05.*

I observed a strong to very strong correlation of the individual 365-day mean concentration with the grid cell-specific mean concentration (Table 8): Pearson's ρ was > 0.85 for all pollutants and was higher than 0.9 for PM_{10} and PN.

Gaseous pollutants

Table 9. Concentrations of gaseous pollutants in the study population at baseline.

Exposure (unit)		Individual 365-day mean			Mean of 2000–2003			Correlation
		Mean ± SD	***Min –Max***	***IQR***	***Mean ± SD***	***Min –Max***	***IQR***	***Pearson's ρ***
O_3	(µg/m³)	35.4 ± 1.6	29.9 – 39.6	2.2	34.7 ± 1.4	30.1 – 38.1	1.9	0.874*[1]
NO_2	(µg/m³)	40.1 ± 4.2	27.6 – 53.3	5.7	41.3 ± 4.0	28.4 – 53.1	5.1	0.935*
NO	(µg/m³)	12.7 ± 4.5	4.8 – 35.7	5.5	13.2 ± 4.4	5.9 – 30.7	5.8	0.964*
SO_2	(µg/m³)	8.7 ± 1.1	5.9 – 16.3	1.5	9.1 ± 1.0	6.7 – 17.1	1.5	0.874*
CO	(µg/m³)	0.3 ± 0.1	0.2 – 0.6	0.1	0.3 ± 0.1	0.3 – 0.6	0.1	0.967*
NH_3	(µg/m³)	2.6 ± 0.4	1.8 – 3.7	0.5	2.7 ± 0.3	2.2 – 3.6	0.4	0.724*

N = 4,584.
[1] *Significance: * = p < 0.05.*

The average modeled long-term concentrations of gaseous pollutants in the analysis sample at baseline are presented in Table 9. The average concentrations of gaseous pollutants 365 days before BP measurement were 35.4 µg/m³ O_3 (SD 1.6 µg/m³), 40.1 µg/m³ NO_2 (SD 4.2 µg/m³), 8.7 µg/m³ SO_2 (SD 1.1 µg/m³), 0.3 µg/m³ CO (SD 0.1 µg/m³), and 2.6 µg/m³ NH_3 (SD 0.4 µg/m³). These values were close to the yearly mean values for 2000 in the Rhein-Ruhr area, as reported by the North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection

(LANUV 2000b): 33 µg/m³ for O_3, 30 µg/m³ for NO_2, 8 µg/m³ for SO_2, and 0.4 µg/m³ for CO. The grid-specific mean concentrations for the 2000–2003 period were quite similar to the individual 365-day mean, though the standard deviation was smaller due to the longer averaging period. The individual 365-day mean was very collinear with the grid cell mean for 2000–2003: Pearson's correlation coefficient varied from 0.724 (observed for NH_3) and 0.967 (observed for CO; Table 9).

Ambient noise, distance to major road, and neighborhood SES indicators (Table 10)

The mean level of 24-hour weighted mean road traffic noise at the residence, defined as the maximum façade value of the 10-m buffer around the geocoded address, was 53.9 dB. Mean level of night-time noise was 45.1 dB. Average tram noise was 12.2 dB.

Table 10. The average levels of ambient noise, distance to major road and neighborhood-level unemployment rate in the baseline analysis sample.

Variable	**(unit)**	**Mean ± SD**
Ambient noise		
L_{den} (traffic)	(dB)[1]	53.9 ± 9.6
L_{night} (traffic)	(dB)[1]	45.1 ± 9.3
L_{den} (tram)	(dB)	12.2 ± 13.6
Distance to major road by vehicle type		
Diesel vehicles	(km)	0.9 ± 0.8
Area socio-economic characteristics		
Population density	(×10³ residents/km²)	3.6 ± 2.1
Proportion of residents ≥ 65 years	(%)	28.3 ± 3.3
Unemployment rate	(%)	12.5 ± 3.4
Social welfare rate	(%)	4.6 ± 2.7

N = 4,584.
[1]Traffic L_{den} and L_{night} variable contain 46 missing values. In the analyses, L_{den} was entered in 5-dB categories, and the missing values were replaced with available noise isophones data.

On average, study participants resided far away from highly trafficked roads (Table 10). The mean distance from participants' residences to major roads with high densities of all-type, diesel, and heavy-duty vehicles was 0.9 km (shown for diesel vehicles only). The mean population density in the 106 neighborhoods in the study area was 36,000 residents per 1 km². Approximately

one-third of each neighborhood residents were older than 65 years of age, 12.5% were unemployed, and 4.6% received social welfare support (Table 10).

Short-term air pollution and meteorology (Table 11)

Short-term concentrations of air pollutants were quite similar to yearly mean concentrations for all pollutants except NH_3, for which the short-term value was substantially smaller than the long-term one. The short-term mean all-source $PM_{2.5}$ concentration was 16.8 μg/m³. The mean temperature 2 days before the BP measurement was 10.3° C; the mean relative humidity was 6.5 g/kg.

Table 11. Description of short-term air pollution and meteorology in the study population.

Variable	Time lag (days)	Unit	Mean ± SD
$PM_{2.5}$	lag 0–6	μg/m³	16.8 ± 6.1
PM_{10}	lag 0–6	μg/m³	20.9 ± 7.3
PN	lag 0–6	$\times 10^4$/L	8.7 ± 2.8
O_3	lag 0–2	μg/m³	36.4 ± 21.1
NO_2	lag 0–6	μg/m³	40.7 ± 15.9
SO_2	lag 0–6	μg/m³	9.1 ± 4.4
CO	lag 0–6	μg/m³	0.3 ± 0.1
NH_3	lag 0–1	μg/m³	0.2 ± 2.6
Humidity	lag 0–1	g/kg	6.5 ± 2.5
Temperature	lag 0–1	°C	10.3 ± 7.6
North-South wind	lag 0–1	m/s	1.3 ± 1.9
East-West wind	lag 0–1	m/s	0.8 ± 2.2
N = 4,584.			

Correlation of exposures at baseline (Table 11)

The different metrics of PM ($PM_{2.5}$, PM_{10}, PM_1, and PN) showed moderate to high correlations (Pearson's ρ 0.625 to 0.990). PM_{coarse} was highly correlated with PM_{10} (ρ = 0.811) and weakly to moderately correlated with smaller particles (ρ 0.328 to 0.519). I observed mostly high correlations between particulate and gaseous pollutants. O_3 was inversely correlated with all PM metrics (ρ -0.752 to -0.434). NO_2, SO_2 and NH_3 were highly positively correlated with PM_{10}, $PM_{2.5}$, PM_1 and PN. NO_2, SO_2 and NH_3 were not or only weakly correlated with coarse particles. NO and CO

were weakly to moderately correlated with $PM_{2.5}$ and PM_1 and were highly correlated with PM_{coarse} and PN. Gaseous compounds correlated moderately to highly with each other. O_3 showed an inverse moderate correlation with all other gaseous compounds (ρ -0.628 to -0.374).

NO_2 was highly positively correlated with SO_2 (0.740) and moderately correlated with NH_3 (0.623), NO (0.443), and CO (0.405). NO, in turn, correlated highly with CO (0.901) and moderately with other gases. A high correlation was also observed between NH_3 and SO_2 (0.734). Short-term concentrations of $PM_{2.5}$ did not correlate with any of the exposures but weakly inversely correlated with short-term humidity. The 24-hour mean traffic noise was weakly correlated with PN, NO, and CO (ρ 0.234, 0.266, and 0.231). A moderate correlation of tram noise was observed with NO (0.451) and CO (0.582). Tram noise was also weakly correlated with PM_{10}, PM_{coarse}, PN, and SO_2 (ρ 0.235, 0.277, and 0.216). Road traffic noise and tram noise were only weakly collinear (ρ 0.249). Distance to major road correlated weakly and inversely with most of the air pollutants. Moderate correlations of distance to major road with traffic noise L_{den} and L_{night} were observed (ρ = -0.382 and -0.415). The area-level unemployment rate correlated moderately with CO (ρ 0.421) and weakly with PM_{coarse}, PN, NO, SO_2, and road traffic noise.

The Shapiro-Wilk test for normality of distribution revealed deviations from normality for all environmental variables at baseline ($p < 0.01$). The parametric statistics were employed for the description of these variables based on the central limit theorem.

Table 12. Correlation matrix of exposures and environmental variables in the study population at baseline.

Variable	**$PM_{2.5}$**	**PM_{10}**	**PM_{coarse}**	**PM_1**	**PN**	**O_3**	**NO_2**	**NO**	**SO_2**	**CO**	**NH_3**	**$PM_{2.5\ (short)}$**	**Humidity**	**$L_{den\ (traffic)}$**	**$L_{night\ (traffic)}$**	**$L_{den\ (tram)}$**	**Road**
PM_{10}[1]	0.819*[5]	1															
PM_{coarse}[1]	0.328*	0.811*	1														
PM_1[1]	0.990*	0.818*	0.337*	1													
PN[1]	0.625*	0.703*	0.519*	0.645*	1												
O_3[1]	-0.572*	-0.618*	-0.434*	-0.567*	-0.752*	1											
NO_2[1]	0.670*	0.506*	0.149*	0.685*	0.732*	-0.628*	1										
NO[1]	0.415*	0.752*	0.814*	0.428*	0.696*	-0.511*	0.443*	1									
SO_2[1]	0.761*	0.588*	0.192*	0.799*	0.684*	-0.558*	0.740*	0.403*	1								
CO[1]	0.262*	0.596*	0.714*	0.298*	0.599*	-0.374*	0.405*	0.901*	0.431*	1							
NH_3[1]	0.840*	0.728*	0.341*	0.824*	0.611*	-0.529*	0.623*	0.502*	0.734*	0.377*	1						
$PM_{2.5\ (short)}$[2]	-0.027	-0.026	-0.016	-0.046*	-0.015	-0.060*	-0.000	0.001	-0.026	-0.010	-0.054*	1					
Humidity	0.002	-0.018	-0.031*	0.026	-0.012	0.051*	0.003	-0.035*	0.004	-0.042*	0.017	-0.316*	1				
$L_{den\ (traf.)}$	0.085*	0.185*	0.218*	0.093*	0.234*	-0.136*	0.140*	0.266*	0.078*	0.231*	0.094*	0.020	-0.012	1			
$L_{night\ (traf.)}$	0.120*	0.212*	0.226*	0.125*	0.255*	-0.163*	0.167*	0.275*	0.087*	0.218*	0.117*	0.021	-0.009	0.994*	1		
$L_{den\ (tram)}$	0.021	0.235*	0.366*	0.050*	0.277*	-0.085*	0.074*	0.451*	0.216*	0.582*	0.123*	-0.019	-0.039*	0.249*	0.208*	1	
Road[3]	-0.162*	-0.298*	-0.325*	-0.170*	-0.330*	0.228*	-0.277*	-0.400*	-0.104*	-0.315*	-0.144*	-0.003	0.006	-0.382*	-0.415*	-0.077*	1
nSES[4]	-0.054*	0.089*	0.201*	0.000	0.340*	-0.115*	0.166*	0.327*	0.224*	0.421*	0.113*	-0.014	-0.006	0.175*	0.155*	0.329*	-0.165*

N = 4,584. Pearson's rho is presented.
[1]Long-term air pollution = individual 365-day mean. [2]Lag of 0–6 days, basic effect (365-day mean) subtracted. [3]Distance to highly trafficked road (upper quartile of diesel vehicle density). [4]Unemployment rate in the neighborhood. [5] = $p < 0.05$.*

4.1.3. Stratified description of participants

Stratification by hypertension status

Table 13. Description of participants by hypertension status.

Variable (unit)	Statistics	Prevalent hypertension		$p_{difference}$ [2]
		No (n = 1,973)	*Yes (n = 2,611)*	
$PM_{2.5}$ (µg/m³)	Mean ± SD	16.7 ± 1.6	16.7 ± 1.6	
PM_{10} (µg/m³)	Mean ± SD	20.7 ± 2.6	20.7 ± 2.6	
PM_{coarse} (µg/m³)	Mean ± SD	4.0 ± 1.5	4.1 ± 1.6	
PM_1 (µg/m³)	Mean ± SD	11.6 ± 1.3	11.6 ± 1.3	
PN ($\times 10^4$/L)	Mean ± SD	8.8 ± 1.9	8.9 ± 1.9	
O_3 (µg/m³)	Mean ± SD	35.5 ± 1.6	35.4 ± 1.6	
NO_2 (µg/m³)	Mean ± SD	40.0 ± 4.2	40.2 ± 4.1	
NO (µg/m³)	Mean ± SD	12.5 ± 4.4	12.8 ± 4.6	*
SO_2 (µg/m³)	Mean ± SD	8.7 ± 1.1	8.7 ± 1.1	
CO (µg/m³)	Mean ± SD	0.3 ± 0.1	0.3 ± 0.1	*
NH_3 (µg/m³)	Mean ± SD	2.6 ± 0.4	2.6 ± 0.4	
L_{den} traffic (dB)	Mean ± SD	53.6 ± 9.4	54.2 ± 9.8	(*)
L_{den} tram (dB)	Mean ± SD	11.9 ± 13.4	12.5 ± 13.7	
Road[1] (km)	Mean ± SD	0.9 ± 0.8	0.9 ± 0.8	
Unemployment (%)	Mean ± SD	12.4 ± 3.4	12.6 ± 3.5	**
SBP (mmHg)	Mean ± SD	119.8 ± 12.2	143.3 ± 20.3	***
DBP (mmHg)	Mean ± SD	76.1 ± 7.4	85.4 ± 11.3	***
PP (mmHg)	Mean ± SD	43.7 ± 8.9	57.8 ± 15.4	***
BPLM intake	%	0.0%	62.4%	***
Age (years)	Mean ± SD	57.0 ± 7.3	61.6 ± 7.5	***
Sex (male)	%	42.6%	54.9%	***
CHD		1.3%	10.4%	***
T2DM	%	6.2%	19.1%	***
BMI (kg/m²)	Mean ± SD	26.5 ± 4.0	29.0 ± 4.7	***
Smoking: Current	%			***
Former		27.9%	19.7%	
Never		30.4%	37.7%	
ETS exposure		41.7%	42.6%	
No sport	%	39.3%	33.8%	***
Education: < 10 yrs	%	40.5%	49.8%	***
BPLM intake	%	8.7%	13.2%	***

N = 4,584.
*[1]Distance to road in upper quartile of diesel vehicle density. [2]Student's T test significance was applied with continuous variables, and χ^2-test was applied with stratum-specific proportions with categorical variables. Test significance: (*) = p < 0.1; * = p < 0.05; ** = p < 0.01; *** = p < 0.001.*

Hypertensive and non-hypertensive participants did not differ with regard to exposure concentrations (although Student's T-test revealed significant differences in concentrations of NO and CO between these groups, the absolute differences were quite small). I observed incrementally higher values of road traffic noise and neighborhood unemployment rate in participants with hypertension (Table 13).

Hypertensive participants differed from the non-hypertensive ones substantially with regard to personal characteristics. Subjects without hypertension had lower BP values, were younger and had lower BMIs (T-test $p < 0.001$ for these characteristics). The hypertensive participants were more frequent alcohol drinkers (not shown) and had lower physical activity and educational levels; there were more men, former smokers, diabetics and subjects with CHD among the hypertensive.

The medicated subjects differed from the non-medicated in the same way as did the hypertensive subjects from the non-hypertensive; in addition, they resided on average closer to major roads (T-test $p < 0.05$; Appendix Table 45).

Stratification by city (Table 14)

I observed differences in exposure and environmental covariates by the city of residence. The highest levels of particulate matter and gaseous pollutants were found in Mülheim, whereas the lowest concentrations were observed in Bochum (T-test $p < 0.001$). In contrast, the noise levels were higher in Essen, especially the tram noise (T-test $p < 0.01$). On average, participants in Mülheim lived nearer to major roads than participants in Essen and Bochum, and the neighborhood-level unemployment rate was lower in Mülheim (for both, T-test $p < 0.001$). The mean values of BP and age were lower in Essen, than in Bochum ($p < 0.05$). Subpopulations in the three cities differed slightly by sex distribution, smoking, alcohol consumption, and physical activity, but these differences were not statistically significant. The proportion of participants with lower education levels was significantly smaller in Essen, than in Bochum ($p < 0.05$).

Table 14. Description of study participants at baseline by city.

Variable (unit)	Statistics	City			$p_{difference}$[2]		
		Bochum (N = 1,331)	***Essen (N = 1,572)***	***Mülheim (N = 1,681)***	***Bo/ Es***[3]	***Es/Mh***	***Bo/Mh***
$PM_{2.5}$ (µg/m³)	Mean ± SD	15.3 ± 0.9	16.4 ± 1.2	18.0 ± 1.3	***	***	***
PM_{10} (µg/m³)	Mean ± SD	18.2 ± 1.3	21.1 ± 2.3	22.3 ± 2.0	***	***	***
PM_{coarse} (µg/m³)	Mean ± SD	2.9 ± 0.7	4.7 ± 2.0	4.4 ± 1.1	***	***	***
PM_1 (µg/m³)	Mean ± SD	10.5 ± 0.9	11.5 ± 1.1	12.6 ± 1.1	***	***	***
PN ($\times 10^4$/L)	Mean ± SD	7.9 ± 1.7	9.1 ± 2.0	9.3 ± 1.7	***	***	***
O_3 (µg/m³)	Mean ± SD	36.5 ± 1.3	35.4 ± 1.3	34.6 ± 1.4	***	***	***
NO_2 (µg/m³)	Mean ± SD	39.0 ± 4.6	39.4 ± 3.9	41.8 ± 3.5	**	***	***
NO (µg/m³)	Mean ± SD	9.8 ± 2.3	14.9 ± 5.6	12.9 ± 3.1	***	***	***
SO_2 (µg/m³)	Mean ± SD	8.1 ± 0.7	8.8 ± 1.3	9.1 ± 1.0	***	***	***
CO (µg/m³)	Mean ± SD	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	***	***	***
NH_3 (µg/m³)	Mean ± SD	2.3 ± 0.2	2.6 ± 0.3	2.8 ± 0.3	***	***	***
L_{den} traffic (dB)	Mean ± SD	52.8 ± 10.5	55.1 ± 9.6	53.7 ± 8.7	***	***	**
L_{den} tram (dB)	Mean ± SD	6.7 ± 9.8	24.6 ± 14.9	5.0 ± 0.0	***	***	***
Road[1] (km)	Mean ± SD	1.1 ± 1.0	0.9 ± 0.8	0.8 ± 0.5	***	***	***
Unemployment (%)	%	13.4 ± 3.0	13.7 ± 3.9	10.7 ± 2.5	*	***	***
SBP (mmHg)	Mean ± SD	132.1 ± 20.6	133.8 ± 21.1	133.4 ± 20.8	*		(*)
DBP (mmHg)	Mean ± SD	80.7 ± 10.4	81.7 ± 11.0	81.7 ± 11.0	*		*
PP (mmHg)	Mean ± SD	51.3 ± 14.8	52.1 ± 15.0	51.7 ± 14.4			
Hypertension	%	56.5%	58.5%	55.9%			
BPLM intake	%	37.0%	35.8%	34.1%			
Age (years)	Mean ± SD	59.2 ± 7.7	59.9 ± 7.8	59.7 ± 7.9	*		(*)
Sex (male)	%	51.2%	47.6%	50.3%			
CHD	%	5.9%	7.7%	5.7%			
T2DM	%	14.0%	14.6%	12.4%			
BMI (kg/m²)	Mean ± SD	28.0 ± 4.6	27.9 ± 4.6	27.9 ± 4.6			
Smoking: Current	%	23.2%	22.5%	18.4%			
Former	%	35.7%	36.5%	28.3%			
Never	%	41.1%	41.0%	32.5%			
ETS exposure	%	37.2%	36.5%	35.1%			
No sport	%	46.3%	47.0%	44.3%			
Education: < 10 yrs	%	11.3%	11.4%	8.9%	*		

N = 4,584.

*[1]Distance to road in upper quartile of diesel vehicle density. [2]Student's T test significance was applied with continuous variables, and χ^2-test was applied with stratum-specific proportions with categorical variables. Test significance: (*) = p < 0.1; * = p < 0.05; ** = p < 0.01; *** = p < 0.001.*

4.1.4. Cross-sectional association of air pollution with BP and hypertension

Particulate matter (Table 15)

Yearly mean concentrations of $PM_{2.5}$ and PM_1 were positively associated with SBP and DBP and showed a positive relationship with PP, independent of lifestyle, personal characteristics, ambient noise, distance to major road, short-term PM, and time trend (Table 15). The IQR increase in the yearly mean $PM_{2.5}$ (2.4 μg/m³) was associated with higher SBP by 1.1 mmHg (95% confidence interval (CI): 0.2, 2.0), higher DBP by 0.7 mmHg (95% CI: 0.2, 1.2), and higher PP by 0.4 mmHg (95% CI: -0.2, 1.0). Estimates with PM_1 (presented per IQR) were almost identical. The PM_{10} exposure was positively associated with DBP and showed a weaker positive relationship with SBP. No clear associations were identified with PM_{coarse} and PN concentrations, although effect estimates were mostly positive. No association between exposure to PM and prevalent hypertension was observed. The yearly mean concentrations of different PM metrics, but not of PN, were linked to lower odds ratios (ORs) for BPLM intake (Table 15).

Gaseous pollutants (Table 15)

The yearly mean concentration of NH_3 was positively associated with BP: per IQR (0.5 μg/m³), the estimated increase in SBP was 1.3 mmHg (95% CI: 0.4, 2.2), the increase in DBP was 0.7 mmHg (95% CI: 0.2, 1.2), and the increase in PP was 0.6 mmHg (95% CI: 0.0, 1.2). NH_3 exposure was related to elevated OR for hypertension and lower OR for BPLM intake. Long-term NO concentration was positively associated with DBP (0.6 mmHg (95% CI: 0.1, 1.0) per 5.5 μg/m³) and related to elevated SBP and OR for hypertension. The findings with other gaseous pollutants were weaker. Exposure to O_3 was inversely related to BP and was positively related to BPLM intake. The yearly mean concentration of NO_2 was weakly positively related to SBP and PP; NO_2, NO and CO concentrations were weakly positively related to the OR for hypertension.

Table 15. The associations of ambient air pollution concentrations (individual 365-day means) with BP and hypertension.

Exposure		**IQR**	**Change in BP, mmHg (95% CI)**			**Hypertension**	**BPLM intake**
			SBP	***DBP*[1]**	***PP***	***Odds ratio (95% CI)***	***Odds ratio (95% CI)***
$PM_{2.5}$	(μg/m³)	2.4	1.1 (0.2, 2.0)	0.7 (0.2, 1.2)	0.4 (-0.2, 1.0)	1.00 (0.90, 1.11)	0.96 (0.86, 1.07)
PM_{10}	(μg/m³)	4.0	0.9 (0.0, 1.8)	0.7 (0.2, 1.2)	0.2 (-0.4, 0.9)	1.00 (0.90, 1.11)	0.95 (0.85, 1.06)
PM_{coarse}	(μg/m³)	1.7	0.3 (-0.4, 1.0)	0.3 (-0.1, 0.7)	0.0 (-0.5, 0.4)	1.00 (0.92, 1.08)	0.96 (0.89, 1.04)
PM_1	(μg/m³)	1.9	1.1 (0.2, 2.1)	0.7 (0.2, 1.2)	0.5 (-0.2, 1.1)	1.01 (0.90, 1.12)	0.96 (0.86, 1.08)
PN	($\times 10^4$/L)	2.7	0.5 (-0.4, 1.3)	0.3 (-0.2, 0.8)	0.2 (-0.5, 0.8)	1.00 (0.90, 1.11)	1.00 (0.90, 1.11)
O_3	(μg/m³)	2.2	-0.7 (-1.5, 0.1)	-0.3 (-0.8, 0.1)	-0.3 (-0.9, 0.2)	1.00 (0.91, 1.10)	1.04 (0.94, 1.14)
NO_2	(μg/m³)	5.7	0.7 (-0.1, 1.5)	0.3 (-0.1, 0.7)	0.4 (-0.2, 0.9)	1.06 (0.96, 1.16)	1.02 (0.93, 1.13)
NO	(μg/m³)	5.5	0.4 (-0.4, 1.2)	0.6 (0.1, 1.0)	-0.1 (-0.7, 0.4)	1.06 (0.96, 1.17)	1.01 (0.92, 1.12)
SO_2	(μg/m³)	1.5	0.4 (-0.4, 1.2)	0.2 (-0.3, 0.6)	0.3 (-0.3, 0.8)	1.01 (0.92, 1.11)	1.01 (0.91, 1.11)
CO	(μg/m³)	0.1	0.3 (-0.4, 1.0)	0.4 (0.0, 0.8)	-0.1 (-0.6, 0.4)	1.05 (0.96, 1.14)	0.99 (0.91, 1.08)
NH_3	(μg/m³)	0.5	1.3 (0.4, 2.2)	0.7 (0.2, 1.2)	0.6 (0.0, 1.2)	1.02 (0.91, 1.13)	0.95 (0.85, 1.06)

N = 4,584. The results are presented per given IQRs of exposure concentrations.

Adjusted for: time trend, short-term exposure, L_{night} traffic (linear, in 5-dB categories), L_{den} tram (linear, in 5-dB categories), distance to major road (upper quartile of diesel vehicle density), age, sex, BMI (linear and squared terms, centered on mean), WHR, blood lipids (lipid ratio, HDL, triglycerides), smoking status, ETS, physical activity (in categories), education (in categories), and economic activity (in categories).

[1]*In the analysis with DBP, age and blood triglycerides were entered as linear and squared terms, centered on the mean.*

4.1.5. Sensitivity analyses

Results with self-reported binary outcomes (Table 16)

Yearly mean concentrations of PM_1 to PM_{10} were related to lower ORs for self-reported hypertension. The strongest association was observed with PM_{10}: OR 0.86 (95% CI: 0.77, 0.95) per IQR of 4 µg/m³. Similarly to results with self-reported hypertension, higher long-term concentrations of PM were related to lower ORs of self-reported BPLM intake, but the confidence intervals were generally wider. No statistically significant associations of gaseous pollutants with hypertension or BPLM intake were observed. Concentration of O_3 was related to an elevated OR for hypertension, and concentrations of NO, SO_2, CO, NH_3 were related to decreased ORs for self-reported hypertension. Exposure to CO and NH_3 were inversely related to ORs for BPLM intake.

Table 16. The cross-sectional associations with self-reported hypertension and BPLM intake.

Exposure	**(unit)**	**IQR**	**Self-reported hypertension[1]** *Odds ratio (95% CI)*	**Self-reported BPLM intake[2]** *Odds ratio (95% CI)*
$PM_{2.5}$	(µg/m³)	2.4	0.90 (0.81, 1.00)	0.94 (0.84, 1.05)
PM_{10}	(µg/m³)	4.0	0.86 (0.77, 0.95)	0.91 (0.81, 1.01)
PM_{coarse}	(µg/m³)	1.7	0.89 (0.83, 0.97)	0.93 (0.86, 1.01)
PM_1	(µg/m³)	1.9	0.90 (0.81, 1.00)	0.94 (0.84, 1.06)
PN	($\times 10^4$/L)	2.7	0.96 (0.87, 1.07)	1.00 (0.90, 1.11)
O_3	(µg/m³)	2.2	1.06 (0.97, 1.17)	1.02 (0.92, 1.13)
NO_2	(µg/m³)	5.7	0.99 (0.90, 1.08)	1.02 (0.92, 1.12)
NO	(µg/m³)	5.5	0.93 (0.85, 1.03)	0.98 (0.89, 1.09)
SO_2	(µg/m³)	1.5	0.95 (0.86, 1.04)	1.02 (0.92, 1.13)
CO	(µg/m³)	0.1	0.93 (0.86, 1.01)	0.97 (0.89, 1.06)
NH_3	(µg/m³)	0.5	0.92 (0.83, 1.02)	0.95 (0.85, 1.06)

The results are presented per given IQRs of exposure concentrations.
Adjusted for: time trend, short-term exposure, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.
[1]Analysis subset: N = 4,574.[2]Analysis subset: N = 4,583.

Models from crude to main (Figure 7)

I calculated results with a range of reduced models: the covariates from the main model were added in steps to observe the changes in the estimate with exposure after adjustment (models 0 to

MAIN, Figure 7 with $PM_{2.5}$ and PM_{10}).

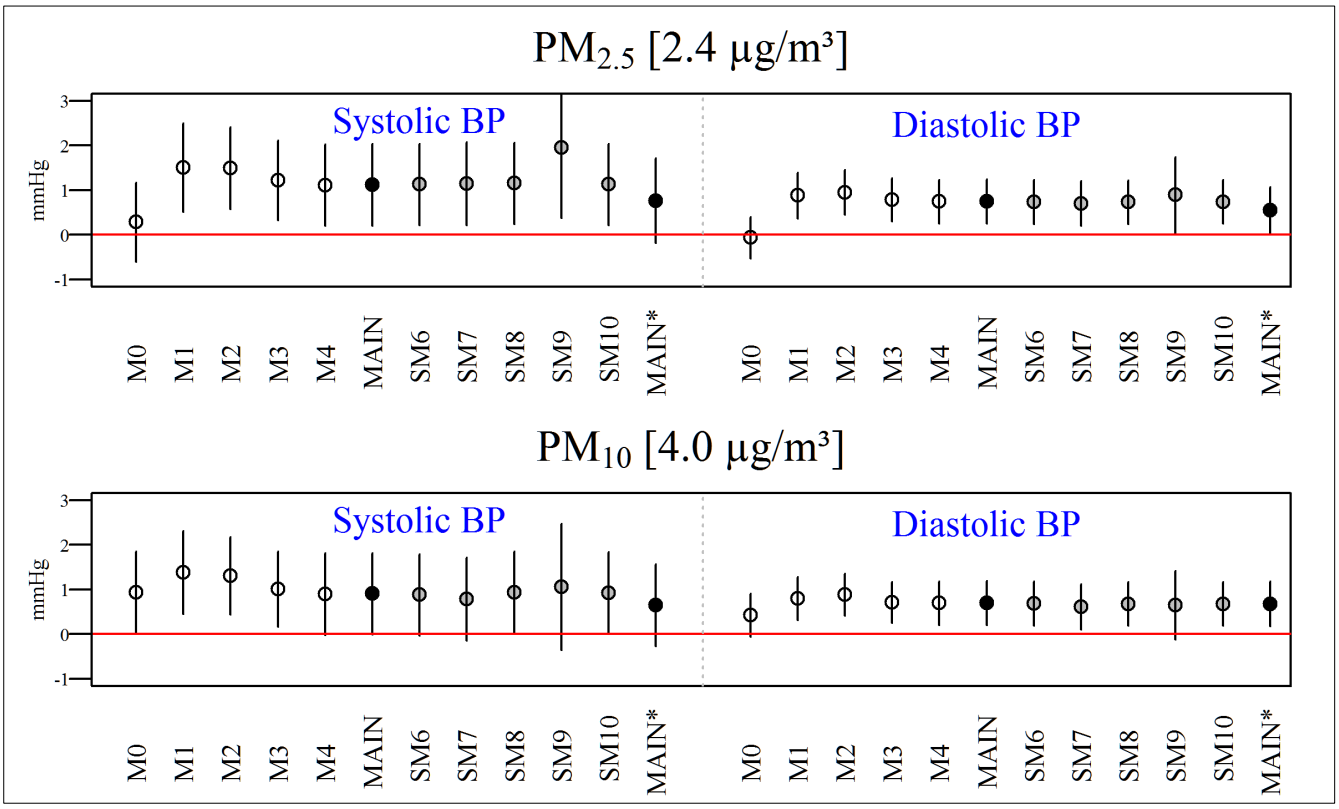


Figure 7. Estimated associations of $PM_{2.5}$ and PM_{10} with SBP and DBP, using different adjustment models.

Legend: N = 4,584 (if not indicated otherwise). The results are presented per given IQRs of exposure concentrations. Reduced models: M0 = crude model (only exposure); M1 = added time trend; M2: added age, sex; M3: added BMI, WHR, blood lipids, smoking, ETS, physical activity, educational level, economic activity; M4: added L_{night} traffic, L_{den} tram; MAIN = M4 with short-term exposure; sensitivity models (MAIN with additional covariates): SM6 = MAIN with short-term humidity, wind speed; SM7 = MAIN with alcohol, pack-years of smoking (N = 4,368); SM8 = MAIN with CHD, T2DM; SM9 = MAIN with city, geographic area; SM10 = MAIN, influential observations (high residual, high leverage excluded, N = 4,581); MAIN = MAIN model, grid-specific mean for 2000-2003 (representing the spatial contrast only) as the exposure.*

The crude association of the yearly mean $PM_{2.5}$ with BP concentration was positive and statistically significant in the presence of the time trend (models M1 to MAIN); without the time trend, a null effect was observed (model M0). PM_{10} showed a crude positive association with BP, which became stronger after addition of time trend to the model. Adjustment for personal risk factors

resulted in decreased estimates (models M2, M3). Further adjustment for road traffic noise and short-term air pollution did not affect the estimates substantially (models M4 and MAIN). Adjustment for the variables in the main model had similar effects on the estimates with gaseous compounds (not shown). In addition to what was observed with PM, the precision of estimates further decreased, and they diminished towards null after adjustment for short-term pollutant concentration. Only the results with NH_3 were robust across different model specifications.

Extended adjustment sets

Adjustment for short-term meteorology (model SM6), additional personal risk factors (alcohol consumption and pack-years of smoking), estimated with a reduced analysis sample due to missing values (model SM7), and co-diseases, namely, CHD and T2DM (model SM8), did not affect the estimates (Figure 7 with $PM_{2.5}$ and PM_{10}). Adjustment for city and geographic location (SM9) led to an increase in the estimate and a wider confidence interval. Exclusion of the observations with high residuals or leverages (SM10) did not affect the estimates substantially.

Spatial versus spatiotemporal variations in exposure

When grid-specific means over the entire baseline period (2000–2003) were used instead of individual 365-day means, without adjustment for time trend, the estimated relationship with BP was slightly weaker than in the main model with 365-day mean. The confidence intervals were wider with SBP. With DBP, the association with grid-specific mean was statistically significant.

Adjusting for neighborhood SES

I calculated the mixed-effects models, which included a neighborhood-level SES parameter and random intercept for the neighborhood, in addition to the long-term air pollution exposure and covariates from the main model. The results for the main exposure remained unchanged after adjustment for various neighborhood SES variables (Figure 8).

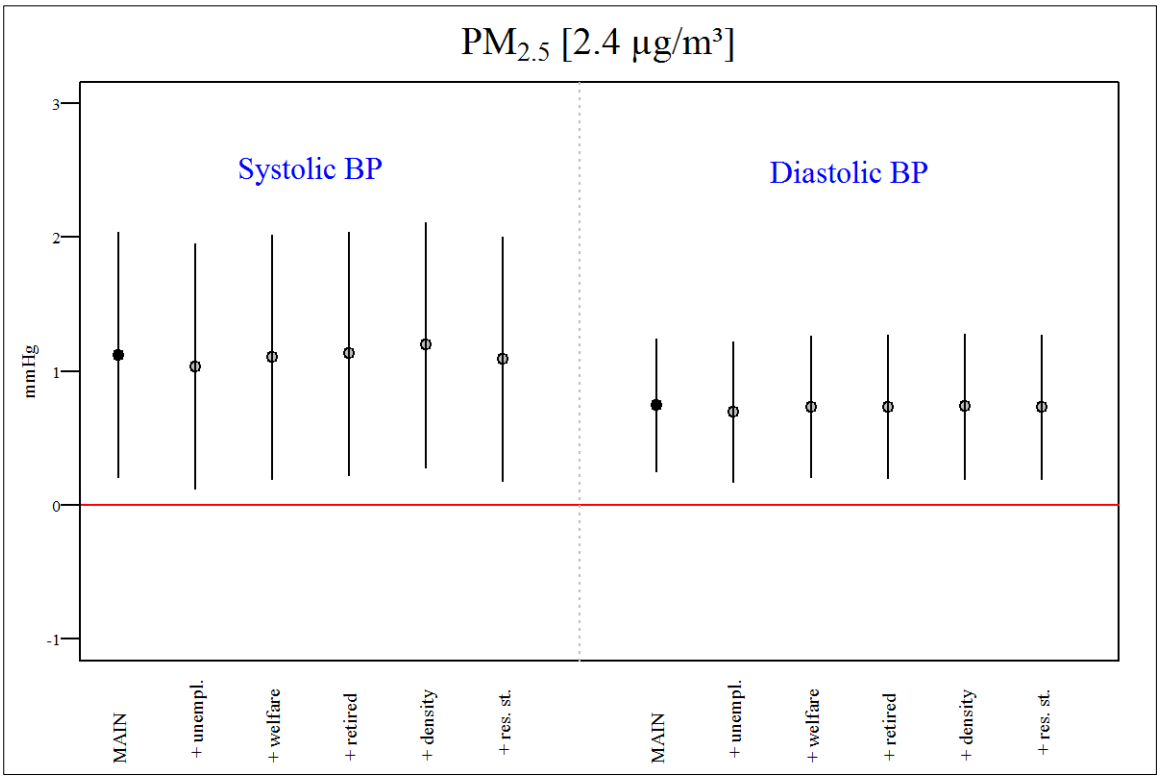


Figure 8. The associations of long-term exposure to $PM_{2.5}$ and PM_{10} with SBP and DBP, adjusted for neighborhood-level SES.

Legend: N = 4,584. The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term exposure, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

4.1.6. Independence of PM effects from gaseous compounds

Multi-pollutant models

I computed multi-pollutant models, adding each of the gaseous compounds to the main model with $PM_{2.5}$ or PM_{10} and SBP (Figure 9). In comparison to the main adjusted model (adjustment set MAS1), adjustment for O_3, NO_2, and SO_2 resulted in slightly lower estimates. Adjustment for NO and CO had no effect on the estimates for $PM_{2.5}$ and PM_{10} but slightly increased confidence intervals. I observed null association of PM with BP after adjustment for NH_3.

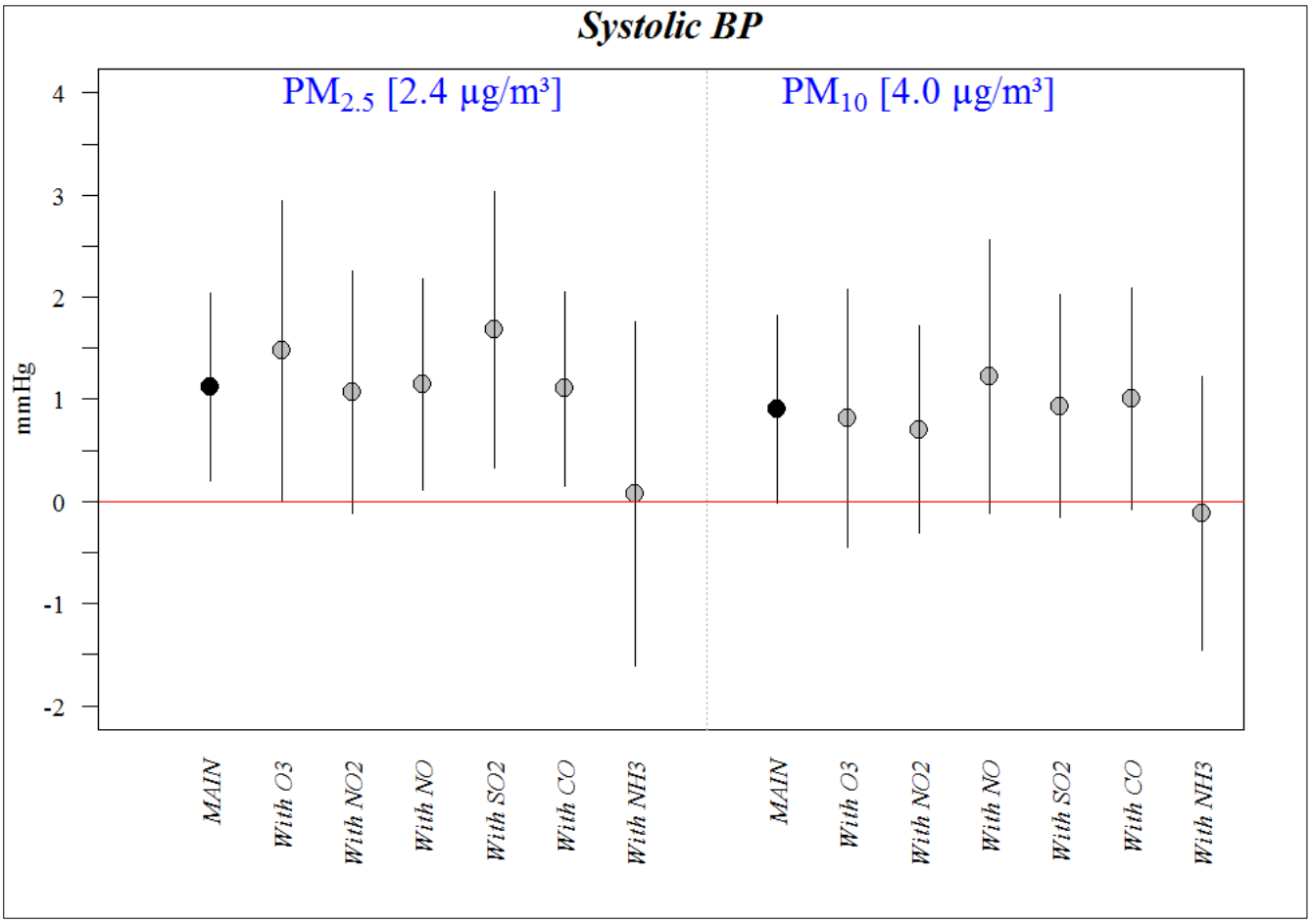


Figure 9. Multipollutant models: association of the 365-day mean $PM_{2.5}$ and PM_{10} concentrations with SBP, adjusted for the 365-day mean concentration of gaseous pollutants.

Legend: N = 4,584. The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term exposure, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

Interaction of PM with gaseous compounds

I dichotomized each of the gaseous compounds at the 75th percentile and computed product terms with $PM_{2.5}$ and PM_{10} to estimate the association of PM with BP at different concentration levels of the gaseous component (Figure 10). For all interactions tested, the estimates at the gas levels below the cut point were quite similar to the main model. At high levels of O_3 and NO_2, the effect estimate for PM was also similar to the main model, although less precise. Close to null or inverse association was observed at high levels of NO and CO; this interaction was statistically significant ($p < 0.05$). The results with interaction by NH_3 were inconsistent for $PM_{2.5}$ and PM_{10}.

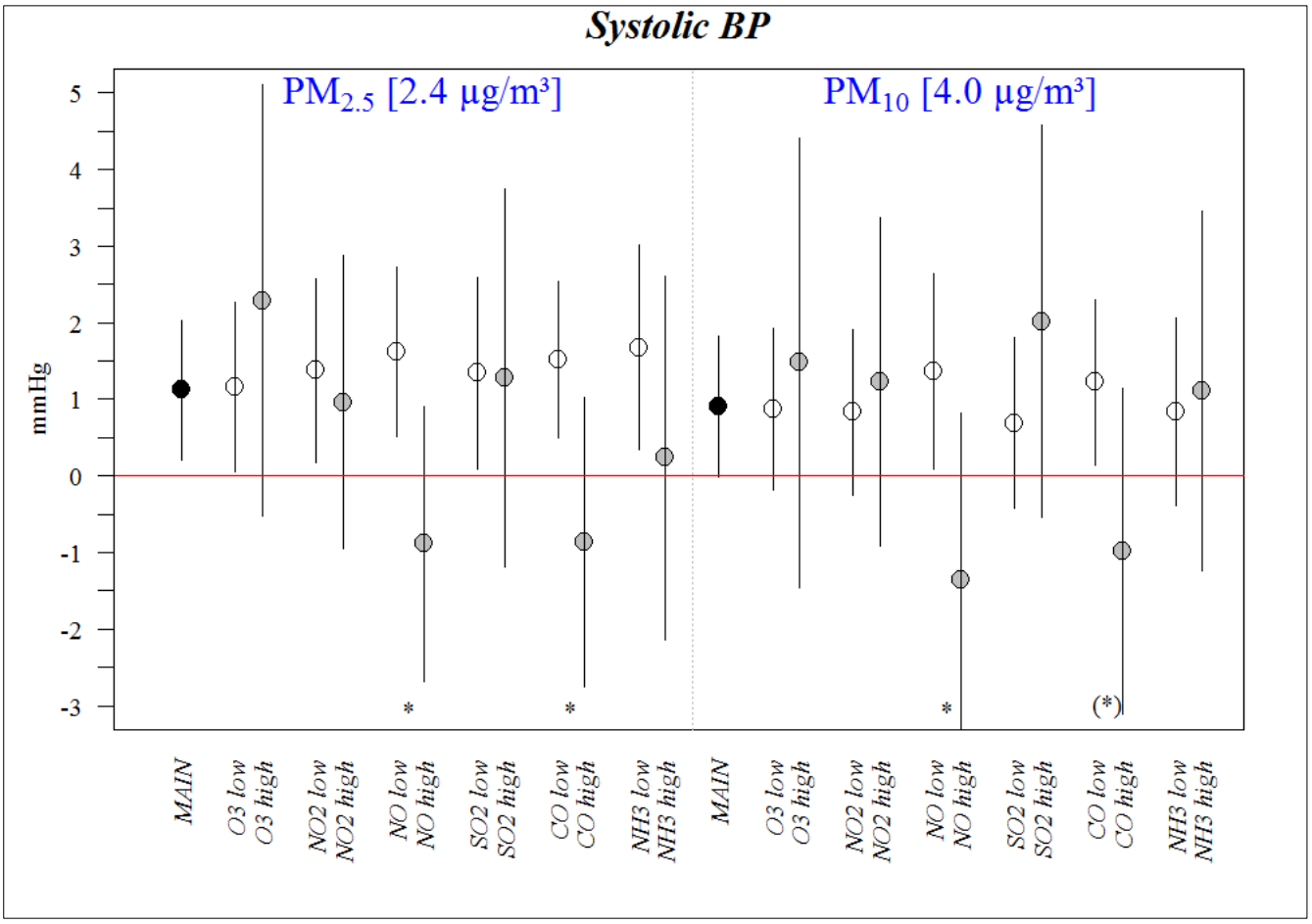


Figure 10. Effect modification of the associations of the 365-day mean $PM_{2.5}$ and PM_{10} concentrations with SBP by 365-day mean concentrations of gaseous pollutants.

Legend: N = 4,584. The results are presented per given IQRs of exposure concentrations. Adjusted *for time trend, short-term exposure, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, economic activity; high = concentration ≥ 75th percentile; low = concentration < 75th percentile. * = $p_{interaction}$ < 0.05; (*) = $p_{interaction}$ < 0.1. Adjusted for time trend, short-term exposure, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.*

4.1.7. Independence of the gaseous compounds from PM

Multi-pollutant models

Adjustment for PM_{10} or $PM_{2.5}$ led to changes of the effect estimate towards zero and also to loss of precision for all gases but NH_3 (Figure 11). With NH_3, the estimates remained unchanged, but

the confidence intervals increased.

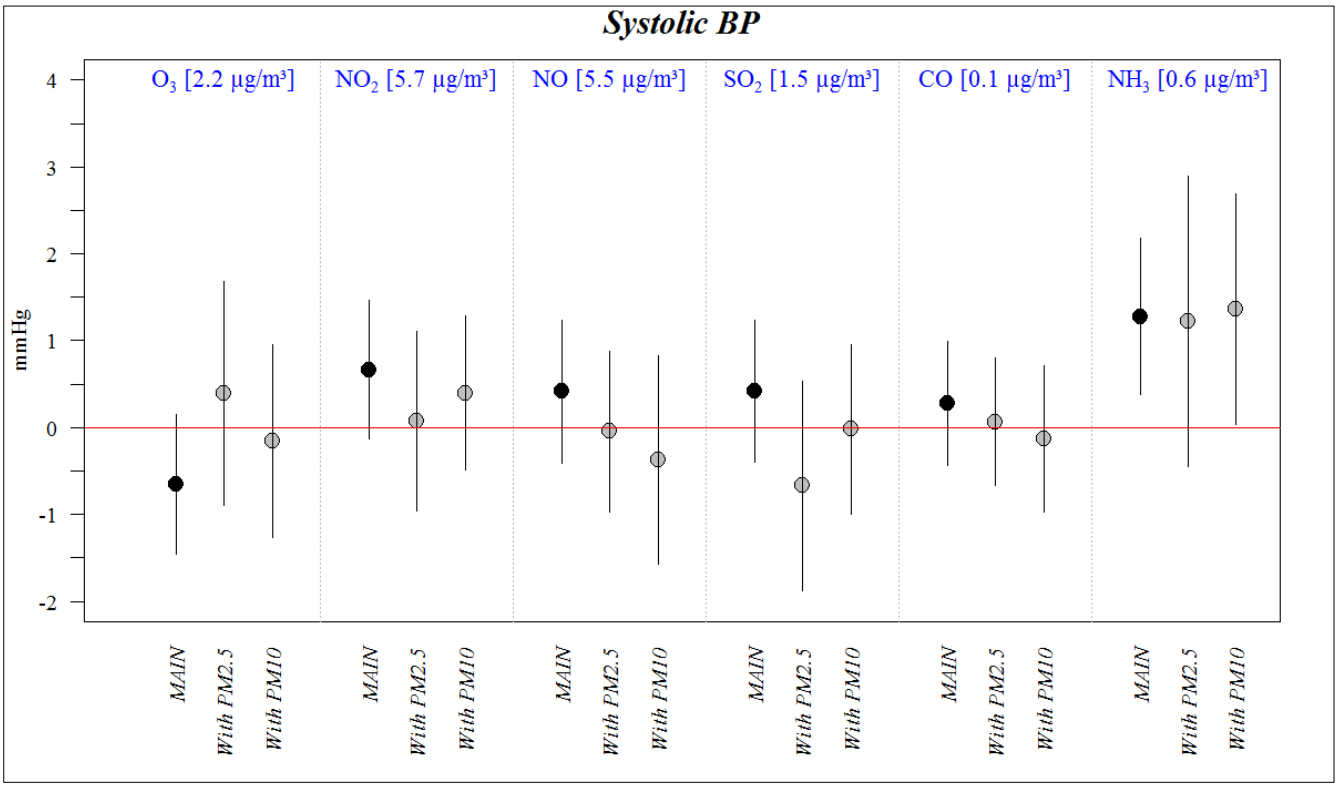


Figure 11. The multipollutant models: association of 365-day mean exposure to gaseous pollutants with SBP, adjusted for the 365-day mean concentrations of $PM_{2.5}$ and PM_{10}.

Legend: N = 4,584. The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term exposure, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

Interaction of gaseous pollutants with dichotomized PM

At lower concentrations of $PM_{2.5}$ or PM_{10}, the estimates for O_3, nitrogen oxides, SO_2, CO and NH_3 were similar to the main model, although the precision of these results was often lower than in the main model (Figure 12). At high levels of PM, I observed mostly null associations of the gaseous compounds with BP. The results with NH_3 were similar to the main model at high levels of PM_{10} but diminished towards null at high levels of $PM_{2.5}$.

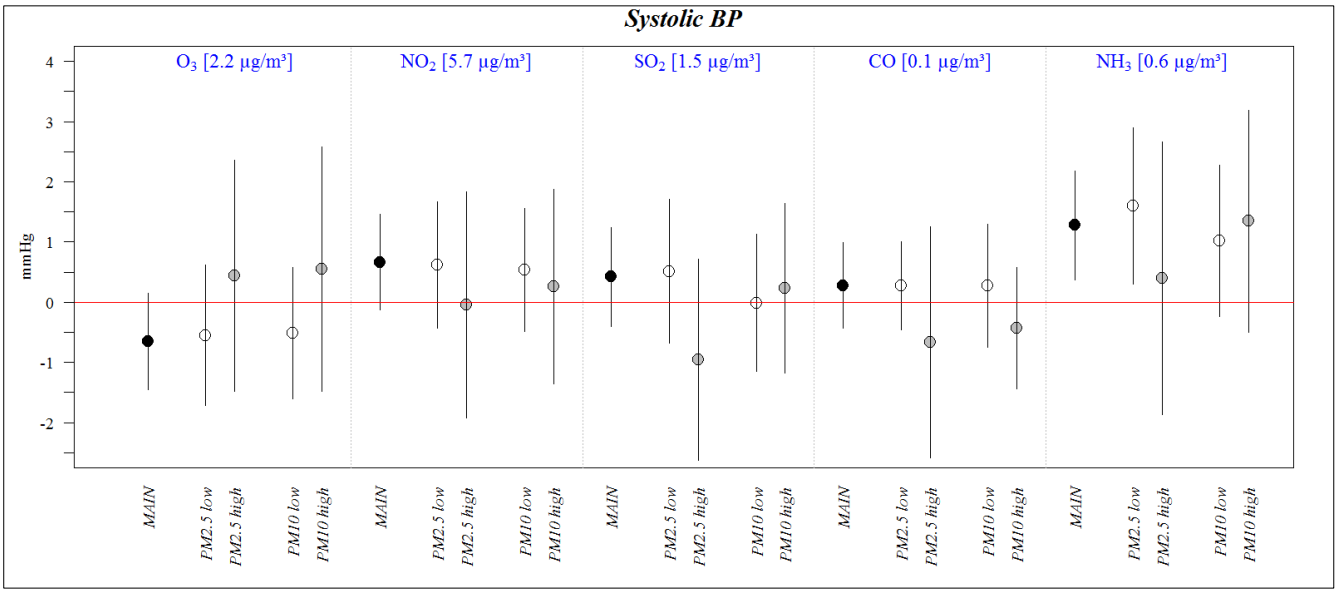


Figure 12. Effect modification of the association of 365-day mean concentrations of gaseous pollutants with SBP by 365-day mean concentrations of $PM_{2.5}$ and PM_{10}.

*Legend: N = 4,584. The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term exposure, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, economic activity; high = concentration ≥ 75th percentile; low = concentration < 75th percentile. * = $p_{interaction}$ < 0.05; (*) = $p_{interaction}$ < 0.1.*

4.1.8. *Linearity of exposure-outcome association*

I divided long-term grid-specific mean exposure concentrations (mean of 2000–2003) into quartiles to check the linearity of the exposure-outcome relationship. A monotonic relationship was observed for PM with DBP; with SBP, the relationship was similarly linear, though the estimate for quartile 3 was slightly lower than for quartile 2 (Figure 13). Similarly to PM, monotonic inverse relationships with both systolic and DBP were observed with O_3 (Figure 14). For the rest of gaseous pollutants, I found a gradual increase in estimates for quartiles 2–4 compared to quartile 1. The estimate for quartile 3 was in some cases lower than the estimate for quartile 2, as was already observed with PM. The results for quartile 4 were either significantly different from 0 ($p < 0.05$) or close to significance ($p < 0.1$) for all pollutants.

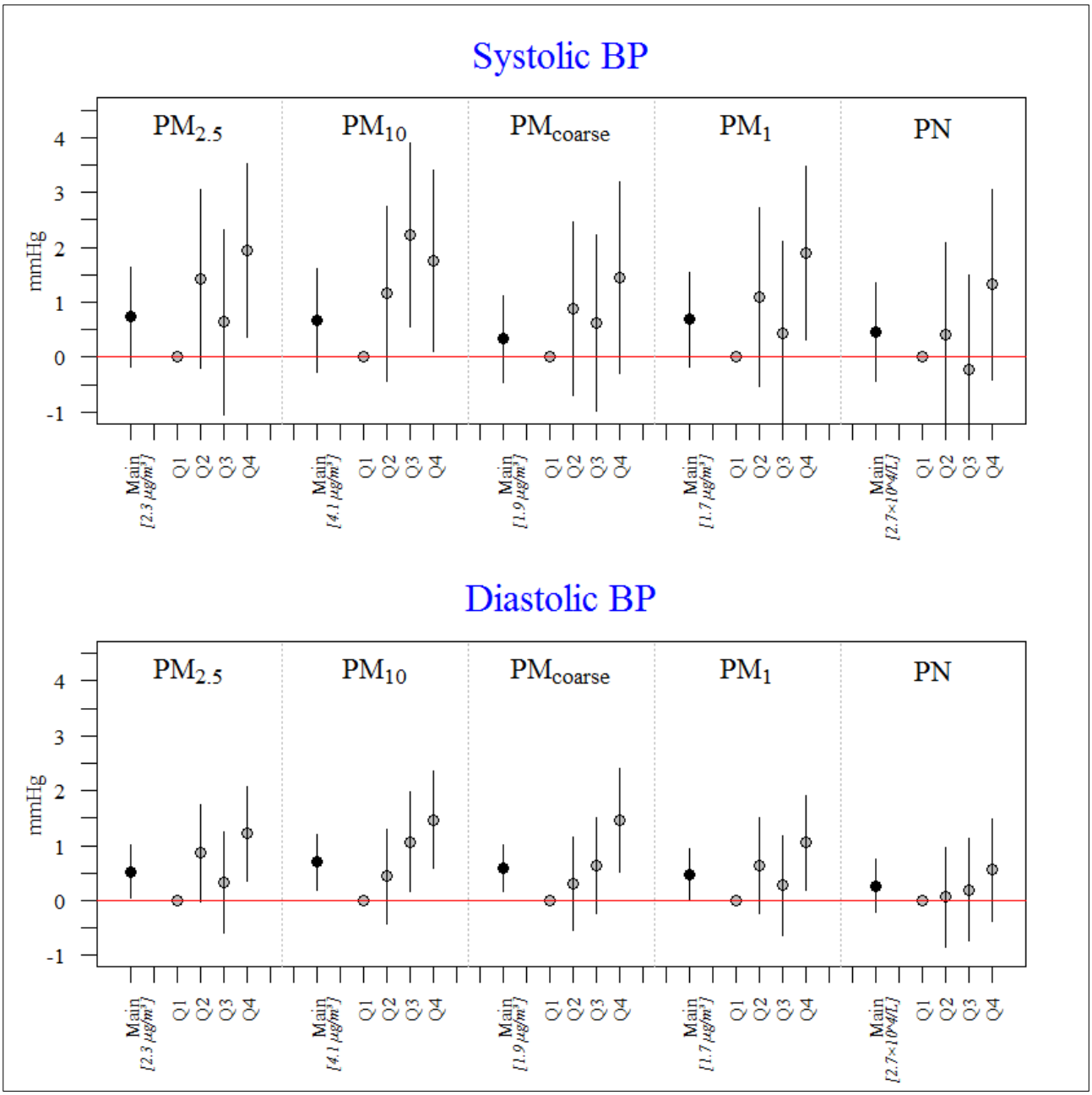


Figure 13. The associations of grid-specific mean concentrations of PM (2000–2003), divided in quartiles, with SBP and DBP.

Legend: N = 4,584. Adjusted for L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

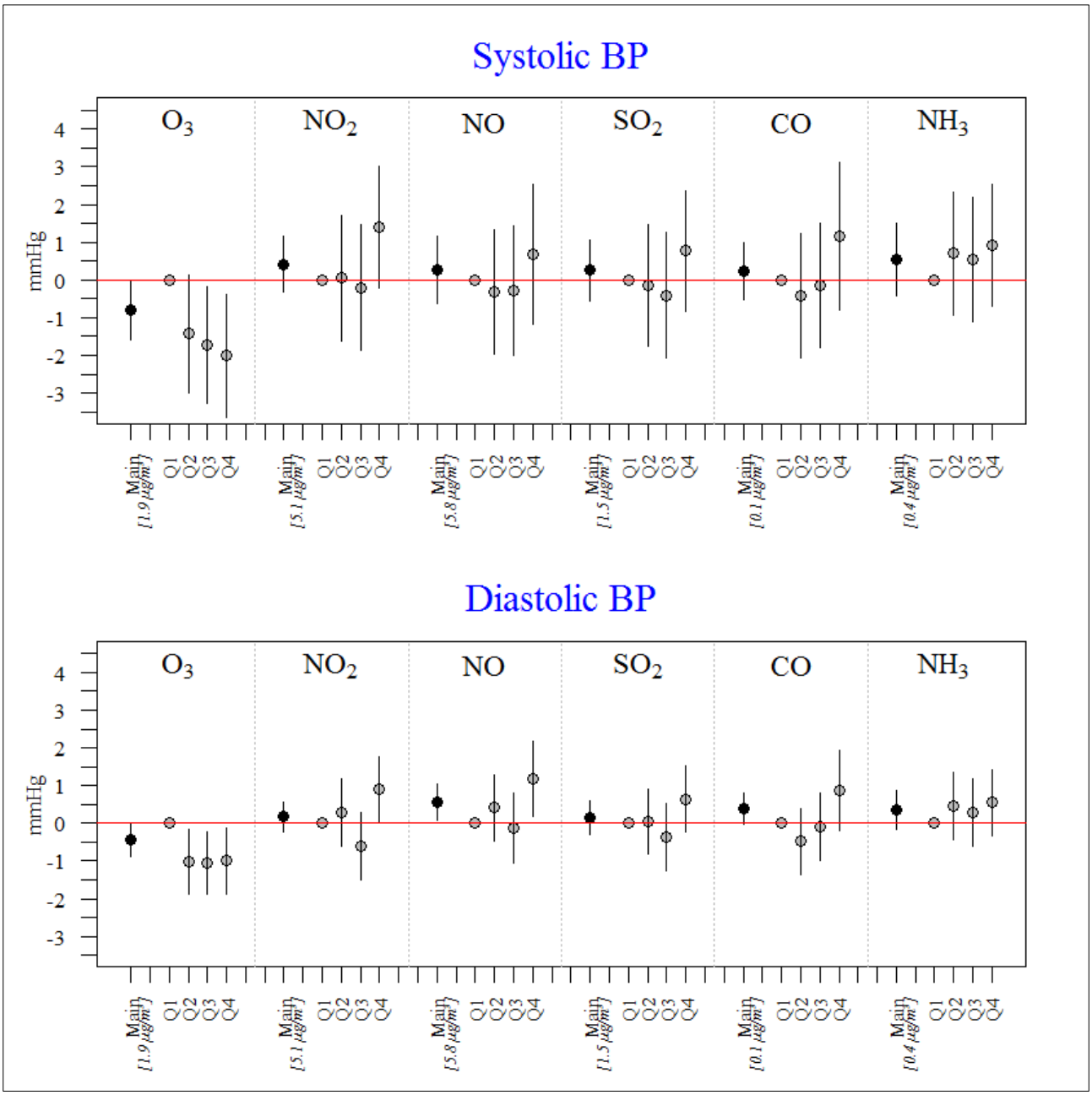


Figure 14. The associations of grid-specific mean concentrations of gaseous pollutants (2000–2003), divided in quartiles, with SBP and DBP.

Legend: N = 4,584. Adjusted for L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

I tested the linearity of exposure effects using penalized splines with 4 knots (Figure 15). The two models – a linear regression with exposure as a continuous covariate and a generalized additive model with a penalized spline – were compared using ANOVA. The exposure-outcome relationships did not significantly deviate from linearity.

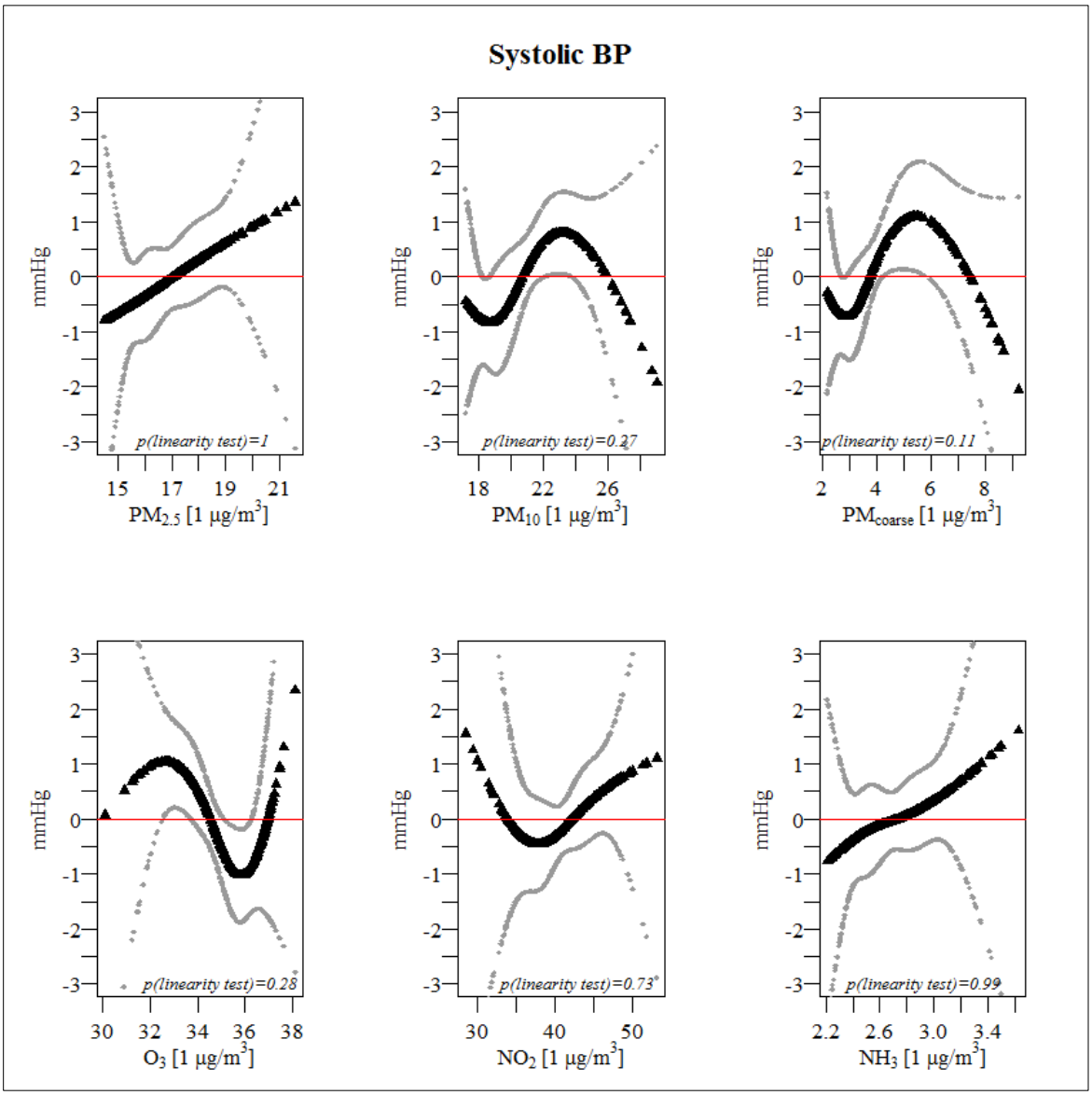


Figure 15. The associations of long-term grid-specific mean air pollution concentrations (2000–2003), entered as penalized splines, with SBP.

Legend: N = 4,584. Adjusted for L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

4.1.9. Association of other environmental exposures with BP and hypertension

Road traffic noise

I analyzed road traffic noise both as a continuous variable (with the original modeled noise value and merged into 5-dB categories) and as a categorical variable (Table 17). I controlled for air pollution and distance to major road in the model.

Table 17. The cross-sectional associations of 24-hour mean road traffic noise with BP, hypertension, and BPLM intake.

Exposure	**N**	**SBP (mmHg)**	**DBP (mmHg)**	**PP (mmHg)**	**Hypertension (OR)**	**BPLM (OR)**
L_{den} (original value, continuous)						
(per 5 dB)	4,538	0.26 (-0.06, 0.58)	0.07 (-0.10, 0.25)	0.19 (-0.03, 0.41)	1.02 (0.99, 1.06)	1.01 (0.97, 1.05)
L_{den} (in 5-dB categories, as continuous)						
(per 5 dB)	4,584	0.31 (0.01, 0.61)	0.10 (-0.07, 0.26)	0.22 (0.01, 0.43)	1.03 (0.99, 1.06)	1.01 (0.98, 1.05)
L_{den} (in 5-dB categories, as categorical)						
< 40 dB	177	3.29 (0.07, 6.50)	1.28 (-0.46, 3.02)	2.05 (-0.18, 4.28)	1.17 (0.80, 1.73)	0.98 (0.67, 1.45)
≥ 40 and < 45 dB	580	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
≥ 45 and < 50 dB	1,028	2.04 (0.11, 3.98)	-0.24 (-1.29, 0.81)	2.32 (0.98, 3.66)	1.05 (0.83, 1.32)	1.02 (0.80, 1.29)
≥ 50 and < 55 dB	901	2.93 (0.89, 4.96)	-0.14 (-1.24, 0.97)	3.06 (1.65, 4.48)	1.00 (0.78, 1.27)	0.95 (0.74, 1.21)
≥ 55 and < 60 dB	599	2.13 (-0.11, 4.37)	-0.33 (-1.54, 0.89)	2.46 (0.91, 4.02)	1.12 (0.86, 1.47)	1.10 (0.84, 1.45)
≥ 60 and < 65 dB	510	2.62 (0.31, 4.93)	0.38 (-0.87, 1.63)	2.29 (0.69, 3.89)	1.00 (0.76, 1.32)	0.99 (0.75, 1.31)
≥ 65 and < 70 dB	492	3.48 (1.14, 5.82)	0.42 (-0.85, 1.69)	3.08 (1.46, 4.70)	1.26 (0.96, 1.67)	1.07 (0.80, 1.42)
≥ 70 dB	251	1.62 (-1.36, 3.66)	0.79 (-0.83, 1.89)	0.87 (-1.20, 2.28)	1.20 (0.84, 1.53)	1.15 (0.80, 1.47)
Adjusted for time trend, 365-day mean $PM_{2.5}$, short-term mean $PM_{2.5}$, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.						

Table 18. The cross-sectional associations of night-time mean road traffic noise with BP, hypertension, and BPLM intake.

Exposure	N	SBP (mmHg)	DBP (mmHg)	PP (mmHg)	Hypertension (OR)	BPLM (OR)
L_{night} (continuous)						
(per 5 dB)	4,538	0.29 (-0.04, 0.62)	0.08 (-0.10, 0.26)	0.22 (-0.01, 0.45)	1.03 (0.99, 1.07)	1.01 (0.97, 1.05)
L_{night} (in 5-dB categories, as continuous)						
(per 5 dB)	4,584	0.23 (-0.04, 0.50)	0.11 (-0.03, 0.26)	0.12 (-0.07, 0.30)	1.02 (0.99, 1.06)	1.01 (0.98, 1.05)
L_{night} (in 5-dB categories, as categorical)						
< 30 dB	121	4.10 (0.39, 7.81)	3.26 (1.25, 5.28)	0.82 (-1.76, 3.39)	1.21 (0.77, 1.89)	0.93 (0.59, 1.45)
≥ 30 and < 35 dB	449	-1.51 (-3.66, 0.63)	0.51 (-0.65, 1.67)	-2.05 (-3.54, -0.57)	1.18 (0.92, 1.52)	1.25 (0.97, 1.62)
≥ 35 and < 40 dB	939	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
≥ 40 and < 45 dB	953	1.62 (-0.08, 3.33)	0.35 (-0.58, 1.27)	1.26 (0.08, 2.45)	1.02 (0.84, 1.25)	1.04 (0.85, 1.28)
≥ 45 and < 50 dB	693	1.00 (-0.87, 2.88)	0.39 (-0.62, 1.41)	0.57 (-0.73, 1.87)	1.00 (0.80, 1.25)	0.89 (0.71, 1.12)
≥ 50 and < 55 dB	551	0.75 (-1.26, 2.75)	0.51 (-0.58, 1.59)	0.26 (-1.13, 1.65)	1.04 (0.82, 1.31)	1.12 (0.88, 1.43)
≥ 55 and < 60 dB	541	2.02 (0.00, 4.04)	0.71 (-0.38, 1.81)	1.28 (-0.13, 2.68)	1.29 (1.01, 1.64)	1.18 (0.93, 1.51)
≥ 60 dB	291	1.24 (-1.33, 2.94)	1.25 (-0.15, 2.17)	0.01 (-1.77, 1.19)	1.16 (0.86, 1.42)	1.04 (0.76, 1.28)
Adjusted for time trend, 365-day mean $PM_{2.5}$, short-term mean $PM_{2.5}$, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.						

Noise as a continuous variable. Road traffic noise values L_{den} and L_{night} were weakly positively related to SBP and PP (Tables 17, 18). When continuous noise values were merged into 5-dB categories, the linear association was significant with SBP and PP. For example, a 5 dB increase in L_{den} was associated with an increase in SBP by 0.31 mmHg (95% CI: 0.01, 0.61). Results with L_{night} and BP were similar. I also detected weak positive relationships of L_{den} and L_{night} with hypertension.

Noise as a categorical variable. I used category 40-45 dB as a reference with L_{den} and category 30-35 dB as a reference with L_{night} (both in 5-dB categories). The following L_{den} traffic categories were associated with elevated SBP and PP compared to the reference (≥ 40 and < 45 dB): ≥ 50 and < 55 dB, ≥ 60 and < 65 dB, ≥ 65 and < 70 dB. The lowest noise level, observed in a very small category of participants, was associated with elevated BP compared to the reference. The ORs for hypertension and BPLM intake were mostly elevated but not for all categories above the reference. There were significant positive associations of L_{night} ≥ 55 and < 60 dB with hypertension: OR 1.29 (95% CI: 1.01, 1.64), compared to the reference group (L_{night} ≥35 and <40 dB).

Tram noise

No associations of tram noise with the study outcomes were detected: the estimates (per 5 dB) were 0.02 mmHg (-0.19, 0.22) with SBP, 0.02 mmHg (-0.09, 0.13) with DBP, and -0.01 mmHg (-0.15, 0.13) with PP. The OR for hypertension was 1.00 (0.97, 1.02), and the OR for BPLM intake was 1.00 (0.97, 1.02).

Traffic indicators (Table 19)

I estimated the associations between distance to high general traffic (continuous and in categories) and BP, hypertension and BPLM intake, independently of co-exposure to background air pollution, road traffic noise, and other relevant confounders. Distance to a major road (high density of all-type vehicles) was weakly inversely associated with BPLM intake: the OR was 0.99 (95% CI: 0.98, 1.00) per 100 m. When I truncated distance to a major road at 400 m, this effect was stronger: the OR was 0.92 (95% CI: 0.87, 0.98) per 100 m. Results with hypertension were weaker. No associations with BP were detected. The findings with distance to major road with high heavy-duty and diesel vehicles were very similar (not shown).

When considering distance to high heavy-duty and diesel traffic, I observed elevated BP in participants living ≤ 50 m from the road, and in other categories ≤ 500 m, compared to the reference > 500 m, but these findings were not statistically significant. The ORs for BPLM intake were elevated for all categories compared to the reference (> 500 m); this association was statistically significant for the categories ≤ 50 m and > 50 and ≤ 100 m compared to > 500 m. There were positive, yet imprecise, relationships of distance > 100 and ≤ 200 m, > 200 and ≤ 300 m with ORs for hypertension.

Table 19. The cross-sectional associations of distance to road with high all-type traffic with BP, hypertension, and BPLM intake.

Exposure	N	SBP (mmHg)	DBP (mmHg)	PP (mmHg)	Hypertension (OR)	BPLM (OR)
Distance to road with high all-type traffic (continuous)						
(per 100 m)	4,584	0.00 (-0.07, 0.08)	0.00 (-0.04, 0.05)	0.00 (-0.05, 0.05)	0.99 (0.98, 1.00)	0.99 (0.98, 1.00)
$Truncated_{400\ m}$ (per 100 m)	4,584	0.05 (-0.48, 0.59)	0.01 (-0.28, 0.30)	0.05 (-0.32, 0.42)	0.97 (0.91, 1.04)	0.92 (0.87, 0.98)
Distance to road with high all-type traffic (in categories)						
> 500 m	2,885	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
≤ 50 m	137	-0.38 (-3.80, 3.04)	0.19 (-1.66, 2.04)	-0.66 (-3.04, 1.71)	1.01 (0.67, 1.50)	1.34 (0.89, 2.00)
> 50 and ≤ 100 m	187	-0.61 (-3.47, 2.25)	-1.17 (-2.72, 0.38)	0.55 (-1.44, 2.55)	1.13 (0.81, 1.59)	1.60 (1.14, 2.23)
> 100 and ≤ 200 m	384	0.13 (-1.92, 2.17)	0.24 (-0.86, 1.35)	-0.13 (-1.55, 1.29)	1.08 (0.84, 1.37)	1.08 (0.85, 1.38)
> 200 and ≤ 300 m	368	-0.14 (-2.21, 1.93)	0.09 (-1.03, 1.20)	-0.16 (-1.60, 1.28)	1.16 (0.91, 1.49)	1.23 (0.96, 1.57)
> 300 and ≤ 500 m	623	-0.34 (-1.99, 1.31)	-0.69 (-1.58, 0.20)	0.39 (-0.75, 1.54)	1.01 (0.83, 1.23)	1.11 (0.91, 1.35)
Distance to road with high heavy-duty traffic (in categories)						
> 500 m	2,884	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
≤ 50 m	118	2.28 (-1.34, 5.90)	1.22 (-0.74, 3.18)	1.01 (-1.51, 3.53)	1.52 (0.98, 2.35)	1.91 (1.26, 2.91)
> 50 and ≤ 100 m	214	0.48 (-2.20, 3.16)	-0.68 (-2.13, 0.77)	1.10 (-0.77, 2.96)	1.00 (0.73, 1.38)	1.37 (1.00, 1.88)
> 100 and ≤ 200 m	359	0.37 (-1.75, 2.48)	-0.06 (-1.20, 1.09)	0.41 (-1.06, 1.88)	1.10 (0.85, 1.41)	1.10 (0.85, 1.42)
> 200 and ≤ 300 m	380	0.32 (-1.72, 2.36)	0.29 (-0.81, 1.39)	0.05 (-1.37, 1.47)	1.12 (0.88, 1.43)	1.12 (0.88, 1.44)
> 300 and ≤ 500 m	629	0.31 (-1.33, 1.95)	-0.47 (-1.36, 0.41)	0.82 (-0.32, 1.96)	0.98 (0.81, 1.19)	1.13 (0.93, 1.37)
Distance to road with high diesel traffic (in categories)						
> 500 m	2,920	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
≤ 50 m	133	0.93 (-2.51, 4.37)	0.72 (-1.14, 2.58)	0.17 (-2.23, 2.56)	1.10 (0.73, 1.66)	1.41 (0.94, 2.11)
> 50 and ≤ 100 m	184	-0.25 (-3.12, 2.63)	-1.07 (-2.62, 0.49)	0.81 (-1.19, 2.81)	1.17 (0.83, 1.64)	1.56 (1.12, 2.19)
> 100 and ≤ 200 m	357	0.24 (-1.87, 2.35)	0.50 (-0.64, 1.64)	-0.25 (-1.72, 1.22)	1.05 (0.82, 1.35)	1.00 (0.78, 1.29)
> 200 and ≤ 300 m	359	0.78 (-1.31, 2.86)	0.59 (-0.53, 1.72)	0.26 (-1.19, 1.71)	1.15 (0.90, 1.47)	1.09 (0.85, 1.41)
> 300 and ≤ 500 m	631	-0.46 (-2.10, 1.18)	-0.67 (-1.55, 0.22)	0.26 (-0.88, 1.40)	0.96 (0.79, 1.17)	1.02 (0.84, 1.24)

Adjusted for time trend, 365-day mean $PM_{2.5}$, short-term mean $PM_{2.5}$, L_{night} traffic, L_{den} tram, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

Neighborhood-level SES (Table 20)

I investigated the association of neighborhood-level SES with BP, adjusting for exposure to background air pollution, road traffic noise, distance to major road, and relevant confounders. Higher residential density and unemployment rate were associated with lower BP. In addition, unemployment rate in the neighborhood was weakly related to elevated OR for BPLM intake. A higher proportion of elderly residents was weakly linked to lower PP (Table 20).

Table 20. The cross-sectional associations of neighborhood-level SES indicators with BP in the study population.

Area-level factor (increment)	**BP change, mmHg (95% CI)**			**Hypertension**	**Medication**
	SBP	***DBP***	***PP***	***OR (95% CI)***	***OR (95% CI)***
Unemployment rate (per 10%)	-1.6 (-3.3, 0.1)	-0.9 (-1.9,0 .1)	-0.7 (-1.9, 0.5)	1.09 (0.88, 1.33)	1.20 (0.97, 1.48)
Social welfare rate (per 10%)	-1.7 (-3.8, 0.5)	-0.9 (-2.1, 0.4)	-0.7 (-2.2, 0.8)	1.18 (0.91, 1.53)	1.14 (0.87, 1.49)
Percentage of elderly residents (per 10%)	-0.7 (-2.4, 1.0)	0.4 (-0.6, 1.3)	-1.2 (-2.3, 0.0)	0.92 (0.75, 1.12)	0.92 (0.74, 1.13)
Population density (per 1,000 residents/km^2)	-0.3 (-0.6, 0.0)	0.0 (-0.2, 0.2)	-0.3 (-0.5, -0.1)	1.01 (0.97, 1.04)	1.00 (0.97, 1.04)
Residential turnover (per 10%)	-0.1 (-0.2, 0.1)	0.0 (-0.1, 0.1)	-0.1 (-0.1, 0.0)	1.00 (0.99, 1.02)	1.01 (0.99, 1.02)

N = 4,584. Adjusted for time trend, 365-day mean $PM_{2.5}$, short-term mean $PM_{2.5}$, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

4.1.10. Analysis of effect modification

The following factors modified the association of certain pollutants with BP: sex, CHD, T2DM, active inflammation, alcohol consumption, educational level, the city of residence, season, and distance to major road (Figures 16 and 17). In particular, a stronger positive association of $PM_{2.5}$ with BP was observed in summer ($p_{interaction} < 0.05$). No effects of $PM_{2.5}$ on BP were detected for participants living < 100 m from the major road ($p_{interaction} < 0.05$), participants with T2DM ($p_{interaction} < 0.1$), and those consuming > 6 drinks per week ($p_{interaction} < 0.1$). No associations of PM_{10} with SBP were found for participants living < 100 m from the major road ($p_{interaction} < 0.1$) and among participants with low education status. Positive associations of PN with SBP were detected in women ($p_{interaction} < 0.1$), participants with T2DM ($p_{interaction} < 0.05$), participants in Mülheim ($p_{interaction} < 0.1$) and among participants living ≥ 100 m from a major road ($p_{interaction} < 0.05$). In addition, a positive association of PN with DBP was found in participants with higher or middle educational status ($p_{interaction} < 0.1$).

The associations of O_3 with BP were stronger in women ($p_{interaction} < 0.05$) and moderate or no alcohol drinkers ($p_{interaction} < 0.05$). No associations of NO_2 with BP were detected in participants with T2DM ($p_{interaction} < 0.05$), heavy drinkers ($p_{interaction} < 0.05$), those with acute inflammation (defined as CRP > 3 mg/L, $p_{interaction} < 0.05$), and participants residing in Essen ($p_{interaction} < 0.05$). There were more precise positive associations of SO_2 with BP in no or moderate drinkers ($p < 0.05$) and in participants living in Bochum. Weak inverse relationships of CO with BP were observed in participants with T2DM and in those residing in Mülheim ($p_{interaction} < 0.05$ and < 0.1, respectively). The associations of NH_3 with BP were more positive in participants with CHD, among those living in Essen and Bochum, and during summer ($p_{interaction} < 0.05$, < 0.1, and < 0.05, respectively).

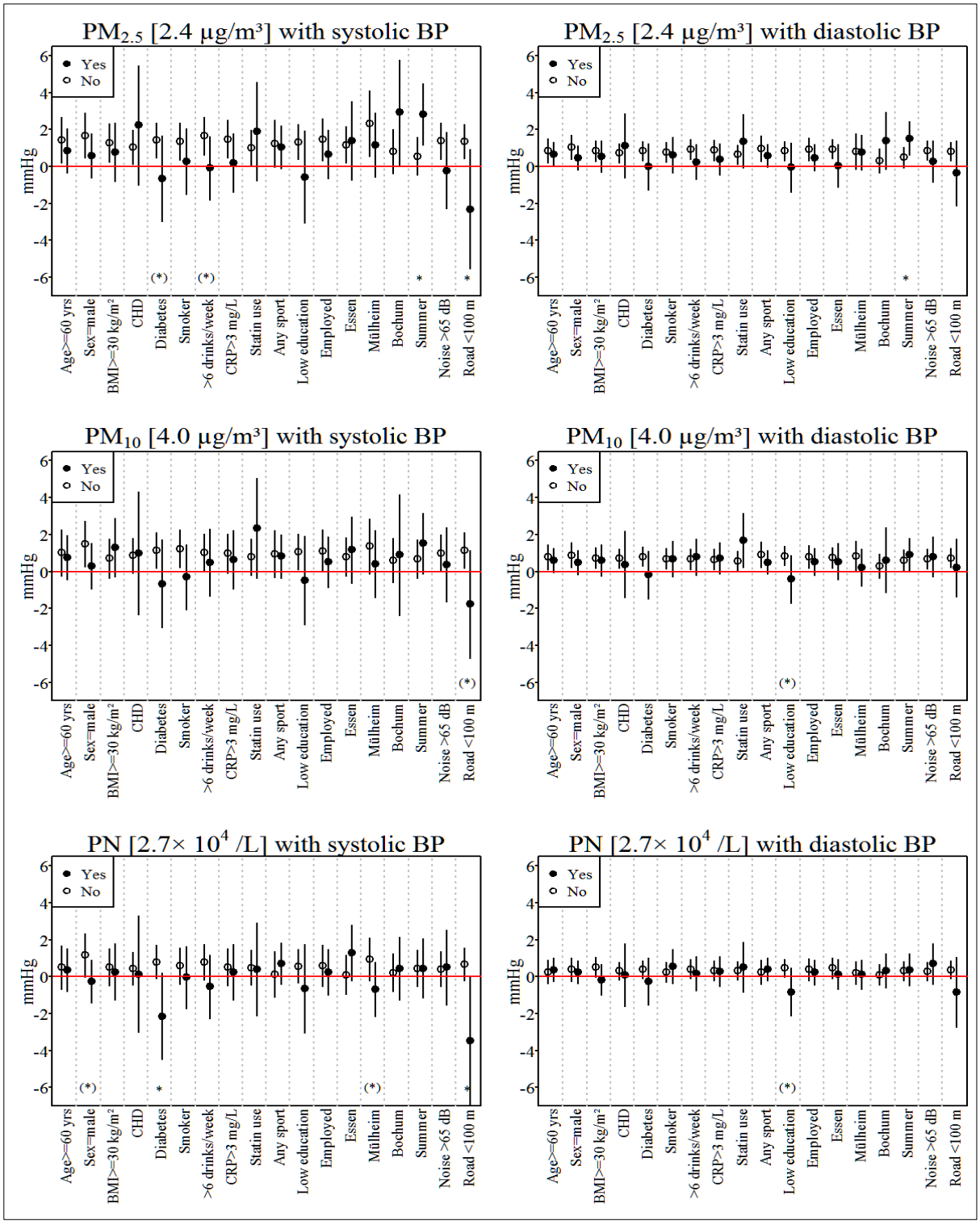


Figure 16. Effect modification analysis: the associations of $PM_{2.5}$, PM_{10} and PN with SBP (mmHg change with 95% CI) in subgroups, using product terms exposure×effect modifier.

*Legend: N = 4,584. The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term mean $PM_{2.5}$, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity. * = $p_{interaction} < 0.05$; (*) = $p_{interaction} < 0.1$.*

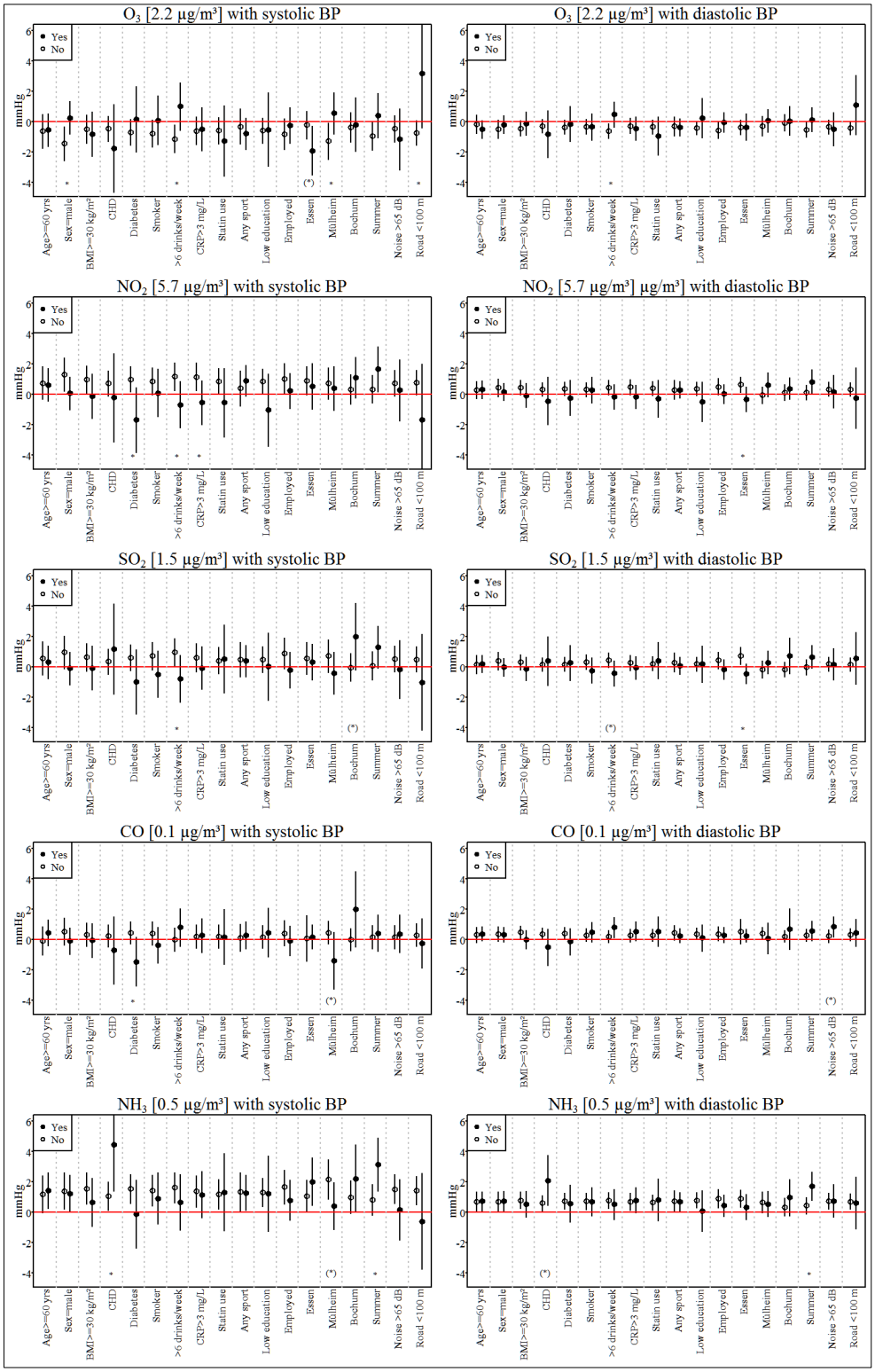


Figure 17. Effect modification analysis: the associations of gaseous pollutants with SBP (mmHg change with 95% CI) in subgroups, using product terms exposure×effect modifier.

*Legend: N = 4,584. The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term mean $PM_{2.5}$, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity. * = $p_{interaction} < 0.05$; (*) = $p_{interaction} < 0.1$.*

4.1.11. Correction for medication effect (Table 21)

I employed the following methods to correct for the intake of BPLM among study participants: (i) adjusted for BPLM in a multivariable regression model; (ii) added a fixed constant (5, 10, 15 mmHg) to the BP values of participants taking BPLM; (iii) calculated a right-censored regression model (censoring on BPLM intake); and (iv) estimated results in medicated and non-medicated participants separately, using an interaction term, and tested for the significance of interaction.

Table 21. The cross-sectional associations of $PM_{2.5}$, PN, NO_2, NH_3 with SBP, estimated using strategies to correct for BPLM intake.

Correction method	**Exposure**			
	$PM_{2.5}$ (2.4 µg/m³)	**PN (2.7×10^4/L)**	**NO_2 (5.7 µg/m³)**	**NH_3 (0.5 µg/m³)**
No correction	1.1 (0.2, 2.0)	0.4 (-0.4, 1.3)	0.7 (-0.1, 1.5)	1.3 (0.4, 2.2)
+ BPLM (covariate)	1.1 (0.2, 2.1)	0.4 (-0.4, 1.3)	0.7 (-0.1, 1.5)	1.3 (0.4, 2.2)
Fixed addition (only in medicated)				
+ 5 mmHg	1.1 (0.2, 2.0)	0.4 (-0.5, 1.3)	0.7 (-0.1, 1.5)	1.3 (0.4, 2.2)
+ 10 mmHg	1.0 (0.1, 2.0)	0.4 (-0.5, 1.3)	0.7 (-0.1, 1.6)	1.2 (0.3, 2.2)
+ 15 mmHg	1.0 (0.0, 2.0)	0.4 (-0.5, 1.4)	0.8 (-0.1, 1.6)	1.2 (0.2, 2.1)
Censored regression	0.9 (-0.3, 2.0)	0.6 (-0.5, 1.7)	1.0 (0.0, 2.0)	0.9 (-0.2, 2.0)
Estimate in subgroups by BPLM intake				
No BPLM intake	0.9 (-0.2, 2.0)	0.5 (-0.5, 1.6)	0.9 (0.0, 1.9)	1.1 (0.1, 2.2)
BPLM intake	1.6 (0.2, 3.0)	0.3 (-1.1, 1.6)	0.2 (-1.1, 1.5)	1.7 (0.3, 3.1)
$p_{interaction}$	0.373	0.737	0.358	0.507

N = 4,584. Results are presented as mmHg change in SBP with 95% CI. Adjusted for time trend, short-term mean air pollution concentration, L_{night} traffic, L_{den} tram, distance to major road, age, sex, BMI, WHR, blood lipids, smoking status, ETS, physical activity, education, and economic activity.

The results with different pollutants and SBP are presented in Table 21. The estimates did not change after adding BPLM as a covariate or fixed addition. The estimates with censored regression, compared to no correction, were lower and less precise with $PM_{2.5}$ and NH_3, higher with PN, and higher and more precise with NO_2.With SO_2 and CO, censored regression produced a higher and more precise effect estimate (not shown). I did not observe any statistically significant effect modifications with BPLM intake. The effect was stronger in medicated participants than in non-medicated participants (with up to 2-fold higher effect estimates) with $PM_{2.5}$, PM_{10} (not shown), O_3 (not shown), SO_2 (not shown) and NH_3 (Table 21). In contrast,

the association of NO_2 in non-medicated participants was stronger than in medicated participants.

4.2. Cross-sectional and longitudinal analysis with BP and hypertension at follow-up

4.2.1. Description of the study population at follow-up (Table 22)

Table 22. Description of the study population at follow-up.

Variable (unit) statistics	Description	N missing
Prevalent binary outcomes		
Hypertension, n (%)	2,157 (66.6%)	–
BPLM intake, n (%)	1,595 (49.2%)	–
Self-reported hypertension[1], n (%)	1,434 (45.4%)	80
Self-reported BPLM intake[1], n (%)	1,391 (43.3%)	25
Incident binary outcomes[2]		
Hypertension, n (%)	520 (36.2%)	1,802
BPLM intake, n (%)	556 (26.1%)	1,108
Self-reported hypertension, n (%)	382 (21.3%)	1,449
Self-reported BPLM intake, n (%)	515 (23.3%)	1,028
Personal characteristics		
Age (years), Mean ± SD	64.6 ± 7.6	–
Sex (male), n (%)	1,630 (50.3%)	–
CHD, n (%)	263 (8.1%)	–
T2DM, n (%)	614 (19.0%)	–
BMI (kg/m²), Mean ± SD	28.3 ± 4.8	–
LDL:HDL ratio, Mean ± SD	2.3 ± 0.8	–
Smoking, n (%)		–
Current	552 (17.0%)	–
Former	1,301 (40.2%)	–
Never	1,387 (42.8%)	–
ETS exposure, n (%)	799 (24.7%)	–
Alcohol (drinks/week), Mean ± SD	7.1 ± 11.2	–
No sport, n (%)	1,379 (42.6%)	–
Education, n (%)		–
< 10 years	289 (8.9%)	–
11–17 years	1,817 (56.1%)	–
≥ 18 years	1,134 (35.0%)	–

N = 3,240.

[1]Self-reported hypertension and self-reported BPLM intake were included to the sensitivity analyses only and therefore had missing values. [2]For incident outcomes, the prevalent cases were set to missing and were excluded from the analysis.

There were 520 incident cases of hypertension (36.2%) and 556 new cases of BPLM intake (26.1%). These values are higher than the estimated 5-year incidence of 20% for men and

women aged 65 years (Chobanian et al. 2003). The incidence of self-reported hypertension was 21.3%, and the incidence of self-reported BPLM intake was 23.3%. Mean follow-up time was 5.1 years (SD 0.4 years). On average, the age at follow-up examination was 5 years higher (mean 64.6 years, SD 7.6 years). The gender distribution remained equal to those at the baseline. The proportion of subjects with CHD was 8.1%, slightly higher than at baseline. The proportion of diabetics increased almost by half (19.1%). The mean BMI increased slightly to 28.3 kg/m² (SD 4.8 kg/m²). The LDL : HDL ratio decreased at the follow-up (mean 2.3, SD 0.8). The percentage of current smokers dropped to 17%, whereas the proportion of ex-smokers increased correspondingly. The mean alcohol consumption decreased to 7.1 drinks per week (SD 11.2 drinks per week). The proportion of participants not practicing sport decreased to (42.6%). The ratio of subgroups by education level remained very similar to baseline.

Table 23. Difference between hypertension and BPLM intake, included as main outcomes according to the study definition, and self-reported hypertension and BPLM intake.

Prevalent outcomes			
Hypertension (JNC7 definition)	***Self-reported hypertension = No***	***Self-reported hypertension = Yes***	***Self-reported hypertension = Missing***
No	971 (89.7%)	72 (6.6%)	40 (3.7%)
Yes	755 (35.0%)	1,362 (63.1%)	40 (1.9%)
BPLM intake (ATC codes)	***Self-reported BPLM intake = No***	***Self-reported BPLM intake = Yes***	***Self-reported BPLM intake = Missing***
No	1,555 (94.5%)	86 (5.2%)	4 (0.2%)
Yes	269 (16.9%)	1,305 (81.8%)	21 (1.3%)
Incident outcomes			
Hypertension (JNC7 definition)	***Self-reported hypertension = No***	***Self-reported hypertension = Yes***	***Self-reported hypertension = Missing***
No	791 (86.2%)	26 (2.8%)	101 (11.0%)
Yes	266 (51.2%)	138 (26.5%)	116 (22.3%%)
BPLM intake (ATC codes)	***Self-reported BPLM intake = No***	***Self-reported BPLM intake = Yes***	***Self-reported BPLM intake = Missing***
No	1,505 (95.5%)	48 (3.0%)	23 (1.5%)
Yes	114 (20.5%)	386 (69.4%)	56 (10.1%)

Agreement of prevalent hypertension by study definition and self-report was 73.8%, Cohen's kappa was 0.49, corresponding to fair to good agreement. Agreement of incident hypertension by the two definitions was 76.1%, Cohen's kappa was 0.36 (poor agreement). Agreement of prevalent BPLM intake with study definition and self-report was 89.0%, Cohen's kappa was

0.78. Agreement of incident BPLM intake with these definitions was 92.1%, Cohen's kappa was 0.78 (excellent agreement).

4.2.2. Progression of BP at follow-up

In the follow-up analysis sample, SBP increased by 0.3 mmHg (SD 3.8 mmHg), DBP decreased by 0.4 mmHg (SD 2.1 mmHg), and PP increased by 0.8 mmHg (SD 2.6 mmHg) (Table 23). The decrease in DBP could be due to the stiffening of the arteries that progresses with age (Chobanian et al. 2003). In the subgroup of participants taking BPLM (N = 1,595), SBP decreased by 0.3 mmHg (SD 4.4 mmHg), DBP decreased by 0.8 (SD 2.3 mmHg), and PP increased by 0.5 mmHg (SD 2.9 mmHg). Approximately half of the study subjects used BPLM. Among non-medicated participants (N = 1,645), SBP increased by 1.0 mmHg (SD 3.0 mmHg) and DBP did not change (0.0 mmHg, SD 1.8 mmHg). On average, men had higher arterial BP at both baseline and follow-up compared to women. The increase in SBP in women was higher than that in men, and the decrease in DBP was lower in women.

I stratified the study participants by hypertension status at baseline and follow-up according to the JNC7 definition (Chobanian et al. 2003). Approximately half of the analysis sample participants were hypertensive at baseline and follow-up (1,637). The SBP and PP values were the highest in this group (Table 24). In the group with incident hypertension at follow-up (N = 520), the increase from baseline to follow-up was the highest. The lowest values of BP were observed in the group with normal BP values at baseline and follow-up (N = 918). The smallest group (N = 165) included subjects who had hypertension at baseline and no hypertension at follow-up. Their BP values at baseline were almost as high as in the group with prevalent hypertension and at follow-up and were similar to BP values in the normotensive group.

Table 24. Progression of BP from baseline to follow-up in the entire analysis sample and in subgroups.

Group	SBP, mmHg			DBP, mmHg			PP, mmHg		
	BL	***FU***	***FU-BL***[5]	***BL***	***FU***	***FU-BL***	***BL***	***FU***	***FU-BL***
All (N = 3,240)	132.9 ± 20.2	134.6 ± 19.8	0.3 ± 3.8	81.5 ± 10.6	79.2 ± 10.5	-0.4 ± 2.1	51.4 ± 14.2	55.4 ± 14.7	0.8 ± 2.6
By BPLM intake									
Yes (N = 1,645)	138.7 ± 20.6	137.2 ± 20.4	-0.3 ± 4.4	83.5 ± 11.0	79.2 ± 11.0	-0.8 ± 2.3	55.2 ± 14.9	58.0 ± 15.5	0.5 ± 2.9
No (N = 1,595)	126.8 ± 17.8	131.9 ± 18.6	1.0 ± 3.0	79.4 ± 9.7	79.3 ± 9.9	0.0 ± 1.8	47.4 ± 12.3	52.7 ± 13.2	1.0 ± 2.2
By sex									
Men (N = 1,630)	137.4 ± 19.0	138.2 ± 19.1	0.2 ± 3.8	83.8 ± 10.3	80.9 ± 10.6	-0.6 ± 2.1	53.6 ± 13.8	57.3 ± 14.7	0.7 ± 2.6
Women (N = 1,610)	128.4 ± 20.4	131.0 ± 19.8	0.5 ± 3.9	79.2 ± 10.5	77.6 ± 10.1	-0.3 ± 2.1	49.3 ± 14.3	53.4 ± 14.5	0.8 ± 2.6
By hypertension									
BL–, FU–[1] (N = 918)	117.2 ± 12.0	120.7 ± 11.2	0.7 ± 2.3	75.0 ± 7.3	74.7 ± 7.3	-0.1 ± 1.4	42.2 ± 8.5	46.1 ± 8.4	0.8 ± 1.7
BL+, FU+[2] (N = 1,637)	143.1 ± 20.3	141.7 ± 20.6	-0.3 ± 4.3	85.5 ± 11.2	81.2 ± 11.4	-0.8 ± 2.3	57.7 ± 15.1	60.5 ± 15.8	0.5 ± 2.9
BL–, FU+[3](N = 520)	126.0 ± 9.8	139.3 ± 17.4	2.6 ± 3.3	78.9 ± 6.4	82.0 ± 10.1	0.6 ± 1.9	47.1 ± 8.6	57.3 ± 12.9	2.0 ± 2.3
BL+, FU–[4] (N = 165)	141.5 ± 15.0	127.3 ± 9.9	-2.8 ± 2.8	86.8 ± 10.4	76.3 ± 7.7	-2.0 ± 2.0	54.7 ± 13.0	51.0 ± 9.5	-0.7 ± 2.3

N = 3,240. BL = baseline; FU = follow-up.

[1]No hypertension at baseline and follow-up measurement. [2]Hypertension both at baseline and follow-up measurements. [3]No hypertension at baseline, incident hypertension at follow-up measurement. [4]Hypertension at baseline, no hypertension at follow-up measurement. [5]Progression of BP, calculated as BP (follow-up measurement) minus BP (baseline measurement) per year of follow-up.

I stratified participants in 5-year age groups (age at baseline) and calculated the mean SBP and DBP at baseline and follow-up (Figure 18). In the entire group, SBP increased from baseline to follow-up in all age groups except for participants aged 65–69 years. Among participants not taking BPLM at follow-up, SBP increased in all age groups. In the non-medicated participants aged 65–69 years, this increase was smaller. Among participants taking BPLM at follow-up, mean SBP decreased in all age groups except for those aged 50–54 years.

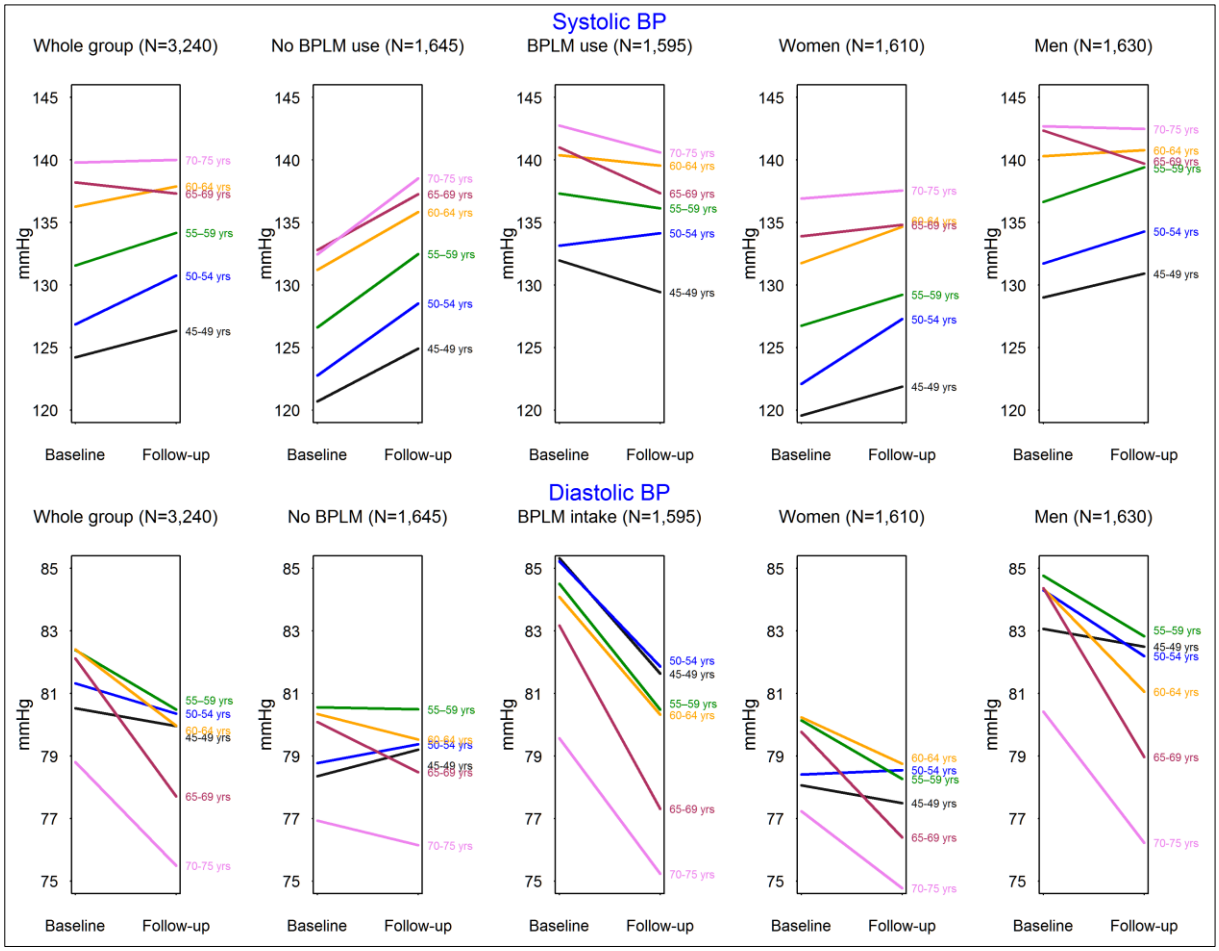


Figure 18. Changes in BP from baseline to follow-up in groups by age at baseline.

Legend: N = 3,200 (. Mean BP values per 5-year age group at baseline and follow-up measurements are presented.

The strongest decrease in BP was observed in the age groups 65–69 and 45–49 years. In women (regardless of medication status), SBP increased; in men, SBP increased in all age groups but two: 65–69 and 70–75 years. DBP decreased in all age groups in the entire cohort. Among participants not taking BPLM, DBP decreased in participants aged 55–59, 60–64, and 70–75 years and increased in the other age groups. In medicated participants, I observed a steep decrease in DBP in all age groups. In women, DBP increased in the 50-54-year age group and decreased in the other age groups. In men, DBP decreased in all age groups.

4.2.3. Description of air pollution exposure at follow-up

The individual 365-day mean values were quite close to the grid-specific mean during 2006–2008 (Table 25). During the 365 days before the follow-up examination, the mean concentrations were as follows: $PM_{2.5}$, 15.6 µg/m³; PM_{10}, 18.1 µg/m³; O_3, 37.6 µg/m³; NO_2, 38.6 µg/m³; SO_2, 7.4 µg/m³; CO 0.3, µg/m³; and NH_3, 2.8 µg/m³. The measured mean values of PM_{10} in 2006 (available from the background stations in Ruhr area) were as follows: BUCH

(Duisburg) 26 µg/m³ and STYR (Mülheim) 27 µg/m³ (LANUV 2006a). The measured mean concentrations of gaseous pollutants for year 2006 in the Ruhr area were as follows: 31 µg/m³ for NO_2, 13 µg/m³ for NO, and 40 µg/m³ for O_3 (LANUV 2006b). The modeled EURAD values were higher for NO_2 and O_3 and lower for NO.

Table 25. Long-term concentrations of particulate air pollution in the study population at follow-up.

Exposure	(unit)	365-day mean			Mean of 2006-2008		
		Mean ± SD	***Min – Max***	***IQR***	***Mean ± SD***	***Min – Max***	***IQR***
$PM_{2.5}$	(µg/m³)	15.6 ± 1.4	12.7 – 19.8	2.1	15.5 ± 1.3	13.3 – 19.1	2.0
PM_{10}	(µg/m³)	18.1 ± 1.8	14.7 – 23.5	2.7	19.5 ± 2.7	15.8 – 28.2	3.8
PM_{coarse}	(µg/m³)	2.4 ± 0.5	1.7 – 4.3	0.7	4.0 ± 2.0	1.9 – 12.4	2.0
PM_1	(µg/m³)	11.0 ± 1.1	8.6 – 14.5	1.6	10.9 ± 1.0	9.0 – 13.8	1.6
PN	($\times 10^4$/L)	7.7 ± 1.7	4.5 – 15.8	2.4	7.7 ± 1.7	4.6 – 15.9	2.4
O_3	(µg/m³)	37.6 ± 2.0	31.9 – 43.5	2.7	37.0 ± 1.5	32.5 – 40.1	2.1
NO_2	(µg/m³)	38.6 ± 4.7	28.6 – 52.6	7.1	38.4 ± 4.2	26.5 – 48.7	5.4
NO	(µg/m³)	7.7 ± 2.2	3.4 – 18.0	3.2	11.5 ± 5.6	4.5 – 36.5	5.7
SO_2	(µg/m³)	7.4 ± 1.1	5.3 – 14.7	1.6	7.4 ± 1.0	5.6 – 15.5	1.5
CO	(µg/m³)	0.3 ± 0.0	0.2 – 0.4	0.0	0.3 ± 0.0	0.2 – 0.4	0.0
NH_3	(µg/m³)	2.8 ± 0.3	2.0 – 3.9	0.5	2.9 ± 0.3	2.3 – 3.8	0.5
N = 3,240							

Correlations of exposure concentrations at follow-up

The correlations of particulate matter of different sizes were higher at follow-up than at baseline (Table 26). For example, the correlation of $PM_{2.5}$ with PM_{coarse} was 0.881 (Pearson's ρ), with PM_{10} 0.993, with PM_1 0.994, and with PN 0.733. The correlations between gaseous pollutants were also higher than at baseline. O_3 was highly inversely correlated to PM metrics and to other gaseous compounds. The correlations of fine particles with long-term gaseous pollutants were also moderate to high, except for carbon monoxide with PM_1. The short-term concentration of $PM_{2.5}$ did not correlate with long-term exposures.

Table 26. Correlation matrix of exposures and environmental variables in the study population at follow-up.

Variable	**$PM_{2.5}$**	**PM_{10}**	**PM_{coarse}**	**PM_1**	**PN**	**O_3**	**NO_2**	**NO**	**SO_2**	**CO**	**NH_3**	**$PM_{2.5\ (short)}$**	**Humidity**	**$L_{den\ (traffic)}$**	**$L_{night\ (traffic)}$**	**$L_{den\ (tram)}$**
PM_{10}[1]	0.993*[6]	1														
PM_{coarse}[1]	0.881*	0.932*	1													
PM_1[1]	0.994*	0.978*	0.842*	1												
PN[1]	0.733*	0.707*	0.564*	0.770*	1											
O_3[1]	-0.787*	-0.786*	-0.711*	-0.770*	-0.645*	1										
NO_2[1]	0.901*	0.882*	0.744*	0.912*	0.858*	-0.770*	1									
NO[1]	0.839*	0.804*	0.626*	0.853*	0.842*	-0.755*	0.965*	1								
SO_2[1]	0.771*	0.719*	0.498*	0.799*	0.793*	-0.644*	0.828*	0.821*	1							
CO[1]	0.567*	0.496*	0.237*	0.613*	0.777*	-0.485*	0.752*	0.787*	0.918*	1						
NH_3[1]	0.913*	0.884*	0.717*	0.916*	0.712*	-0.760*	0.883*	0.852*	0.884*	0.740*	1					
$PM_{2.5\ (sh.)}$[2]	-0.005	-0.008	-0.018	0.002	-0.017	0.008	-0.011	-0.016	-0.021	-0.010	-0.011	1				
Humidity	-0.037*	-0.049*	-0.083*	-0.022	0.017	0.150*	0.030	0.041*	0.035*	0.087*	-0.035*	-0.200*	1			
$L_{den\ (traf.)}$	0.103*	0.088*	0.035*	0.114*	0.218*	-0.073*	0.165*	0.169*	0.140*	0.193*	0.126*	-0.002	0.004	1		
$L_{night\ (traf.)}$	0.136*	0.125*	0.080*	0.145*	0.241*	-0.099*	0.199*	0.198*	0.145*	0.193*	0.152*	-0.004	0.002	0.994*	1	
$L_{den\ (tram)}$	0.054*	0.005	-0.146*	0.085*	0.251*	-0.026	0.102*	0.112*	0.378*	0.435*	0.192*	-0.008	0.025	0.246*	0.209*	1
Road[3]	-0.166*	-0.154*	-0.104*	-0.174*	-0.305*	0.147*	-0.278*	-0.275*	-0.166*	-0.250*	-0.185*	0.033	-0.011	-0.382*	-0.414*	-0.083*
$BL_{365}\times FU_{365}$[4]	0.759*	0.733*	0.309*	0.755*	0.978*	0.634*	0.697*	0.479*	0.662*	0.627*	0.400*	–	–	–	–	–
$BL_{grid}\times FU_{grid}$[5]	0.998*	0.917*	0.845*	0.998*	0.990*	0.964*	0.917*	0.759*	0.966*	0.785*	0.991*	–	–	–	–	–

N = 3,240. [1]Long-term air pollution = individual 365-day mean. [2]$PM_{2.5}$ short-term (lag 0–6), basic effect (365-day mean) subtracted.[3]Distance to major road (upper quartile of diesel vehicle density). [4]Correlation between individual 365-day mean concentrations at baseline and follow-up. [5]Correlation of long-term grid cell means at baseline and follow-up. [6] = $p < 0.05$.*

I observed collinearity in the long-term air pollutant concentrations at follow-up with baseline concentrations of the respective pollutants (Table 26). With 365-day mean concentrations, all pollutants except PM_{coarse} and NH_3 showed moderate to high ($\rho > 0.6$) correlation of follow-up and baseline concentration. With long-term grid cell means, the correlation of follow-up concentration with baseline concentration was high ($\rho > 0.7$) to very high ($\rho > 0.9$) for all pollutants. Neither tram nor road traffic noise correlated with PM or PN. I detected weak positive correlations between road traffic and tram noise and PN. Distance to major road correlated weakly and inversely with air pollutants. Similarly to baseline, tram and traffic noise were weakly correlated with each other (ρ 0.246), and road traffic noise correlated moderately and inversely with distance to a major road (ρ -0.382).

Changes in exposure during the follow-up period

Table 27. Changes in exposure during follow-up, calculated as differences in the long-term grid-specific mean concentrations at follow-up (2006–2008) and at baseline (2000–2003) per year of follow-up.

Exposure (unit)		**Description**		**Correlation with long-term exposure[1]**			
		Mean ± SD	***Min – Max***	$\times BL_{365}$[2]	$\times FU_{365}$	$\times BL_{grid}$	$\times FU_{grid}$
$PM_{2.5}$	(µg/m³ per year)	-0.31 ± 0.04	-0.68 – 0.03	-0.85	-0.76	-0.85	-0.82
PM_{10}	(µg/m³ per year)	-0.30 ± 0.21	-1.11 – 0.86	-0.07	-0.25	-0.06	0.35
PM_{coarse}	(µg/m³ per year)	0.00 ± 0.04	-0.10 – 0.25	0.15	-0.22	0.20	0.68
PM_1	(µg/m³ per year)	-0.21 ± 0.03	-0.54 – 0.08	-0.83	-0.74	-0.83	-0.80
PN	($\times 10^4$/L per year)	-0.22 ± 0.07	-0.88 – 0.24	-0.72	-0.63	-0.75	-0.65
O_3	(µg/m³ per year)	0.45 ± 0.08	-0.17 – 0.85	0.18	0.28	0.11	0.36
NO_2	(µg/m³ per year)	-0.56 ± 0.33	-1.81 – 1.18	-0.14	0.15	-0.14	0.26
NO	(µg/m³ per year)	-0.31 ± 0.71	-1.70 – 3.10	-0.02	0.01	-0.02	0.64
SO_2	(µg/m³ per year)	-0.33 ± 0.06	-0.76 – 0.00	-0.50	-0.14	-0.45	-0.21
CO	(µg/m³ per year)	-0.01 ± 0.01	-0.05 – 0.00	-0.90	-0.41	-0.90	-0.45
NH_3	(µg/m³ per year)	0.03 ± 0.01	-0.04 – 0.08	0.46	0.58	0.56	0.67

N = 3,240.
[1]$p < 0.05$ for all correlation coefficients. [2]BL = baseline measurement; FU = follow-up measurement; 365 = 365-day mean exposure concentration; grid = long-term grid-specific mean exposure concentration (mean of 2000–2003 at baseline and of 2006–2008 at follow-up).

I calculated the changes in long-term exposure using grid-specific means of the whole baseline (2000–2003) and follow-up (2006–2008) periods. The average concentrations decreased

for all pollutants except for PM_{coarse} (no change), O_3 and NH_3 (both increased). The average decreases in $PM_{2.5}$, PM_{10}, NO and SO_2 were 0.3 μg/m³ per year (Table 27). The decrease in NO_2 was 0.6 μg/m³ per year. The concentration of O_3 increased by 0.45 μg/m³ per year, and the concentration of NH_3 increased by 0.03 μg/m³ per year on average.

I calculated the correlations of change during follow-up with the follow-up and baseline exposure values (Table 27). The changes in $PM_{2.5}$ were highly inversely correlated with baseline and follow-up concentrations (Pearson's ρ -0.85 to -0.76). In contrast, the changes in PM_{10} correlated weakly with follow-up concentrations as follows: inversely with the 365-day mean (ρ -0.25) and positively with the long-term grid cell mean (0.35); no correlation was detected with the baseline value. The findings with PM_{coarse} were similar to PM_{10}, except the correlation with the follow-up grid cell mean was higher (ρ 0.68). The correlations of change in PM_1 and PN with their long-term concentrations were similar to $PM_{2.5}$. The changes in O_3 during the follow-up period correlated weakly and positively with the baseline and follow-up values. NO_2 and NO were positively correlated with their follow-up grid-specific mean concentration (ρ 0.26 and 0.64, respectively) but not with other long-term concentrations. The changes in SO_2 and CO were negatively correlated with baseline and follow-up concentrations. The change in NH_3 was moderately positively correlated with baseline and follow-up concentrations.

4.2.4. Cross-sectional associations of air pollution at follow-up with the study outcomes

Air pollution at follow-up and BP (Table 28)

The observed cross-sectional associations of air pollution with BP at follow-up were stronger and more consistent across pollutants compared to the cross-sectional analysis with baseline data (Table 28). The 365-day mean concentrations of PM_1 to PM_{10} at follow-up were positively associated with SBP and DBP in the cross-sectional analysis; the same pollutants were also positively related to PP. When compared per IQR, the estimates were quite similar for different PM metrics. For example, per increase in $PM_{2.5}$ by 2.1 μg/m³, I detected increases in SBP of 1.2 mmHg (95% CI: 0.2 to 2.2), in DBP of 0.7 mmHg (95% CI: 0.2 to 1.2), and in PP of 0.4 mmHg (95% CI: -0.3 to 1.2). The results with PN and BP were inconclusive. Analysis with 365-day NH_3 as a main exposure yielded results quite similar to those observed with PM: positive associations with SBP and DBP and a positive relationship with PP. Follow-up concentrations of NO_2, NO, SO_2, and CO were positively associated with DBP and more weakly positively related to SBP.

Table 28. The cross-sectional associations of 365-day mean air pollution at follow-up with BP at follow-up among participants with no change in address from baseline to follow-up.

Exposure (IQR)		**Blood pressure change, mmHg (95% CI)**		
		SBP	***DBP***	***PP***
$PM_{2.5}$	(2.1 µg/m³)	1.2 (0.2, 2.2)	0.7 (0.2, 1.2)	0.4 (-0.3, 1.2)
PM_{10}	(2.8 µg/m³)	1.2 (0.1, 2.2)	0.7 (0.2, 1.3)	0.4 (-0.3, 1.2)
PM_{coarse}	(0.7 µg/m³)	1.1 (0.0, 2.1)	0.7 (0.1, 1.3)	0.3 (-0.4, 1.1)
PM_1	(1.6 µg/m³)	1.1 (0.1, 2.0)	0.7 (0.1, 1.2)	0.4 (-0.3, 1.1)
PN	(2.4×10^4/L)	0.2 (-0.8, 1.2)	0.5 (-0.1, 1.0)	-0.3 (-1.0, 0.5)
O_3	(2.7 µg/m³)	-0.9 (-2.0, 0.1)	-0.8 (-1.4, -0.3)	-0.1 (-0.9, 0.6)
NO_2	(7.1 µg/m³)	1.0 (0.0, 2.1)	0.9 (0.3, 1.4)	0.1 (-0.6, 0.9)
NO	(3.2 µg/m³)	0.9 (-0.1, 2.0)	0.9 (0.3, 1.5)	0.1 (-0.7, 0.8)
SO_2	(1.6 µg/m³)	0.8 (-0.3, 1.8)	0.7 (0.1, 1.2)	0.1 (-0.6, 0.9)
CO	(0.04 µg/m³)	0.5 (-0.6, 1.5)	0.6 (0.0, 1.2)	-0.1 (-0.9, 0.7)
NH_3	(0.5 µg/m³)	1.4 (0.4, 2.5)	0.8 (0.3, 1.4)	0.6 (-0.2, 1.3)

N = 3,240. The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term exposure, traffic and tram noise, distance to major road, age, sex, BMI, WHR, blood lipids, smoking, ETS, physical activity, and educational level.

Air pollution at follow-up and prevalent hypertension and BPLM intake (Table 29)

The concentrations of PM_{10} and PM_{coarse} were related to lower ORs for prevalent hypertension, whereas the concentrations of PN, NO_2, and CO exhibited positive relationships; however, neither of the estimates was statistically significant. $PM_{2.5}$, PM_{10} and PM_{coarse} were associated with lower ORs for BPLM intake. Weaker inverse relationships with OR for BPLM intake were observed for PM_1, PN, NO_2, NO, and NH_3. I also observed inverse associations of PM_1 to PM_{10} with self-reported hypertension and BPLM intake.

Air pollution at follow-up and incident hypertension and BPLM intake (Table 30)

I detected no significant associations with incidence of hypertension. Exposures to 365-day mean concentrations of $PM_{2.5}$, PM_{10}, PM_1, PN, NO_2, NO, SO_2, and CO at follow-up were related to higher relative risk (RR) for incident hypertension, though these estimates were imprecise. Relative risk (RR) for BPLM intake was decreased at higher levels of PM, NO_2 and NH_3. RRs for incident self-reported hypertension were inversely related to different PM metrics and were positively related to O_3, NO_2, NO and CO concentrations. The results with incident self-reported BPLM intake were almost identical to the results with incident BPLM intake according to the study definition.

Table 29. The cross-sectional associations of 365-day mean air pollution concentrations at follow-up with the prevalent outcomes hypertension and BPLM intake.

Exposure (unit)		IQR	Main study outcomes		Self-reported outcomes	
			Hypertension *OR (95% CI)*	**BPLM intake** *OR (95% CI)*	**Self-reported hypertension** *OR (95% CI)*	**Self-reported BPLM intake** *OR (95% CI)*
$PM_{2.5}$	(μg/m³)	2.1	0.98 (0.86, 1.11)	0.89 (0.79, 1.00)	0.88 (0.78, 0.99)	0.85 (0.76, 0.96)
PM_{10}	(μg/m³)	2.7	0.97 (0.85, 1.10)	0.88 (0.78, 0.99)	0.88 (0.78, 0.99)	0.85 (0.75, 0.96)
PM_{coarse}	(μg/m³)	0.7	0.94 (0.82, 1.07)	0.85 (0.75, 0.97)	0.88 (0.78, 1.00)	0.85 (0.75, 0.97)
PM_1	(μg/m³)	1.6	0.98 (0.87, 1.11)	0.90 (0.80, 1.01)	0.89 (0.79, 1.00)	0.86 (0.77, 0.97)
PN	($\times 10^4$/L)	2.4	1.03 (0.91, 1.17)	0.97 (0.86, 1.09)	1.00 (0.89, 1.12)	1.01 (0.90, 1.14)
O_3	(μg/m³)	2.7	1.00 (0.88, 1.14)	1.13 (1.00, 1.27)	1.09 (0.96, 1.23)	1.13 (1.00, 1.28)
NO_2	(μg/m³)	7.1	1.01 (0.89, 1.15)	0.92 (0.82, 1.04)	0.96 (0.85, 1.09)	0.94 (0.83, 1.07)
NO	(μg/m³)	3.2	1.05 (0.92, 1.20)	0.95 (0.84, 1.08)	0.99 (0.88, 1.12)	0.98 (0.86, 1.11)
SO_2	(μg/m³)	1.6	1.02 (0.90, 1.17)	0.98 (0.87, 1.11)	1.00 (0.88, 1.13)	0.99 (0.87, 1.12)
CO	(μg/m³)	0.0	1.04 (0.91, 1.19)	1.01 (0.89, 1.15)	1.03 (0.91, 1.17)	1.05 (0.92, 1.19)
NH_3	(μg/m³)	0.5	1.00 (0.87, 1.14)	0.90 (0.79, 1.02)	0.90 (0.80, 1.02)	0.86 (0.76, 0.97)

N = 3,240. The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term exposure, traffic and tram noise, distance to major road, age, sex, BMI, WHR, blood lipids, smoking, ETS, physical activity, and educational level.

Table 30. The cross-sectional associations of 365-day mean air pollution concentrations at follow-up with the incident outcomes hypertension and BPLM intake.

Exposure (unit)		IQR	Main study outcomes		Self-reported outcomes	
			Hypertension[1] *RR (95% CI)*	BPLM intake[2] *RR (95% CI)*	Hypertension[3] *RR (95% CI)*	BPLM intake[4] *RR (95% CI)*
$PM_{2.5}$	(μg/m³)	2.1	1.04 (0.91, 1.19)	0.92 (0.81, 1.05)	0.95 (0.81, 1.11)	0.89 (0.77, 1.02)
PM_{10}	(μg/m³)	2.7	1.03 (0.89, 1.18)	0.91 (0.79, 1.04)	0.94 (0.79, 1.10)	0.88 (0.76, 1.01)
PM_{coarse}	(μg/m³)	0.7	0.99 (0.86, 1.14)	0.86 (0.75, 1.00)	0.91 (0.77, 1.08)	0.86 (0.74, 0.99)
PM_1	(μg/m³)	1.6	1.04 (0.91, 1.19)	0.93 (0.82, 1.06)	0.96 (0.82, 1.12)	0.90 (0.78, 1.03)
PN	($\times 10^4$/L)	2.4	1.07 (0.93, 1.22)	0.99 (0.87, 1.14)	1.08 (0.92, 1.27)	1.04 (0.91, 1.20)
O_3	(μg/m³)	2.7	0.94 (0.82, 1.08)	1.07 (0.93, 1.24)	1.02 (0.87, 1.21)	1.09 (0.94, 1.25)
NO_2	(μg/m³)	7.1	1.07 (0.93, 1.23)	0.96 (0.83, 1.10)	1.03 (0.87, 1.21)	0.97 (0.84, 1.12)
NO	(μg/m³)	3.2	1.11 (0.96, 1.28)	0.99 (0.86, 1.14)	1.06 (0.89, 1.26)	1.02 (0.88, 1.18)
SO_2	(μg/m³)	1.6	1.06 (0.92, 1.21)	1.02 (0.89, 1.17)	1.12 (0.95, 1.32)	1.01 (0.88, 1.17)
CO	(μg/m³)	0.0	1.09 (0.94, 1.26)	1.06 (0.92, 1.22)	1.16 (0.98, 1.38)	1.07 (0.92, 1.23)
NH_3	(μg/m³)	0.5	1.06 (0.92, 1.23)	0.96 (0.84, 1.11)	0.98 (0.83, 1.16)	0.91 (0.79, 1.05)

The results are presented per given IQRs of exposure concentrations. Adjusted for time trend, short-term exposure, traffic and tram noise, distance to major road, age, sex, BMI, WHR, blood lipids, smoking, ETS, physical activity, and educational level.
[1]Analysis subset (prevalent cases and missing values excluded): N = 1,438. [2]Analysis subset: N = 2,132. [3]Analysis subset: N = 1,791. [4]Analysis subset: N = 2,212.

Sensitivity analyses

I adjusted for baseline BP values in the analyses. This resulted in attenuated effect size and precision, which was expected because adjustment for such a major predictor as a baseline BP value removes most of the outcome variability. In another sensitivity analysis, I used the assimilated (corrected with measured value) PM_{10} concentration. Assimilation was not uniformly performed for the air pollutants at follow-up, which is why this value was not included in the main analysis. In contrast to a non-assimilated value, the assimilated PM_{10} concentration was not clearly associated with BP, although the effect estimates were generally positive. For example, the change in SBP per increase in PM_{10} by an IQR of 4.1 µg/m³ was 0.5 mmHg (95% CI: -0.5, 1.5).

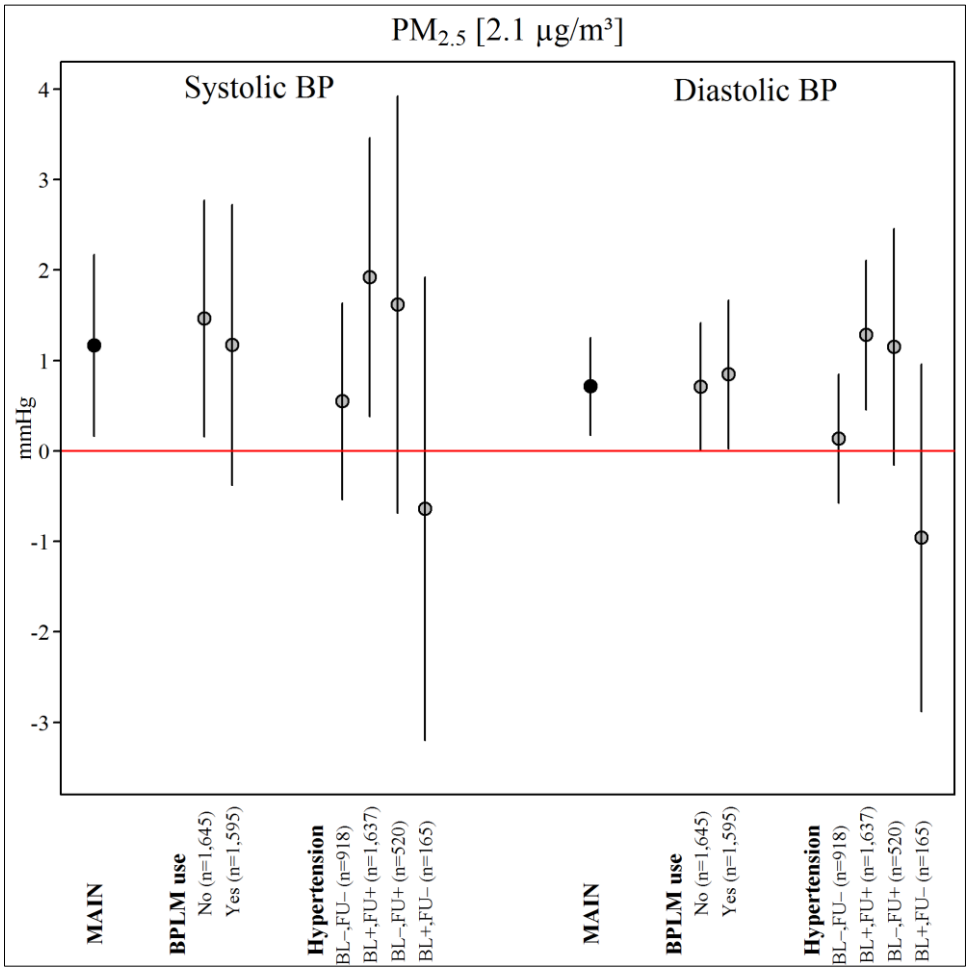


Figure 19. The cross-sectional associations of 365-day mean $PM_{2.5}$ concentration with BP among participants with no change in address since baseline, stratified by BPLM intake at follow-up and by hypertension status at baseline and follow-up.

Legend: N = 3,320. Results are presented per given IQR and are adjusted for time trend, short-term exposure, traffic and tram noise, distance to major road, age, sex, BMI, WHR, blood lipids, smoking, ETS, physical activity, and educational level.

I performed a stratified analysis by BPLM intake at follow-up (Figure 19 with $PM_{2.5}$; not shown with other pollutants). The results in medicated and non-medicated participants were very similar for $PM_{2.5}$, PM_{10}, PN (DBP only) and NH_3. The results in medicated participants were closer to null or inverse compared to non-medicated participants for PN (SBP only), O_3, NO_x, SO_2, and CO. The estimates in medicated and non-medicated participants differed less for DBP than for SBP.

I also stratified participants by hypertension status at baseline and follow-up (results with $PM_{2.5}$ in Figure 19). Among the four groups considered, the most consistent findings were among participants with prevalent hypertension at baseline and follow-up. The estimated associations in this group were higher than in the main model, with higher statistical significance. In the group with incident hypertension, the results were similar to the estimate in the entire analysis sample but not statistically significant (most likely due to small number of participants in this group). In the groups with no hypertension at baseline and follow-up and with hypertension only at baseline, the estimates were closer to a null effect compared to the entire analysis sample and were less precise.

4.2.5. Longitudinal associations of air pollution with study outcomes

BP as outcome

I investigated the association of exposure at baseline with the progression of hypertension at follow-up. To eliminate temporary contrasts, I investigated this association using the long-term grid-specific mean exposure (2000–2003 at baseline and 2006–2008 at follow-up). I used 2 outcomes, the BP at follow-up measurement (2006–2008) and the change in BP (ΔBP) during the follow-up period, calculated as:

$$\Delta BP = \frac{(BP_{follow-up} - BP_{baseline})}{Duration\ of\ follow-up\ in\ years} \quad [16]$$

Concentrations of $PM_{2.5}$, PM_{10}, PM_1, NO, and NH_3 at baseline were positively associated with BP at follow-up. These results were very similar to those obtained with the cross-sectional analysis at follow-up (Table 28). For example, an increase in $PM_{2.5}$ by 1 μg/m³ was associated with an increase in SBP by 0.6 mmHg (95% CI: 0.2, 1.1). The baseline concentration of O_3 was inversely associated with BP; per increase in O_3 by 1 μg/m³, SBP decreased by 0.4 mmHg (95% CI: -0.9, 0.0). When I excluded participants who used BPLM at follow-up (leaving 1,645 participants in the analysis), the associations observed in the remaining group did not change. In addition, borderline positive associations with SBP were observed with CO and NO_2.

Table 31. The longitudinal associations of long-term grid-specific mean exposures at baseline (2000-2003) with progression of BP during follow up in participants not taking BPLM.

Exposure (unit)		IQR	Progression of BP[1], mmHg per year (95% CI)		
			ΔSBP	ΔDBP	ΔPP
$PM_{2.5}$	(μg/m³)	2.5	0.2 (0.0, 0.4)	0.1 (0.0, 0.2)	0.2 (0.0, 0.4)
PM_{10}	(μg/m³)	4.0	0.1 (-0.1, 0.4)	0.0 (-0.1, 0.2)	0.1 (-0.1, 0.4)
PM_{coarse}	(μg/m³)	2.0	0.2 (-0.1, 0.4)	0.1 (0.0, 0.2)	0.2 (-0.1, 0.4)
PM_1	(μg/m³)	1.7	-0.1 (-0.2, 0.1)	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.1)
PN	($\times 10^4$/L)	2.8	0.1 (-0.2, 0.3)	0.0 (-0.2, 0.1)	0.1 (-0.2, 0.3)
O_3	(μg/m³)	2.2	0.1 (-0.1, 0.3)	0.1 (0.0, 0.2)	0.1 (-0.1, 0.3)
NO_2	(μg/m³)	5.9	0.1 (-0.1, 0.3)	0.1 (0.0, 0.2)	0.1 (-0.1, 0.3)
NO	(μg/m³)	5.4	0.1 (-0.1, 0.3)	0.0 (-0.1, 0.1)	0.1 (-0.1, 0.3)
SO_2	(μg/m³)	1.5	0.1 (-0.1, 0.3)	0.1 (0.0, 0.2)	0.1 (-0.1, 0.3)
CO	(μg/m³)	0.1	0.1 (-0.1, 0.3)	0.0 (-0.1, 0.1)	0.1 (-0.1, 0.3)
NH_3	(μg/m³)	0.5	0.1 (-0.1, 0.3)	0.1 (-0.1, 0.2)	0.1 (-0.1, 0.3)

N = 1,625. The results are presented per given IQRs of exposure concentrations. Adjusted for L_{night}, L_{den}, distance to major road, age, sex, BMI, WHR, blood lipids, smoking, ETS, physical activity, and educational level. For personal risk factors (BMI, blood lipids, smoking, physical activity), changes in follow-up were included as separate variables.
[1]*Calculated according to the formula [16].*

I analyzed the longitudinal associations of the long-term grid-specific mean exposures at baseline (2000–2003) with progression of BP at follow-up (Table 31) in participants not taking BPLM at follow-up (N = 1,645). The concentration of $PM_{2.5}$ at baseline was imprecisely positively related to BP: an increase of the mean baseline $PM_{2.5}$ concentration by 2.5 μg/m³ (the IQR of this sample) was associated with an increase in SBP by 0.2 mmHg per year of follow-up (95% CI: 0.0, 0.4). A slightly weaker positive relationship was detected with the PM_{coarse} concentration at baseline. Exposures to NO_2 and SO_2 at baseline were positively related to DBP. For the rest of the pollutants, no conclusive associations with changes in BP in the follow-up period were detected.

I also calculated the longitudinal associations of changes in exposure with progression of BP during the follow-up period (Table 32). Increases in $PM_{2.5}$, PM_{10}, PN and NH_3 were associated with decreases in BP. For example, per increase in $PM_{2.5}$ by 0.27 μg/m³ per year, SBP decreased by 0.2 mmHg per year (95% CI: -0.4, 0.0). The results with O_3 were similar, but not statistically significant. The inverse associations of change in PM with change in BP may be because air pollution concentrations decreased over the follow-up period. I also detected a

strong negative correlation of the changes in $PM_{2.5}$, PM_1, PN concentration during follow-up with the concentrations at either baseline or follow-up. Although such a correlation was not observed with NH_3, the inverse association of changes in NH_3 concentration with changes in BP may be due to high correlations of NH_3 with PM. An increase in SO_2 concentration, which did not correlate with baseline or follow-up values substantially, was associated with increased DBP: a change of 0.1 mmHg per year (95% CI: 0.0, 0.2) occurred per 0.10 μg/m³ per year.

Table 32. The longitudinal associations of change in long-term grid-specific mean exposures during follow-up with change in BP during follow-up among participants no taking BPLM at follow-up.

Exposure (unit)		**IQR**	**Progression of BP[1], mmHg per year (95% CI)**		
			ΔSBP	***ΔDBP***	***ΔPP***
$PM_{2.5}$	(μg/m³ per year)	0.27	-0.2 (-0.4, 0.0)	-0.2 (-0.3, -0.1)	-0.2 (-0.4, 0.0)
PM_{10}	(μg/m³ per year)	0.49	-0.2 (-0.4, 0.0)	-0.2 (-0.3, -0.1)	-0.2 (-0.4, 0.0)
PM_{coarse}	(μg/m³ per year)	0.04	-0.1 (-0.1, 0.0)	0.0 (-0.1, 0.0)	-0.1 (-0.1, 0.0)
PM_1	(μg/m³ per year)	0.05	-0.1 (-0.3, 0.1)	-0.1 (-0.2, 0.0)	-0.1 (-0.3, 0.1)
PN	($\times 10^4$/L per year)	0.14	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.0)	-0.1 (-0.2, 0.0)
O_3	(μg/m³ per year)	0.02	-0.1 (-0.1, 0.0)	0.0 (-0.1, 0.0)	-0.1 (-0.1, 0.0)
NO_2	(μg/m³ per year)	0.04	-0.1 (-0.3, 0.1)	-0.1 (-0.2, 0.1)	-0.1 (-0.3, 0.1)
NO	(μg/m³ per year)	0.08	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.1)	0.0 (-0.2, 0.2)
SO_2	(μg/m³ per year)	0.10	0.1 (-0.1, 0.3)	0.1 (0.0, 0.2)	0.1 (-0.1, 0.3)
CO	(μg/m³ per year)	0.35	-0.1 (-0.2, 0.1)	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.1)
NH_3	(μg/m³ per year)	0.43	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.0)	-0.1 (-0.2, 0.0)

N = 1,625. The results are presented per given IQRs of exposure concentrations. Adjusted for L_{night}, L_{den}, distance to major road, age, sex, BMI, WHR, blood lipids, smoking, ETS, physical activity, and educational level. For personal risk factors (BMI, blood lipids, smoking, and physical activity), changes in follow-up were included as separate variables.
[1]*Calculated according to the formula [16].*

Incident hypertension and BPLM intake as outcomes

Using Poisson regression with robust variance estimation, I analyzed the association of air pollution exposure at baseline and incident hypertension or BPLM intake (Table 33). $PM_{2.5}$, PN, NO_2, NO and CO concentrations were positively related to RR for incident hypertension. Concentrations of SO_2 and CO at baseline were associated with increased RR for incident BPLM intake; for example, per increase in baseline concentration of SO_2 by 1.5 μg/m³, the estimated RR of incident BPLM intake was 1.13 (95% CI: 1.01, 1.27). PM_{coarse}, PN, NO_2 and

NO had imprecise positive relationships with incident BPLM intake. Findings for self-reported incidence of hypertension were discordant from those with the main outcome, hypertension, though not statistically significant; RR was below 1 with PM and above 1 with gaseous pollutants. Findings with incident self-reported BPLM intake were very similar to those with the main study outcome, BPLM intake; NO_2, SO_2 and CO were positively associated with incident BPLM intake (RRs 1.10, 1.13 and 1.13, respectively), and positive, though not statistically significant, effect estimates were observed with most exposures.

Association of change in exposure from baseline to follow-up with incident outcomes is presented in Table 34. The change in NO from baseline to follow-up was related to a lower risk of incident hypertension: RR 0.88 (95% CI 0.82, 0.95) per 0.08 µg/m³ NO per year. Similar, though not precise, inverse relationships were detected with PM_{10}, PM_1 and NO_2. Incident intake of BPLM was inversely associated with NO: RR 0.82 (95% CI: 0.75, 0.90) per 0.08 µg/m³ per year; a similar inverse relationship was detected with $PM_{2.5}$. Changes in PN, O_3, SO_2 and NH_3 concentrations were associated with elevated RR for BPLM intake. For example, a yearly increase in O_3 by 0.02 µg/m³ per year was associated with RR for BPLM intake 1.04 (95% CI: 1.01, 1.08) after 5 years of follow-up. The results with self-reported incidence of hypertension and BPLM intake were quite similar to the ones with the main outcome, BPLM intake.

Table 33. The longitudinal associations of long-term air pollution concentrations at baseline (grid-specific means of 2000-2003) with incident binary outcomes at follow-up.

Exposure		IQR	Main study outcomes		Self-reported outcomes	
			Hypertension[1] *RR (95% CI)*	BPLM intake[2] *RR (95% CI)*	Hypertension[3] *RR (95% CI)*	BPLM intake[4] *RR (95% CI)*
$PM_{2.5}$	(μg/m³)	2.5	1.03 (0.93, 1.14)	1.01 (0.88, 1.16)	0.91 (0.82, 1.02)	1.02 (0.91, 1.15)
PM_{10}	(μg/m³)	4.0	1.02 (0.91, 1.15)	1.02 (0.88, 1.18)	0.91 (0.80, 1.02)	0.98 (0.86, 1.11)
PM_{coarse}	(μg/m³)	2.0	1.02 (0.92, 1.14)	1.04 (0.90, 1.19)	0.93 (0.83, 1.04)	1.05 (0.93, 1.17)
PM_1	(μg/m³)	1.7	1.00 (0.92, 1.09)	1.01 (0.92, 1.12)	0.96 (0.88, 1.05)	0.95 (0.87, 1.04)
PN	($\times 10^4$/L)	2.8	1.10 (0.99, 1.22)	1.12 (0.97, 1.28)	1.01 (0.90, 1.14)	1.07 (0.95, 1.21)
O_3	(μg/m³)	2.2	0.98 (0.89, 1.07)	0.99 (0.87, 1.12)	1.05 (0.95, 1.16)	1.06 (0.96, 1.19)
NO_2	(μg/m³)	5.9	1.06 (0.97, 1.17)	1.10 (0.97, 1.25)	1.00 (0.90, 1.11)	1.11 (0.99, 1.23)
NO	(μg/m³)	5.4	1.08 (0.98, 1.19)	1.12 (0.99, 1.27)	1.02 (0.92, 1.14)	1.09 (0.97, 1.21)
SO_2	(μg/m³)	1.5	1.02 (0.93, 1.11)	1.13 (1.01, 1.27)	1.01 (0.92, 1.11)	1.12 (1.01, 1.23)
CO	(μg/m³)	0.1	1.03 (0.94, 1.12)	1.13 (1.02, 1.25)	1.04 (0.95, 1.13)	1.08 (0.99, 1.19)
NH_3	(μg/m³)	0.5	1.02 (0.92, 1.13)	1.02 (0.89, 1.16)	0.93 (0.83, 1.03)	1.06 (0.95, 1.19)

The results are presented per given IQRs of exposure concentrations. Adjusted for L_{night}, L_{den}, distance to major road, age, sex, BMI, WHR, blood lipids, smoking, ETS, physical activity, and educational level. For personal risk factors (BMI, blood lipids, smoking, and physical activity), change in follow-up was included as a separate variable.

[1]Analysis subset (prevalent cases and missing values excluded): N = 1,438. [2]Analysis subset: N = 2,132. [3]Analysis subset: N = 1,791. [4]Analysis subset: N = 2,212.

Table 34. The longitudinal associations of changes in exposure from baseline to follow-up with incident binary outcomes at follow-up.

Exposure (unit)		**IQR**	**Main study outcomes**		**Self-reported outcomes**	
			Hypertension[1]	**BPLM intake[2]**	**Hypertension[3]**	**BPLM intake[4]**
			RR (95% CI)	***RR (95% CI)***	***RR (95% CI)***	***RR (95% CI)***
$PM_{2.5}$	(μg/m³ per year)	0.27	0.99 (0.91, 1.08)	0.91 (0.82, 1.02)	1.02 (0.94, 1.11)	0.83 (0.76, 0.91)
PM_{10}	(μg/m³ per year)	0.49	0.96 (0.88, 1.05)	1.01 (0.91, 1.12)	1.03 (0.95, 1.12)	0.93 (0.85, 1.02)
PM_{coarse}	(μg/m³ per year)	0.04	0.98 (0.94, 1.02)	1.03 (0.99, 1.07)	1.01 (0.97, 1.05)	1.01 (0.97, 1.05)
PM_1	(μg/m³ per year)	0.05	0.95 (0.87, 1.05)	0.96 (0.84, 1.08)	1.06 (0.95, 1.18)	0.99 (0.90, 1.09)
PN	($\times 10^4$/L per year)	0.14	1.00 (0.95, 1.04)	1.06 (1.01, 1.12)	1.05 (1.01, 1.09)	1.05 (1.00, 1.10)
O_3	(μg/m³ per year)	0.02	1.00 (0.97, 1.03)	1.04 (1.01, 1.08)	1.03 (1.00, 1.06)	1.03 (1.00, 1.07)
NO_2	(μg/m³ per year)	0.04	0.94 (0.86, 1.04)	0.92 (0.81, 1.05)	1.03 (0.92, 1.15)	0.96 (0.87, 1.06)
NO	(μg/m³ per year)	0.08	0.88 (0.82, 0.95)	0.82 (0.75, 0.90)	0.87 (0.80, 0.94)	0.84 (0.77, 0.91)
SO_2	(μg/m³ per year)	0.10	1.04 (0.95, 1.13)	1.20 (1.08, 1.33)	1.12 (1.03, 1.22)	1.17 (1.07, 1.28)
CO	(μg/m³ per year)	0.35	1.00 (0.93, 1.08)	1.02 (0.92, 1.12)	0.97 (0.89, 1.05)	0.98 (0.90, 1.06)
NH_3	(μg/m³ per year)	0.43	1.01 (0.97, 1.05)	1.06 (1.01, 1.11)	1.03 (0.99, 1.08)	1.05 (1.01, 1.09)

The results are presented per given IQRs of exposure concentrations. Adjusted for L_{night}, L_{den}, distance to major road, age, sex, BMI, WHR, blood lipids, smoking, ETS, physical activity, and educational level. For personal risk factors (BMI, blood lipids, smoking, and physical activity), change in follow-up was included as a separate variable.

[1]Analysis subset (prevalent cases and missing values excluded): N = 1,438. [2]Analysis subset: N = 2,132. [3]Analysis subset: N = 1,791. [4]Analysis subset: N = 2,212.

5. EXPERIMENTAL STUDY RESULTS

5.1. Expression of housekeeping genes

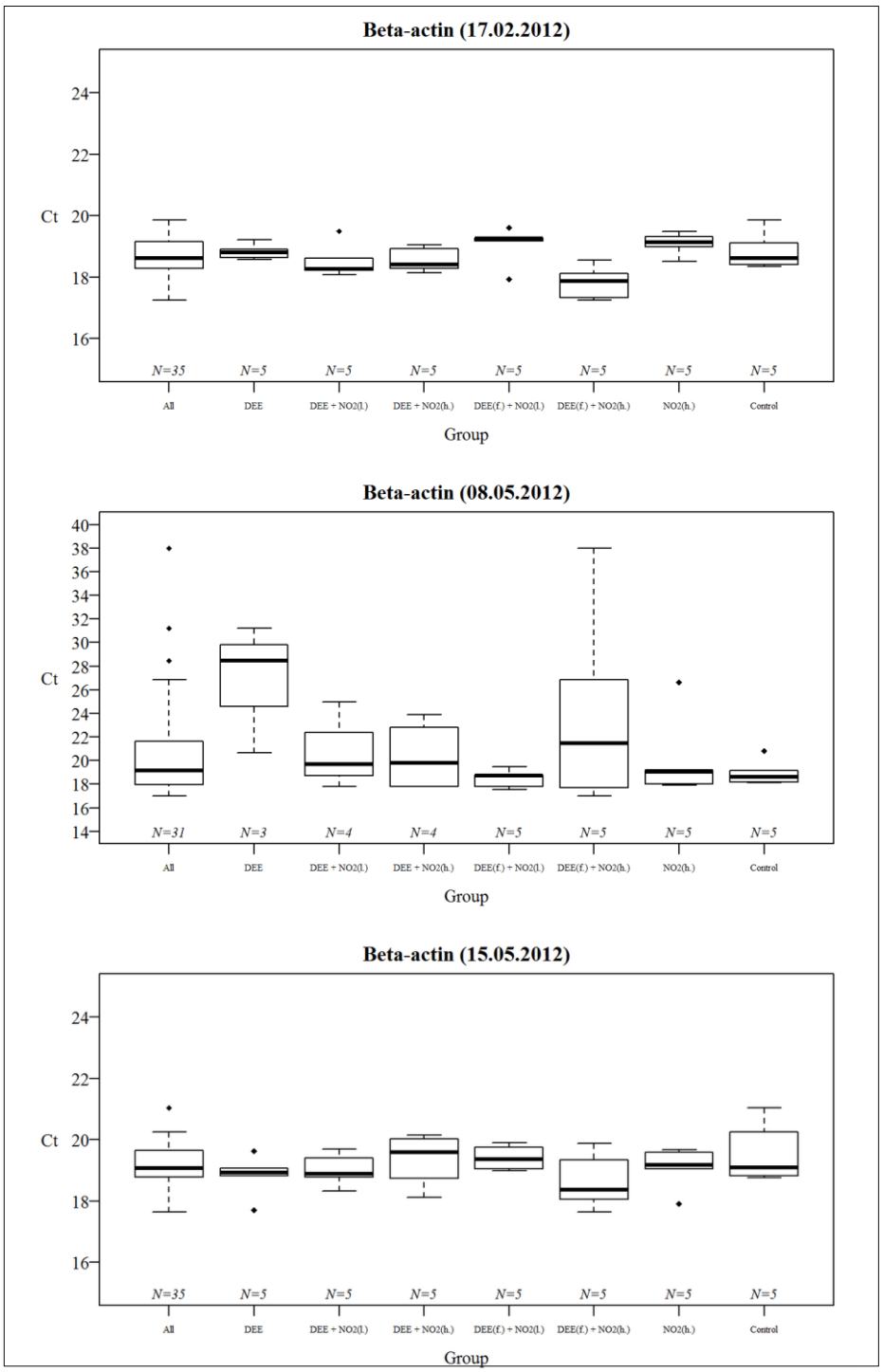


Figure 20. The expression of beta-actin, measured at three time points.

Legend: results are presented as Tukey box plots. Solid horizontal line represents a group-specific mean. Ends of the whiskers are within 1.5 IQR of the lower and upper quartile.

Beta-actin. I performed 3 PCR runs with beta-actin (17.02.2012, 08.05.2012, 15.05.2012). Results are presented in Figure 20. C_t stands for threshold cycle, a relative measure of beta-

actin concentration: higher C_t indicates lower concentration. The run from 08.05 demonstrated much lower expression and much higher variability than other two runs and was not used in the analyses.

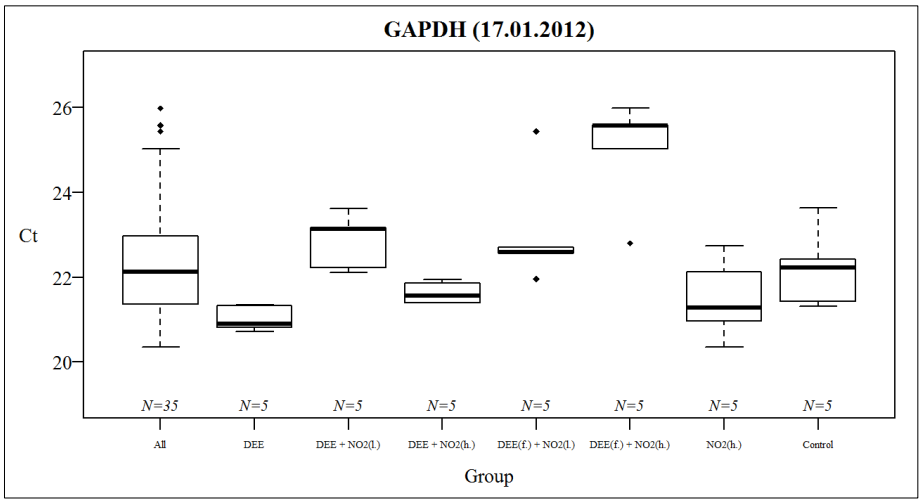


Figure 21. The measured expression of GAPDH.

Legend: results are presented as Tukey box plots. Solid horizontal line represents a group-specific mean. Ends of the whiskers are within 1.5 IQR of the lower and upper quartile. N= number of samples included.

GAPDH (Figure 21). Measured expression of GAPDH demonstrated high variability in different groups. I observed much lower expression in the group DEE + NO_2 (high), compared to other groups. Therefore it was decided that the results would not be normalized for GAPDH.

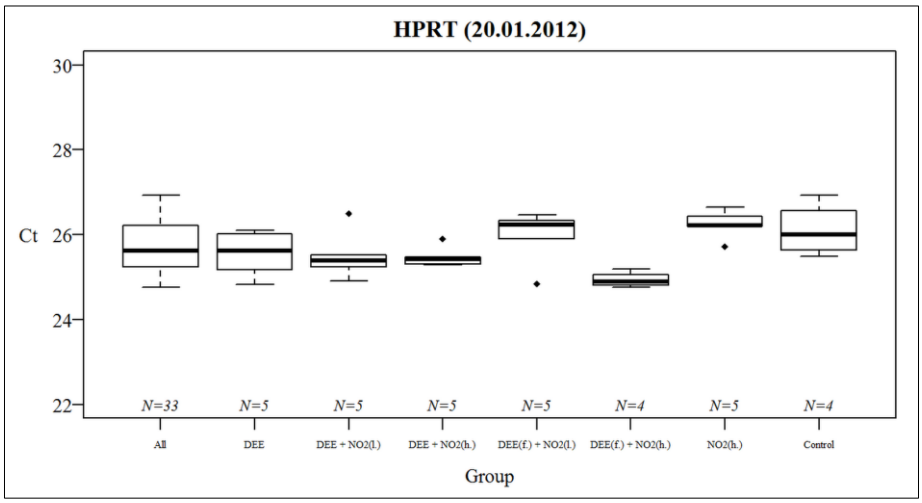


Figure 22. The measured expression of HPRT.

Legend: results are presented as Tukey box plots. Solid horizontal line represents a group-specific mean. Ends of the whiskers are within 1.5 IQR of the lower and upper quartile. N= number of samples included.

HPRT. Expression of HPRT is depicted at Figure 22. I observed more homogeneity between exposed groups and control, than with expression of GAPDH. However, I found slightly lower expression levels in groups DEE (filtered) + NO_2 (low) and NO_2 (high), than in other groups.

5.2. The relative expression levels of the studied genes

I measured the expression levels of the following genes in lung tissue: CYP1A1, iNOS, ICAM1, NQO1, and TNFα. Expression in exposed groups was compared with control group. Results were expressed as fold change in expression compared to control. I presented results with measured expression, not normalizing for housekeeping genes, and also normalized for beta-actin and HPRT concentration.

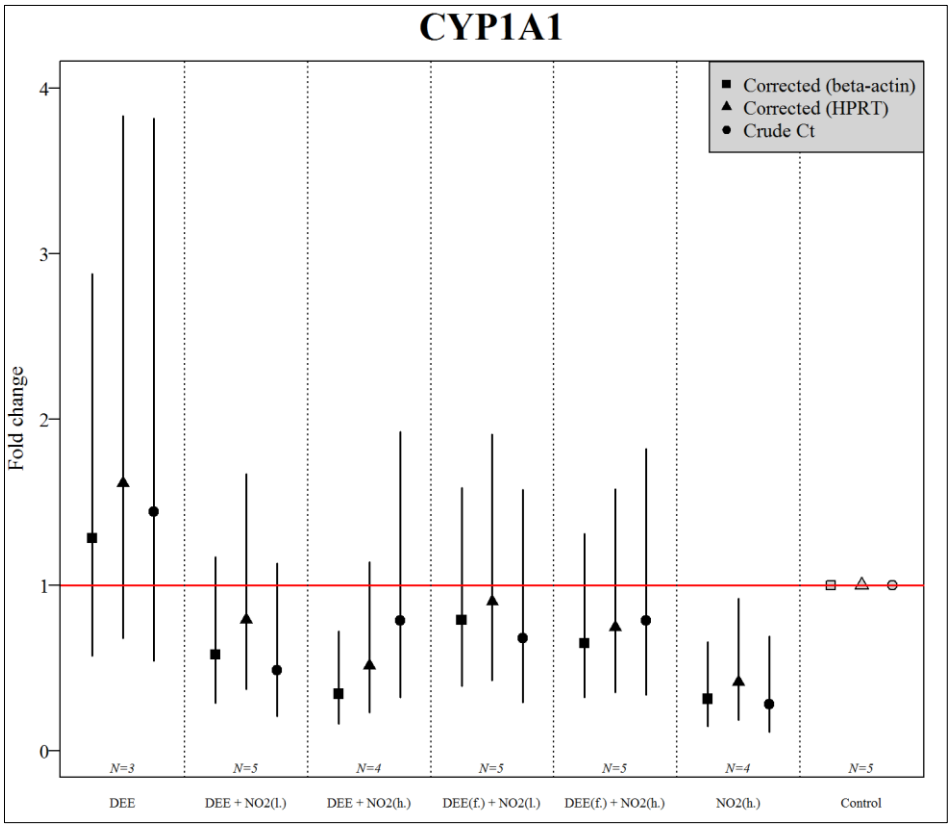


Figure 23. The relative expression of CYP1A1, presented as fold change in an average expression per group, compared to the average expression in control group.

Legend: Corrected = gene expression normalized for the given housekeeping gene; N = number of animals in the group, included to the analysis.

The relative expression of CYP1A1 is presented at Figure 23. I observed significantly lower expression levels in the group NO_2 (high). Fold change was about 0.5 in this group, which corresponds to an expression lower by 50% compared to the control group. In addition to this

finding, CYP1A1 expression in all groups with NO_2 tended to be lower, than in the control group. Expression in the DEE group was higher than in the control group. However, these results were not statistically significant.

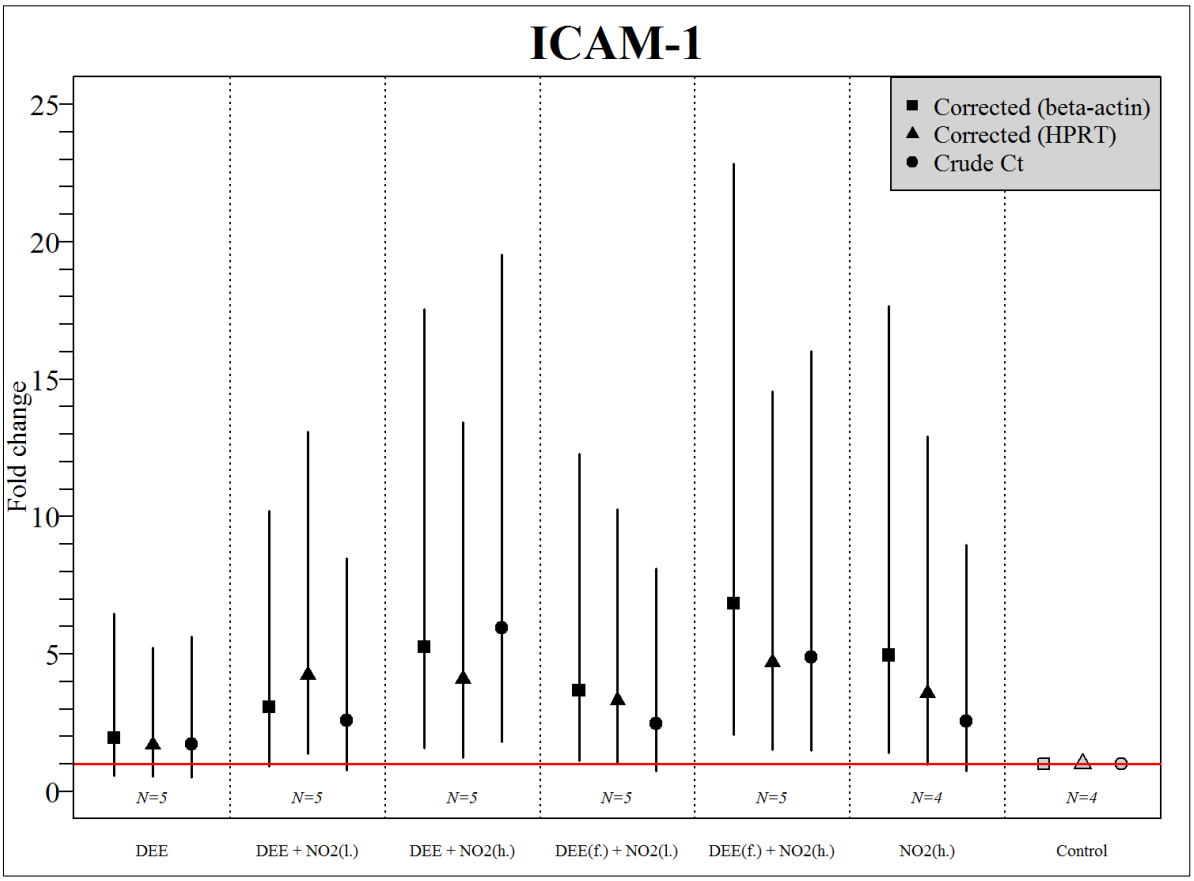


Figure 24. The relative expression of ICAM, presented as fold change in an average expression per group, compared to the average expression in control group..

Legend: Corrected = gene expression normalized for the given housekeeping gene; N = number of animals in the group, included to the analysis.

Measured The relative expression of ICAM1 is shown at Figure 24. In all exposed groups, gene expression was 2- to 7-fold higher than in the control group. In groups with higher NO_2 concentrations, the expression levels tended to be elevated as well. The estimated fold change did not differ substantially, when calculated with or without correction for housekeeping genes. However, statistical significance of results in some groups (e.g., DEE + NO_2 (low), NO_2 (high) differed by correction method or housekeeping gene choice.

Measured The relative expression of iNOS (Figure 25). I observed lower iNOS expression levels in the following groups: DEE (filtered) + NO_2 (high) and NO_2 (high). In the group DEE (filtered) + NO_2 (high) expression was about twice lower than in the control group. In the group NO_2 (high) iNOS expression was close to null, with low variation in results, possibly

indicating a measurement error (although no obvious problem was detected). In the group DEE + NO_2 (high) crude expression of iNOS was about two times higher, than in the control group. However, after correction for a housekeeping gene the expression attenuated towards one. In other exposure groups, iNOS expression of iNOS was not different from the control group.

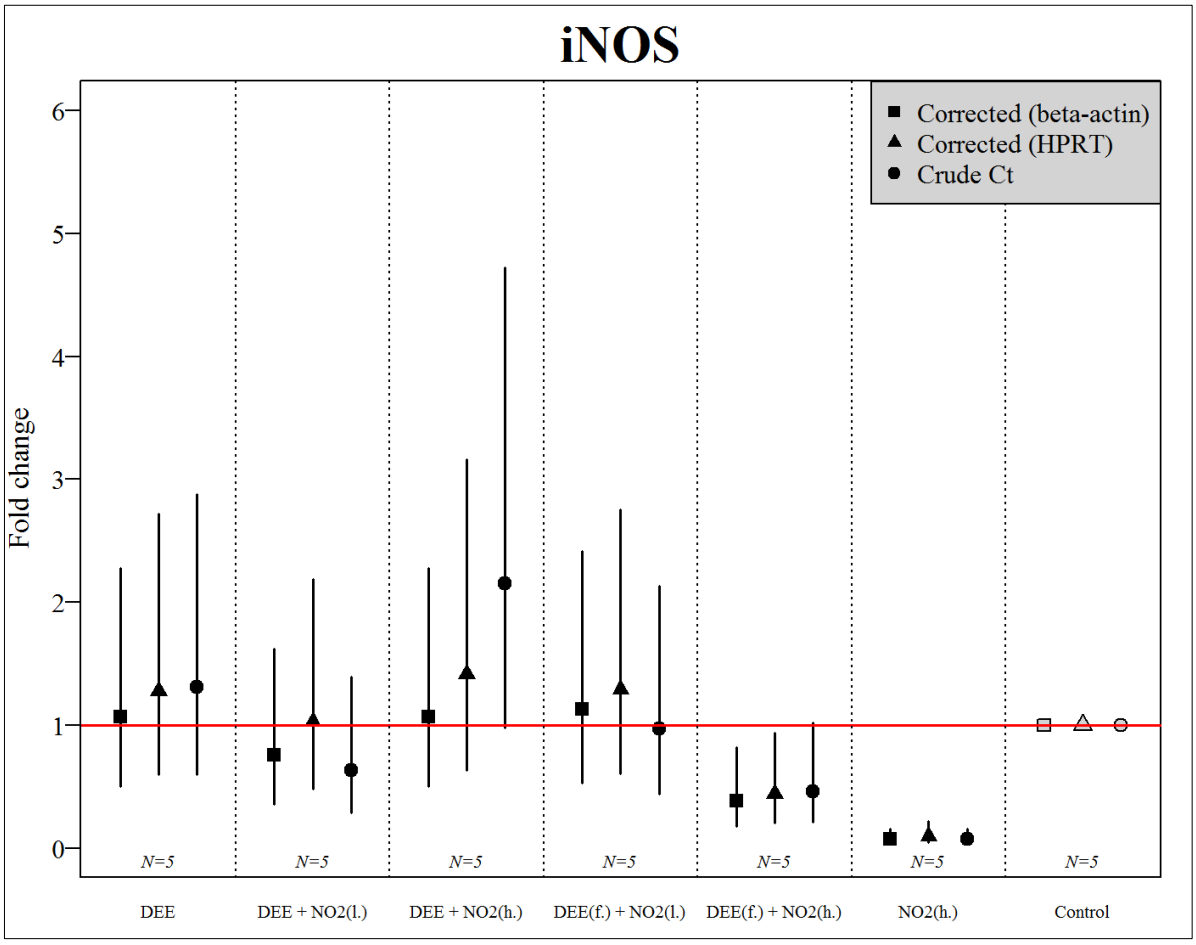


Figure 25. The relative expression of iNOS, presented as fold change in an average expression per group, compared to the average expression in control group.

Legend: Corrected = gene expression normalized for the given housekeeping gene; N = number of animals in the group, included to the analysis.

Measured The relative expression of NQO1 is presented at Figure 26. Groups with higher concentrations of NO_2 (with or without DEE and filtered DEE) showed 2- to 3-fold higher expression levels than the control group. The estimated expression in these groups was higher when presented as crude or corrected for beta-actin as housekeeping gene, than when corrected for HPRT. Expression of NQO1 in the groups DEE and DEE + NO_2 (low) was 1.5 to 2 times higher than in the control group.

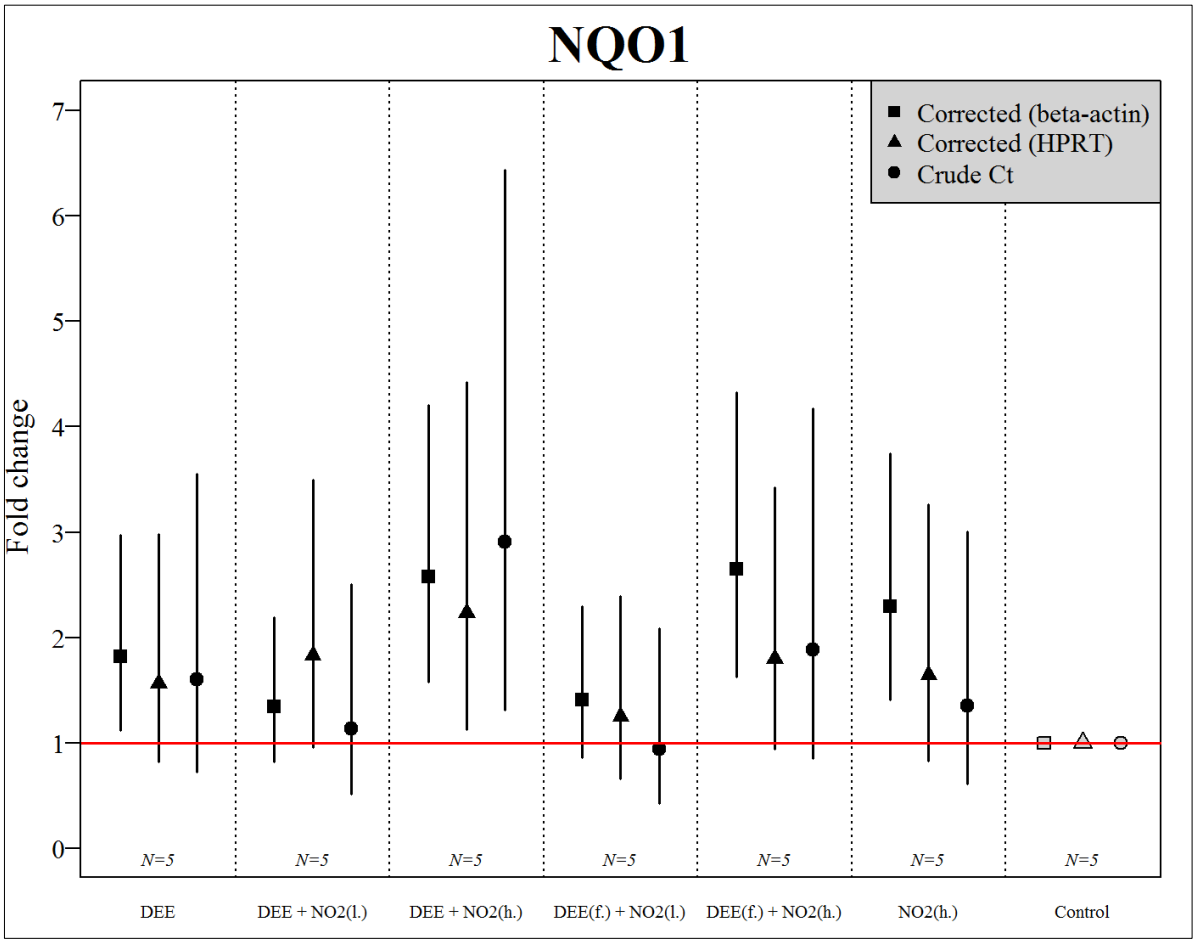


Figure 26. The relative expression of NQO1, presented as fold change in an average expression per group, compared to the average expression in control group.

Legend: Corrected = gene expression normalized for the given housekeeping gene; N = number of animals in the group, included to the analysis.

Measured The relative expression of TNFα (Figure 27). The width of CIs, including 1, did not allow to reject the null hypothesis of no difference with the control group. However, expression of TNFα in the group DEE (filtered)+NO_2 (low), as well as in groups with higher concentrations of NO_2 was lower than the control group. Estimated The relative expression of TNFα was mostly homogenous when corrected for housekeeping genes and crude, except for one group (DEE + NO_2 (low)), where the estimates differed.

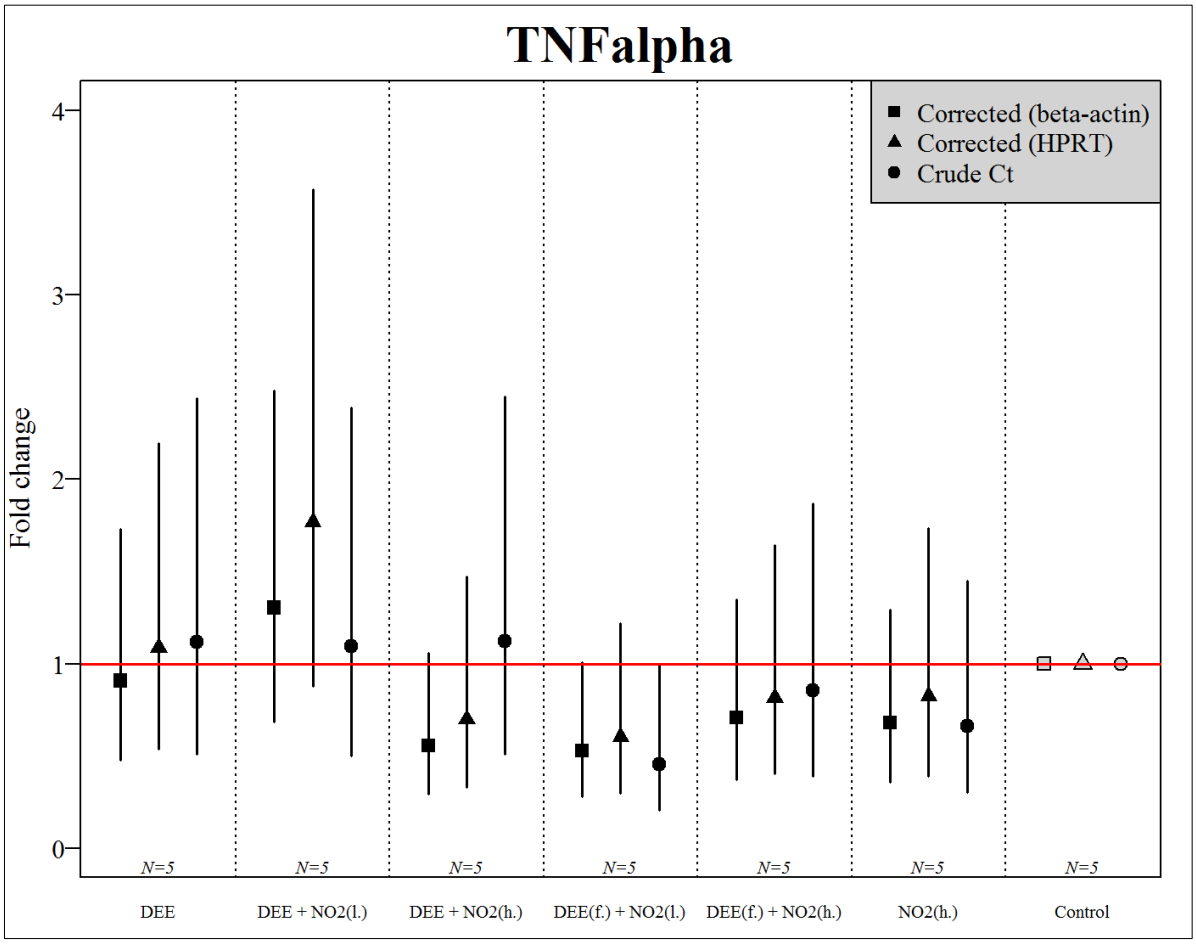


Figure 27. The relative expression of TNFα, presented as fold change in an average expression per group, compared to the average expression in control group.

Legend: Corrected = gene expression normalized for the given housekeeping gene; N = number of animals in the group, included to the analysis.

5.3. Sensitivity analyses

Dose-response analysis with NO_2 concentration

Table 35. The association of NO_2 concentration with gene expression in all exposure groups combined, normalized for beta-actin.

Gene	Fold increase per 1 ppm NO_2 (95% CI)
CYP1A1	0.94 (0.92, 0.98)
ICAM-1	1.09 (1.04, 1.14)
iNOS	0.91 (0.87, 0.96)
NQO1	1.05 (1.03, 1.07)
TNF	0.97 (0.95, 1.00)

I included the concentration of NO_2 in the exposure group as a continuous predictor to check whether a linear exposure-response relationship could be detected (Table 35). Linear trends

were significant for CYP1A, ICAM-1, iNOS and NQO1. The expression levels of CYP1A1 and iNOS were inversely associated with NO_2 concentration, and the expression levels of ICAM-1 and NQO1 were positively associated with NO_2 concentration.

6. DISCUSSION

6.1. Summary of findings

This study was conducted to investigate the effects of long-term air pollution and its specific components on BP and hypertension in humans and to investigate possible pathophysiologic mechanisms in an animal model. Using an observational study setting, I investigated the long-term effects of fine particulate matter urban background air pollution on arterial BP in a population-based cohort. I also studied the effects of different components of an air pollution mixture – e.g., smaller and bigger particles, and gases – and tested the independence of the effects in multi-pollutant models. I detected consistent positive associations of long-term urban residential concentrations of $PM_{2.5}$ and PM_1 with BP in a cross-sectional analysis with baseline and follow-up data, independent of co-exposures and relevant confounders. The associations with other PM sizes were positive, but less consistent compared to $PM_{2.5}$ and PM_1. Among gaseous compounds, long-term NH_3 concentration was positively associated with BP, independent of co-exposure PM and relevant confounders. The results with binary outcomes were less conclusive than with BP. I observed no associations with prevalent or incident hypertension (defined with BP value and BPLM intake). BPLM intake was inversely associated with $PM_{2.5–10}$ in the cross-sectional analysis with follow-up data. In the longitudinal analysis, incident BPLM intake was positively associated with traffic-related gaseous pollutants (O_3, NO, CO). Self-reported hypertension and BPLM intake were inversely related to PM exposure levels at baseline in cross-sectional analysis and in longitudinal analysis, though less consistently.

Under the experimental conditions, long-term (13 weeks) exposure to traffic-related particles and nitrogen oxides modulated the activity levels of five genes, the products of which are involved in xenobiotic metabolism, inflammation, oxidative stress and vascular tone regulation: CYP1A1, NQO1, iCAM, iNOS, and TNFα. I observed dose-response relationships between NO_2 concentration and expression of CYP1A1, NQO1, iCAM, and iNOS.

In summary, with the observational study results I was able to confirm the first part of hypothesis 1: that long-term residential exposure to fine PM is linearly associated with an increase in arterial BP, independently of co-exposures and potential confounders. However, I could not confirm the second part: that long-term exposure to fine PM is positively associated with the risk of hypertension in the general population. Using the experimental data, I provided some supportive evidence for hypothesis 2: that traffic-related air pollution can alter expression of genes related to elevated BP and hypertension.

6.2. $PM_{2.5}$ as a responsible pollutant

The observed positive association between urban background air pollution and arterial BP in the population-based cohort should likely be attributed to $PM_{2.5}$. Findings with $PM_{2.5}$ were more consistent than with other PM fractions (PM_{10}, PM_{coarse}, PM_1 and PN, representing ultrafine particles). These findings were robust to adjustment for co-exposure to noise, short-term exposure and various confounders. Additionally, the association of $PM_{2.5}$ with BP was independent of long-term exposure to other traffic- and industry-related emissions, represented with NO_2, NO, SO_2, CO, and O_3. As expected, in the experimental study design, DEE (both particulate and gaseous components) with NO_2 was associated with increased expression of NQO1, an enzyme involved in the production of ROS.

There are certain parameters of $PM_{2.5}$ toxicity that support these findings. Particles in $PM_{2.5}$ belong to the inhalable fraction and can be deposited in the lungs after inhalation. $PM_{2.5}$ consists of primary combustion particles and secondary particles (Kelly and Fussell 2012). The fine and ultrafine primary particles, directly emitted from combustion processes (such as traffic), can trigger redox reactions, leading to oxidative stress and inflammation (Li et al. 2002, 2009). I found that at high levels of NO or CO, no association of $PM_{2.5}$ with BP was observed (and vice versa – no association with the gas was found at high levels of $PM_{2.5}$). These results indicate that at high concentrations of $PM_{2.5}$ or traffic-related gases (NO and CO), which likely represent traffic "hot spots" with direct emissions of fresh combustion particles, the effects of the background air pollution mixture cannot be disentangled from the local "hot spot". The results of this study indicate a major role of the secondary particles in the PM mixture. No consistent association with PN, representing primary ultrafine particles, was detected in the observational study part; thus, it is likely that the larger particles in the $PM_{2.5}$ mixture were associated with BP. The secondary particles within $PM_{2.5}$ may be more toxic due their longer life span compared to primary particles (Kelly and Fussell 2012). The chemical composition of air pollution is also important. The major sources of PM in the study area were traffic and industry; therefore, the particles can contain many redox-active compounds, such as PAH, transition metals, and others.

The presented results with $PM_{2.5}$ are well in line with the existing evidence (Tables 36, 37). The association of long-term exposure to $PM_{2.5}$ with SBP was investigated in two studies (Auchincloss et al. 2008; Chuang et al. 2011). The 365-day mean concentration of $PM_{2.5}$ in this study was identical to the 60-day mean concentration reported by Auchincloss et al. (2008) and was 2-fold lower than the 365-day mean in the study by Chuang et al. (2011). When presented per 1 $\mu g/m^3$, the estimate in the study by Chuang et al. (2011) was the high-

est: a 1.57 mmHg increase in SBP, compared to the estimated changes of 0.31 mmHg in the study by Auchincloss et al., and 0.47 mmHg in the current study. The association of PM_{10} with SBP was also investigated in two other studies (Chuang et al. 2011; Dong et al. 2013). The levels of PM_{10} were the highest in the study by Dong et al. (2013), approximately 6 times higher than in the current study. The estimated changes of SBP per 1 μg/m³ of PM_{10} were 0.23 In this study 0.34 in the study by Chuang et al., and 0.05 in the study by Dong et al. Similarly to DBP, the highest changes of DBP to $PM_{2.5}$ or PM_{10} were observed in the study by Chuang et al. (2011) and the lowest in the study by Dong et al. (2013). In addition, a relatively high increase in BP per 1 μg/m³ increase in black carbon (a marker of traffic-related air pollution) was reported in a U.S. cohort of 853 elderly men (Schwartz et al. 2012).

I detected no association of $PM_{2.5}$ or PM_{10} with the prevalence of hypertension (OR = 1.000 per 1 μg/m³) and a weak positive relationship with the incidence of hypertension (RR = 1.012 per 1 μg/m³). The prevalence of self-reported hypertension was positively associated with annual $PM_{2.5}$ exposure in a study with 132,224 participants: OR 1.005 per 1 μg/m³ (Johnson and Parker 2009). Thus far, the only two studies that used incidence of hypertension as an outcome reported elevated risk. In one of those studies, the estimate was larger than in the current study (RR = 1.040 per 1 μg/m³), but it was also imprecise (Coogan et al. 2012). In another study, the risk estimate was similar (hazard ratio (HR) = 1.014) but was much more precise than in the current study, reaching statistical significance (Chen et al. 2013). Both studies reporting significant associations of $PM_{2.5}$ with prevalent or incident hypertension analyzed much larger populations than the one used in this observational study: 132,224 and 35,303 compared to 4,584 participants in the analysis with prevalent hypertension and 1,471 participants in the analysis with incident hypertension.

6.3. Findings with gaseous compounds (except ammonia)

With the observational study design, I did not find any independent associations of long-term exposure to gaseous compounds (O_3, NO_2, NO, SO_2, and CO) with BP; the weak associations observed in one-pollutant models became null after adjustment for $PM_{2.5}$ or PM_{10}.

With the experimental design, the expression of the studied genes was most affected by the animals' exposure to NO_2, with or without diesel exhaust; in addition, linear relationships of NO_2 concentration with gene expression were detected for most of the studied genes.

I compared the results of one-pollutant models with gaseous compounds of this study with the existing evidence (Tables 36, 37). Three other studies from Taiwan, China and Denmark have investigated the effects of long-term exposure to gaseous compounds on BP or hypertension

(Chuang et al. 2011; Dong et al. 2013; Sørensen et al. 2012). In the Danish study, nitrogen oxides were assessed as NO_x; their concentration was much lower than the concentrations of NO_2 and NO in the current observational study. The concentrations of all gaseous pollutants were higher in the Asian studies. In the Chinese cohort, the concentration of SO_2 was more than 4 times higher than in the Taiwanese study and was approximately 7 times higher than in the current study. Results with gaseous pollutants were more divergent across studies than the results with PM. For example, O_3 was inversely related to BP in my study (a change of -0.30 mmHg SBP per 1 $\mu g/m^3$), whereas in the Taiwanese cohort, O_3 showed a strong positive association with BP (1.57 mmHg SBP); a similar, but weaker, positive association was observed in the Chinese cohort (0.03 mmHg SBP). NO_2 was positively associated with BP in the Taiwanese study (0.55 mmHg SBP per 1 $\mu g/m^3$) and was weakly related to BP in the current study (0.12 mmHg SBP); however, it was not associated with BP in the Chinese cohort (0.03 mmHg). In the Danish study, the NO_x concentration was inversely associated with BP. The results with SO_2 indicated weak positive relationships in this study and in the Taiwanese cohort, but not in the Chinese study.

Table 36. The associations of long-term air pollution with systolic and DBP in the current study and in published studies, presented per increase in a pollutant concentration by 1 μg/m³.

Results	Current study N = 4,584	Auchincloss et al. 2008 N = 5,112	Chuang et al. 2011 N = 1,023	Dong et al. 2013 N = 24,845	Sørensen et al. 2012 N = 44,436	Schwartz et al. 2012 N = 853
Exposure characteristics						
Averaging period (days)	365 days	60 days	365 days	3 years	365 days	365 days
Daily mean (hours)	24-hour	24-hour	8–24 hours[1]	8-hour	24-hour	24-hour
Mean exposure levels (μg/m³)						
BC	–	–	–	–	–	0.61
PM_{10}	20.70	–	67.84	123.06	–	–
$PM_{2.5}$	16.70	16.70	35.31	–	–	–
O_3	35.40	–	48.50	49.40	–	–
NO_x	–	–	–	–	19.60	–
NO_2	40.10	–	49.69	35.28	–	–
SO_2	8.70	–	13.90	54.42	–	–
Association with SBP: change, mmHg (95% CI) (per 1 μg/m³)						
BC	–	–	–	–	–	8.25 (4.59, 11.88)
PM_{10}	0.23 (0.00, 0.46)	–	0.34 (0.26, 0.43)	0.05 (0.03, 0.07)	–	–
$PM_{2.5}$	0.47 (0.09, 0.86)	0.31 (-0.05, 0.22)	1.57 (1.06, 2.09)	–	–	–
O_3	-0.30 (-0.67, 0.07)	–	1.14 (0.89, 1.38)	0.03 (0.02, 0.05)	–	–
NO_x[1]	–	–	–	–	-0.50 (-0.84, -0.16)	–

Results	Current study	Auchincloss et al. 2008	Chuang et al. 2011	Dong et al. 2013	Sørensen et al. 2012	Schwartz et al. 2012
	N = 4,584	N = 5,112	N = 1,023	N = 24,845	N = 44,436	N = 853
NO_2	0.12 (-0.02, 0.26)	–	0.55 (0.42, 0.69)	0.03 (-0.02, 0.08)	–	–
SO_2	0.28 (-0.27, 0.84)	–	0.11 (-0.58, 0.80)	0.02 (0.01, 0.03)	–	
Association with DBP: change, mmHg (95% CI) (per 1 μg/m³)						
BC	–	–	–	–	–	7.53 (5.53, 9.53)
PM_{10}	0.18 (0.05, 0.30)	–	0.31 (0.27, 0.35)	0.02 (0.00, 0.03)	–	–
$PM_{2.5}$	0.31 (0.10, 0.52)	–	1.53 (1.25, 1.82)	–	–	–
O_3	-0.16 (-0.36, 0.04)	–	1.09 (0.96, 1.22)	0.02 (0.01, 0.03)	–	–
NO_x[1]	–	–	–	–	-0.24 (-0.42, -0.07)	–
NO_2	0.05 (-0.02, 0.13)	–	0.48 (0.41, 0.55)	0.02 (-0.01, 0.05)	–	–
SO_2	0.13 (-0.17, 0.43)	–	0.06 (-0.30, 0.43)	0.02 (0.01, 0.03)	–	–
[1]*Estimate is calculated per doubling of exposure concentration.*						

Table 37. The associations of long-term air pollution with prevalent and incident hypertension in the current study and in published studies, presented per increase in a pollutant concentration by 1 μg/m³.

Exposure	Current study	Current study	Dong et al. 2013	Johnson and Parker 2009	Coogan et al. 2012	Sørensen et al. 2012		Chen et al. 2013
	N = 4,584	N = 1,471	N = 24,845	N = 132,224	N = 3,236	N = 50,721	N = 33,275	N = 35,303
Mean exposure levels (μg/m³)								
PM_{10}	20.70	19.80	123.06	–	–	–	–	
$PM_{2.5}$	16.70	15.60	–	Not presented	20.70	–	–	10.70
O_3	35.40	37.60	49.40	–	–	–	–	

Exposure	Current study	Current study	Dong et al. 2013	Johnson and Parker 2009	Coogan et al. 2012	Sørensen et al. 2012		Chen et al. 2013
	N = 4,584	N = 1,471	N = 24,845	N = 132,224	N = 3,236	N = 50,721	N = 33,275	N = 35,303
NO_x	–	–	–	–	43.30	19.60	19.60	
NO_2	40.10	38.60	35.28	–	–	–	–	
SO_2	8.70	7.40	54.42	–	–	–	–	
Outcome (%)	Prevalence 57.0%	Incidence 36.2%	Prevalence 40.6%	Prevalence 25.0%	Incidence 16.4%	Prevalence 16.2%	Incidence 9.6%	Incidence 24.5%
Estimated association								
PM_{10}	OR 1.000 (0.900, 1.123)	RR 1.005 (0.912, 1.166)	1.006 (1.004, 1.008)	–	–			
$PM_{2.5}$	OR 1.000 (0.900, 1.123)	RR 1.012 (0.932, 1.151)	–	OR 1.005 (1.000, 1.010)	RR 1.040 (0.995, 1.087)			HR 1.014 (1.007, 1.022)
O_3	OR 1.000 (0.910, 1.110)	RR 0.991 (0.888, 1.079)	1.006 (1.003, 1.008)	–	–			
NO_x[1]	–	–	–	–	RR 1.005 (1.001, 1.009)	OR 0.96 (0.91, 1.00)	RR 1.01 (0.95, 1.08)	
NO_2	OR 1.010 (0.962, 1.167)	RR 1.006 (0.990, 1.022)	1.010 (1.000, 1.020)	–	–			
SO_2	OR 1.007 (0.921, 1.120)	RR1.013 (0.931, 1.119)	1.005 (1.002, 1.008)	–	–			

[1] *Estimate is calculated per doubling of exposure concentration.*

6.4. Findings with ammonia

In the current study the long-term exposure to NH_3 was positively associated with BP and positively related to hypertension. PM and NH_3 were highly correlated, which is why it was not possible to completely disentangle their effects. The results with NH_3 were robust to adjustment with PM, whereas the association of PM with BP diminished to null after adjustment with NH_3. Ammonia is an important component of secondary particles in $PM_{2.5}$ (such as ammonium nitrate or sulfate). Therefore, the positive association of ammonia with BP may be indicative of the health effect of secondary particles.

Ammonia is a toxic component of air pollution. The acute health effects of NH_4 in animals include respiratory and eye irritation, deeper and slower breathing, lung inflammation, reduced smelling capacity, lethargy, immune response, and increased bacterial susceptibility in the respiratory tract (WBK and Associates Inc. 2004). Chronic exposure to NH_3 results in affected lung function, coughing, phlegm, wheezing, and dyspnea (WBK and Associates Inc. 2004).

However, it is not clear whether NH_3 can increase BP or whether the observed association is due to collinearity of exposures. To date, there are no studies on the association of NH_3 with BP. Nevertheless, it is biologically not implausible that NH_3 can increase BP. Atmospheric NH_3 is an odorant chemical. Malodors such as NH_3, hydrogen sulfide (H_2S), and VOC, can potentially affect BP through a stress-related mechanism (Wing et al. 2013). In a panel study with healthy nonsmoking adults, self-perceived and measured (as H_2S) malodor from the industrial swine operations was strongly and positively associated with SBP and DBP (Wing et al. 2013). More studies on the biological plausibility of the association of ammonia with BP are needed.

6.5. Potential biological mechanisms: analyzing experimental results

I found some indication that long-term exposure to diesel exhaust and NO_2 affects the activity levels of genes involved in inflammatory and immune responses, cell signaling, and cardiovascular pathology in mouse lung tissue. The results with these genes were summarized and compared with other studies.

CYP1A1

In the controlled exposure study, I observed decreased expression of CYP1A1 in the murine lung at higher levels of NO_2. The effect of diesel exhaust was inconclusive. The product of CYP1A1, cytochrome P450, takes part in the metabolism of xenobiotics and in the synthesis

of cholesterol, steroids and other lipids (National Center for Biotechnology Information 2014a). CYP1A1 takes part in the production of arachidonic acid-derived vasoactive substances. Some of these substances influence renal function, peripheral vascular tone and BP (Newton-Cheh et al. 2009). The products of the metabolism of omega-3 polyunsaturated fatty acids with cytochrome P450, such as 17,18-epoxyeicosatetraenoic acid and 19,20-epoxydocosapentaenoic acid, are strong vasodilators, and the loss of these vasodilators resulted in increased BP in CYP1A1 knockout mice (Agbor et al. 2012). The decreased activity of CYP1A1 may also result in increased BP.

Some studies link CYP1A1 activity to BP. In a genome-wide association study with up to 84,114 individuals of European and Indian Asian ancestry, few loci were associated with SBP and DBP, including genes coding the cytochrome P450 proteins (Newton-Cheh et al. 2009). Another study showed that CYP1A1 mediates endothelial dysfunction and hypertension after exposure to xenobiotics, such as dioxin-like halogenated aromatic hydrocarbons (Kopf et al. 2010). Therefore, the alteration of CYP1A1 activity may change cardiovascular risk. In a case-control study with Turkish participants, different genetic variants of CYP1A1 contributed to inter-individual variability in smoking- and hypertension-induced ischemic stroke risk (Demirdöğen et al. 2013). CYP1A1 activity was positively associated with the incidence of stroke in patients with essential hypertension (Lança et al. 2004).

The organic component in the diesel exhaust and the PM mixture can activate CYP1A1 (Dieme et al. 2012; Ma and Ma 2002). Other studies showed that combustion-derived particles stimulated the expression of CYP1A1 through the activation of the aryl hydrocarbon receptor and increased binding to xenobiotic response elements (Chan et al. 2013; Matsumoto et al. 2007; Rouse et al. 2008; Wenger et al. 2009). In an in vitro model with human cells, short-term exposure to $PM_{2.5}$ from a highly industrialized area induced significant increases in the mRNA expression levels of CYP1A1 and other related enzymes (Billet et al. 2007). Chan et al. showed that combustion-derived ultrafine particles were capable of a rather weak increase in AhR activity and altered CYP1A1 expression differently according to age, lung compartment, and recovery time after exposure (Chan et al. 2013). Expression of CYP1A1 in rat lung rapidly increased after exposure to diesel exhaust particles (Van Berlo et al. 2010).

The downregulation of CYP1A1 may be a result of the activation of host defense mechanisms. The administration of inflammatory cytokines, such as TNFα, inhibited CYP1A1 activity in vitro (Gharavi and El-Kadi 2007; Paton and Renton 1998). Another possible explanation of the reduced activity of CYP1A1 following NO_2 inhalation may be that NO (assuming that NO_2 also contains some quantity of NO), also a potent vasodilator, competes with

CYP1A1 metabolites, the vasoactive substances, and downregulates its activity. The inhibition of CYP1A1 by NO was demonstrated in vitro (Gharavi and El-Kadi 2007; Stadler et al. 1994; Vuppugalla and Mehvar 2004).

The potential health impact of cytochrome P450 activity inhibition is not clear. The process of metabolizing the foreign chemical, CYP1A1, may lead to the formation of highly toxic metabolites, such as dihydroxyl epoxide, a potential carcinogen emerging during the transformation of VOCs, in particular, benzo(a)pyrene. The finding that NO_2 decreased CYP1A1 expression could indirectly also mean that the capacity of the lung to address the metabolism of such xenobiotics could be affected during co-exposure to NO_2 in addition to diesel exhaust. This finding might be specific to long-term exposure because short-term exposure with diesel exhaust upregulated CYP1A1 in a rat lung (Van Berlo et al. 2010)

ICAM-1

I observed elevated expression of ICAM-1 with diesel engine exhaust and NO_2. The product of this gene is involved in leukocyte adhesion and trans-migration to the endothelium; it is also involved in vascular inflammation and signal transduction. Expression of this gene correlates with systemic inflammation and cardiovascular risk, rheumatoid arthritis, hypertension, and growth of atherosclerotic lesions (Lawson and Wolf 2009; Witkowska and Borawska 2004). Immune cell recruitment, activated by ICAM-1, is an important event in atherosclerosis development (Wang et al. 2013).

The activity of ICAM-1 is related to both the inflammatory process and endothelial function. It has been suggested that hydrogen peroxide (H_2O_2) can activate ICAM-1 gene transcription (Zadeh et al. 2000). H_2O_2 is produced by leukocytes during vascular response to injury; its production is increased by cytokines such as TNFα (Zadeh et al. 2000). Correspondingly, TNFα upregulates ICAM-1 expression (Wang et al. 2013). At the same time, induction of iNOS expression can inhibit ICAM-1 expression to protect the vascular endothelium (Zadeh et al. 2000).

ICAM-1 activity is affected by smoking, antioxidant intake and vasoconstriction agents, such as angiotensin (Witkowska and Borawska 2004). Air pollution exposure can increase ICAM-1 production. For example, in a cohort study with 704 elderly men, traffic and secondary particles were associated with ICAM-1 and vascular cellular adhesion molecule (VCAM-1) concentrations (Bind et al. 2012b). In the same cohort, exposures to O_3 and sulfate were associated with changes in ICAM-1 gene methylation (Bind et al. 2014). In diabetic patients, outdoor exposures to $PM_{2.5}$, black carbon and sulfate were positively related to ICAM-1 expression;

stronger associations were observed for $PM_{2.5}$ and black carbon with VCAM-1 (O'Neill et al. 2007).

Similar findings have been reported in animal and in vitro studies. In an ischemic stroke model in rats, the inhalation of SO_2 activated gene expression in a dose-dependent manner compared to the control for ICAM-1 and other genes, such as iNOS, cyclooxygenase 2, and ET-1 (Sang et al. 2010). In an animal model with hyperlipidemic rats, exposure to PM_{10} induced ICAM-1 and VCAM-1 expression in the aorta endothelium and caused the progression of atherosclerosis (Yatera et al. 2008). Elevated ICAM-1 levels were reported in lung tissue in rats exposed to O_3 (Bhalla and Gupta 2000). NO_2 was positively associated with ICAM-1 expression in human bronchial cells in vitro (Ayyagari et al. 2007; Pathmanathan et al. 2003). Diesel exhaust particles increased the production of inflammatory mediators, including ICAM-1, in human respiratory epithelial cells in vitro (Bayram et al. 1998; Yang et al. 2009).

INOS

INOS expression was somewhat increased in the DEE+NO_2(high) group, but this increase was not statistically significant. I detected strong decreases of iNOS activity in two groups: DEE(filtered) + NO_2(high) and NO_2(high). In the latter group, the gene expression was almost null, and the variances were very low compared to other groups. Therefore, these observations might be a chance finding and should await confirmation in further studies. There was a linear trend in the association of NO_2 concentration with gene activity: higher concentration of NO_2 was associated with lower expression of iNOS.

iNOS is involved in the immune defense response but also plays a role in vascular pathology, such as hypertension (Lee and Yen 2008). This dual role is provided by NO: a potent oxidant on the one hand, protecting against infectious organisms, and a vasodilatory agent on the other hand, triggering smooth muscle relaxation (Wang et al. 2013). Under normal conditions, iNOS is not expressed or is expressed at a minimal level. Inflammatory cytokines can activate iNOS expression in vascular cells (Lee and Yen 2008). INOS upregulation might play a role in vascular dysfunction and atherogenesis (Bai et al. 2011).

Thus far, two studies have investigated the effect of nitrogen oxides (only NO) on iNOS activity, with different results. In one study, NO downregulated iNOS gene transcription in vitro; the authors hypothesized that this inhibition might be a regulatory mechanism to limit NO overproduction during the inflammatory response (Taylor et al. 1997). In another study, gaseous NO activated iNOS in a mouse model of lung cancer and promoted lung cancer development (Chen et al. 2008). It is possible that duration of exposure additionally modifies iNOS

activity: acute exposure results in an inflammatory response, characterized by iNOS upregulation, similar to the study by Chen et al. (2008), whereas during long-term exposure, a compensatory protective mechanism is activated, and iNOS activity is inhibited, as suggested by Taylor et al. (1997).

Other air pollutants have also shown heterogeneous effects on iNOS activity. In a controlled exposure study with healthy volunteers, exposure to fine and coarse concentrated ambient particles (CAPs) was related to the decreased methylation of Alu, TLR4, and iNOS (for the latter, not statistically significant) (Bellavia et al. 2013). Increased iNOS expression after exposure to CAPs and O_3 was observed in an animal study (Sun et al. 2013). In an ApoE knockout mouse model, exposure to diesel exhaust increased iNOS expression (Bai et al. 2011). In an animal study in which murine lung tissues were investigated after long-term exposure to diesel exhaust, the expression levels of iNOS in the lung and of TNFα and some interleukins in lung macrophages were suppressed after low- and high-dose diesel exhaust exposure; because iNOS, TNFα and IL play important roles in defense against infections, the authors hypothesized that exposure to diesel exhaust may cause increased infectivity (Saito et al. 2002). In a rat model of with O_3-induced lung inflammation, short-term exposure to particles resulted in increased iNOS expression; the authors hypothesized that the free radical NO may have caused the observed endothelial damage (Ulrich et al. 2002). Chauhan et al. (2004) detected in vitro modulation of iNOS activity according to differences in the PM mixture: urban particles (SRM-1648, SRM-1649, EHC-93) downregulated iNOS activity, whereas $PM_{2.5}$ or cristobalite (SRM-1879) were associated with higher iNOS activity levels.

NQO1

In the experimental part of the current study, high NO_2 concentrations (alone or combined with DEE) were associated with elevated NQO1 expression compared to the control. The relationship of NO_2 concentration to gene expression was linear. NQO is involved in redox reactions: it catalyzes the reduction of endogenous (vitamin E) or environmental (benzene) quinones (Nebert et al. 2002). Polymorphisms in NQO1 and other genes involved in oxidative stress genes increase respiratory symptoms, which affects lung function (Yang et al. 2009).

Elevations of NQO-1 expression may work to both protect and exacerbate vascular injury. The inhibition of NQO1 activity increases the risk of toxicity or cancer (Nebert et al. 2002). At the same time, NQO1 is a potent protecting factor against adverse cardiovascular conditions, such as cardiovascular injury, atherogenesis, and others. (Zhu and Li 2012). The gene NQO1 is under control of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcription

factor pathway, and this transcription factor is emerging as a potentially important player in particle-induced lung diseases (Araujo and Nel 2009).

To date, there are no studies on the association of ambient NO_2 with NQO1 expression. However, it is possible that NO_2 increases NQO1 activity, similarly to other air pollutants. For example, organic chemicals in the $PM_{2.5}$ mixture (such as PAH) induced the expression of CYP1A11 and NQO1 in vitro (Dergham et al. 2012). The organic component of the $PM_{2.5}$ mixture activated NQO1 expression and, to a greater extent, the cytochrome P450 genes in vitro (Dieme et al. 2012). The activity of the NQO-1 gene may be mediated through antioxidant responses (Li 1992) and can interact with smoking status and benzene exposure (Kim et al. 2007).

TNFα

TNFα encodes an acute-phase cytokine that regulates multiple processes: apoptosis, inflammation, proliferation, lipid metabolism, and coagulation (National Center for Biotechnology Information 2014b). Similarly to NQO1, it has a dual role; both adverse and protective effects have been reported for its activity (Tracey and Cerami 1993). I did not detect statistically significant differences in TNFα activity in exposed groups compared to the control. However, the expression of TNFα tended to be inhibited in some groups with NO_2. This is in line with at least one study, where $PM_{2.5}$ and PM_{coarse} inhibited TNFα activity, whereas diesel exhaust did not alter it in vitro (Becker et al. 2005).

However, most studies report activation of TNFα induced by air pollution exposure. For example, in a panel study with healthy individuals, TNFα activity was positively associated with zinc in urban air pollution (Wu et al. 2012). In a case-control study on myocardial infarction, Panasevich et al. (2013) found that different genetic variants of TNFα modified the effects of short-and long-term exposure of air pollution on inflammatory marker levels and the risk of infarction. In an in vitro model with human cells, short-term exposure to $PM_{2.5}$ induced apoptosis, which involved TNFα activation and cytochrome c release from the mitochondria (Dagher et al. 2006); these results were preceded by an inflammatory response involving changes in iNOS activity and NO release (Garçon et al. 2006). In another in vitro model, traffic-related particles induced IL-6, TNFα and NO (Lindbom et al. 2007). Future research could shed more light on the role of TNFα in adverse cardiovascular effects of air pollution.

6.6. Clinical relevance of the observed BP change

The estimated change in BP following exposure to air pollution is rather small. However,

even small changes of arterial BP are of high public health importance. The increase of BP by only a few mmHg is not substantial at the individual level; however, it implies that larger proportions of individuals in the population are hypertensive. The rates of hypertension are increasing worldwide in all countries where economic growth is accompanied by epidemiological transition (with non-communicable diseases becoming a more urgent issue than communicable), increasing life expectancy, and population aging. Unhealthy diet, low physical activity level, overweight and psychosocial stress also contribute to the increasing prevalence of hypertension worldwide (WHO 2013). Hypertension is a major risk factor for CVD, stroke, kidney failure, premature mortality and disability (WHO 2013). According to the Global Burden of Disease Study in 2010, high BP was the leading risk factor for mortality (Lim et al. 2013).

The prevention of hypertension is a crucially important issue for public health. A reduction in SBP of only 2 mm leads to reductions in stroke mortality by 5%, in CHD mortality by 4%, and in total mortality by 3% (Whelton et al. 2002). A reduction of DBP of 2 mmHg has been linked to a 6% lower risk of CHD and a 15% lower risk of stroke and transient ischemic attack (Cook et al. 1995). It has been estimated that a 2.2 mm Hg lower SBP (resulting from, e.g., a lower habitual population sodium intake of 100 mmol/day) corresponds to a 4% lower risk of coronary death and a 6% lower risk of stroke death among the middle aged in the US and UK populations (Stamler et al. 1989).

The current levels of urban background air pollution can be seen, with regards to the results of this study, as a target for population-based preventive strategies (Rose 1985). Since a monotonic linear relationship of PM with BP was observed, reduction of PM at any levels is important. Prevention of air pollution-related increases in BP is especially important because hypertension may be a linking mechanism between air pollution exposure and cardiovascular events. Exposure to air pollution may affect vasomotor tone and cause a pro-hypertensive response, which, in turn, could trigger ischemic cardiac events and, thus, could increase the risk for heart failure and stroke (Brook et al. 2009; Tofler and Muller 2006). High BP and hypertension are important intermediates in atherosclerosis progression, an underlying condition for many cardiac events. Air pollution-related increase in BP could be one of the mechanisms by which PM air pollution may lead to atherosclerosis, as shown in recent studies (Bauer et al. 2010; Hoffmann et al. 2007; Künzli et al. 2010).

6.7. The impact of different definitions of hypertension

Availability of measured BP data defines the definitions of hypertension, used in different studies: if no BP data are not accessible, self-reported hypertension status or hospital records

could be used. However, using different definitions limits the studies' comparability. Of 5 studies on air pollution and hypertension, the JNC7 definition, based on both BP values and medication (used as the main outcome definition in this work), was used in one study (Dong et al. 2013). One study used the hospital registry with physician-defined hypertension (Chen et al. 2013). Three studies used self-reported diagnosis of hypertension (Coogan et al. 2012; Johnson and Parker 2009; Sørensen et al. 2012). Coogan et al., who used a self-report of doctor-diagnosed hypertension and the concurrent use of BPLM as an outcome definition, ascertained this definition in the subset of hypertensive participants (139 cases) with the help of medical records or physician checklists; 99% of the cases were confirmed (Coogan et al. 2012).

I compared two outcomes in this study: the "measured" hypertension (included as main study outcome and defined with BP values and BPLM intake status) and the self-reported hypertension. Both outcomes were obtained in most of HNR participants, which provided a unique possibility of comparison. The definitions overlapped quite well: 73% to 76% of participants with information on both outcomes were characterized equally. However, Cohen's kappa, a measure of agreement, was somewhat worse for the incident hypertension, than for prevalent hypertension at baseline or follow-up. Results of the analysis with air pollution were somewhat different with "measured", than with self-reported hypertension. Although not statistically significant, the relationship of exposures with "measured" hypertension was mostly positive, similar to the observed associations with BP, whereas the relationship of exposures with self-reported hypertension was null to slightly negative, similar to the results observed with BPLM intake as an outcome.

The observed difference between results with "measured" and self-reported hypertension might indicate a group of subjects with variable BP, which increased during the measurement and, correspondingly, the overestimation of hypertension prevalence when using the JNC7 definition in the current study. Indeed, there were 5% of participants classified as hypertensive at baseline and as normotensive at follow-up. Benetos et al. (2003) have shown that a single BP measurement could overestimate the proportion of subjects receiving BPLM and yet having high BP values. Possibly, the definition of hypertension based on a single measurement (although standardized and repeated 3 subsequent times) is insensitive towards short-term elevation of BP, which does not indicate an underlying vascular pathology. Indeed, in the group of participants with prevalent hypertension at baseline and follow-up, which constituted approximately half of the follow-up sample, the association of air pollution with BP was stronger than in the entire sample.

An alternative explanation to the observed phenomenon would be a relatively low overlap of known and "measured" hypertension may imply inadequate coverage of hypertensive patients by medical services. Therefore, it is not recommended to favor self-reported hypertension over the information provided by BP values and medication. However, it is advisable to repeat measurements of BP over some period of time for more validity of the "measured" hypertension definition, and to check the "measured" outcome using the self-reported information.

6.8. The role of BPLM intake

I employed a few strategies to assess the potential bias because of the BPLM intake. No correction and adjustment for BPLM intake as a covariate, labeled as "fundamentally flawed" in a methods article on correction for medication (Tobin et al. 2005), delivered very similar results. Moreover, the results remained unchanged when one of the methods recommended by Tobin et al. (Tobin et al. 2005) was employed, namely the addition of a fixed value to the BP of medicated subjects. Right-censored regression, another recommended strategy, yielded slightly different results: some estimates were higher, others were lower, and the precision of the estimates differed somewhat. Estimates with right-censored regression mimicked the effect in non-medicated participants.

In addition to the aforementioned methods, I assessed the association with BP separately in medicated and non-medicated individuals. To avoid power loss, I calculated the effects in the model with interaction terms. In addition, BPLM intake was also included as an outcome. This method has the advantage of implying no assumptions regarding, for example, the treatment effect. Despite the BP-lowering effect of medication, the BP values in medicated individuals were substantially higher than in non-medicated individuals. This observation, together with the finding that known hypertension status has only a 66.4% overlap with the JNC7 definition, possibly hints at the fact that the medication was not effective in some participants. With regard to this finding, it is advisable to assess the effectiveness of medication when making the decision on the strategy to correct for the BPLM intake in the analysis of BP.

I observed the most consistent association of PM with BP in the group of medicated individuals, at both baseline and follow-up. In non-medicated individuals, the effect estimate was slightly lower and less precise than in medicated individuals. The results with NH_3 were very similar, with the only difference being that the association with BP was statistically significant in medicated and non-medicated individuals. The concentrations of PM and NH_3 were also related to lower risk for BPLM intake in the study participants (the estimated associations were statistically significant with the follow-up subcohort only). The hypothesis that air pollu-

tion-induced BP increase leads to BPLM intake and that one would see a positive association of PM with BPLM intake and a negative association of PM with BP was not confirmed with the results with PM and NH_3. Therefore, the mediation of the effect of background $PM_{2.5}$ by BPLM is less likely than confounding with noise. More likely, BPLM intake could be seen as a susceptibility factor in these subjects.

The situation with traffic-related pollutants, such as NO_2, CO, was slightly different. In contrast, the association of NO_2 with BP was robust only in non-medicated individuals. NO_2, O_3 and CO were imprecisely positively related to an elevated risk for BPLM intake. In agreement with this finding, I also observed a positive association of living close to traffic and in disadvantaged neighborhoods with BPLM intake. Traffic noise was imprecisely positively linked to BPLM intake. All exposures associated with higher BPLM intake are traffic-related. Therefore, it is possible that noise annoyance and low socio-economic status (personal or neighborhood-level) contribute to this association and confound the association of air pollution with BP. In this case, one should use BPLM as a covariate in the adjustment set. The exposure to noise, different from air pollution, can be perceived personally and can lead to elevated BP through the stressor response mechanism (Babisch et al. 2014).

Self-reported BPLM intake had a very good overlap with the BPLM intake according to the study definition, and showed similar relationship with the studied exposures. This gives the reason to consider that self-report is quite valid source of information regarding BPLM intake. It is, however, not to forget, that some drugs, prescribed for conditions other than hypertension, may decrease BP was well (diuretics, for example). Therefore, if BPLM intake is used in the analysis to correct for BP values in medicated subjects, self-report may not be sufficient.

6.9. Confounding of long-term associations with temporal variation

Both the exposure and the outcome in the observational study are subject to temporal variation. The individual 365-day mean was used as a main exposure definition, representing long-term air pollution concentration. Over the three years of baseline or follow-up, the 365-day mean demonstrated temporal variation in addition to spatial variation. For example, the 365-day mean concentrations of two participants who lived close to each other but were scheduled for BP measurements on different dates would most likely differ. Such temporal changes might bias the analysis of long-term spatial differences in exposure, which was the primary interest in this study. Therefore, to overcome this problem, the time trend (a count of days from the first baseline or follow-up measurement date to the last, entered as a linear and squared term) was used in this analysis to adjust for confounding from temporal variation.

The results of this analysis, with and without adjustment for time trend, differed dramatically; without time trend, no association of exposure with outcome was observed. Therefore, time trend was always included as a covariate. In addition, season was an effect modifier: the association of $PM_{2.5}$ with BP was stronger in the summer than in other seasons. BP values are also variable over time: BP tends to be higher in cold periods and lower in warm periods (Alpérovitch et al. 2009; Rose 1961). In our data, seasonality was also observed and was more prominent for SBP than for DBP (Appendix Figure 29).

To verify whether temporal variation biased the analysis, an additional exposure definition was used: the grid-specific mean over the entire baseline or follow-up period. This definition did not contain the temporal variation component and did not require adjustment for time trend. The concentrations of grid-specific mean exposure were similar, but not identical, to the individual 365-day mean (for example, there was a correlation of 0.875 for $PM_{2.5}$ at baseline). I also observed a positive relationship of $PM_{2.5}$ with SBP that was close to, though slightly less precise than, the estimate with the 365-day mean, adjusted for time trend. A weaker association may be detected because the grid-specific mean is averaged over a longer time period; therefore, the variation in exposure concentration is smaller. The association of the grid-specific mean was more precise with DBP, possibly because DBP did not vary much over time and, in contrast to SBP, was not confounded by time trend.

6.10. Application of the longitudinal analysis in the study setting

I performed longitudinal analysis with BP in addition to cross-sectional one to test the causal inference. This analysis was done with three different strategies: analysis with baseline exposure and BP at the follow-up measurement, analysis with baseline exposure and change in BP from baseline to follow-up, and analysis with change in exposure and change in BP from baseline to follow-up.

It is shown that SBP increases with life span (Whelton 1994). I observed different rates of increase or even a decrease in certain subgroups of the study population. For example, SBP increased most rapidly among participants not taking BPLM, especially in the older age groups. I observed mostly a decrease in SBP from baseline to follow-up among participants taking BPLM. To take the different rates of progression into account, I performed the longitudinal analysis in the group of participants not taking BPLM.

DBP, opposite to SBP, decreases after certain age due to growing arterial stiffness (Whelton 1994). I observed a decrease in DBP in the study population, regardless of medication status. Therefore, the analysis with change in DBP might be particularly challenging in this study:

the decrease in air pollution from baseline to follow-up, possibly resulting in better cardiovascular health and lower BP, coincides with age-related decrease in DBP, also observed in the follow-up period.

Considering this, I used PP as an additional outcome. It reflects increase in SBP and decrease in DBP simultaneously, and have been shown an independent risk factor for cardiovascular disease, possibly more important than SBP or DBP, in older subjects (Franklin et al. 2001). Results with PP were generally very similar to the results with SBP and are in line with a reported positive association of long-term residential traffic exposure with PP (Rioux et al. 2010).

Longitudinal analysis with exposure at baseline and BP at follow-up has shown quite similar results to the cross-sectional analysis. This finding strengthens the hypothesis of causal association of air pollution with hypertension and BP related cardiovascular system pathology. However, it is necessary to consider an alternative explanation for this finding. I observed a high positive correlation between air pollution concentrations at baseline and after 5 years of follow-up, especially with grid cell means, representing the spatial contrasts in exposure. BP at follow-up is expectedly positively correlated with BP at baseline, especially among participants who underwent no intervention, such as BPLM intake or weight loss. It is therefore to expect that the analysis with baseline exposure and follow-up BP would produce results quite similar to cross-sectional analyses with baseline or follow-up data, which was observed in this study.

I have employed two other strategies, both of which included change in BP from baseline to follow-up as an outcome. I observed different results with these strategies: whereas exposure at baseline was mostly positively related to change in BP at follow-up, change in exposure from baseline to follow-up was negatively associated with change in BP. I also observed a high negative correlation of exposure at baseline with change in exposure during follow-up period for $PM_{2.5}$, PM_1, PN, and CO. Taken together, these results may reflect decreases in exposure, possibly a result of focused actions in the zones with especially high concentrations. The contra-intuitive findings of negative associations may also point out the fact that under these specific study conditions – when air quality improved but cardiovascular health of population with aging worsened – analysis of change with blood pressure is not an optimal analysis method.

It is recommended to use categorical outcomes, such as hypertension (possibly also divided to stages 1, 2, and isolated systolic hypertension) in the longitudinal analysis with BP. These

outcomes would possibly better reflect chronic deterioration in cardiovascular health, than change in BP as outcome. However, using categorical outcomes implies less power to detect significant associations, which was also observed in the current analysis as wider confidence intervals, not allowing to reject the null hypothesis of no association.

6.11. Different BP measures as outcomes

Apart from different progression with aging, discussed in 7.10, SBP and DBP also differ in terms of clinical meaning. SBP represents the heart function, whereas DBP can be seen a marker of arterial aging. For almost three quarters of the twentieth century – starting from the first measurements in early 1900s and until 1970s – DBP was considered a more important measure of risk then SBP (Franklin et al. 2001). In 1971, results of Framingham Heart Study follow-up assessment showed the "declining relative importance of diastolic and a corresponding increase in the importance of systolic pressure with advancing age" (Kannel et al. 1971). It has been shown that SBP better predicts cardiovascular risk in elderly, and DBP – in younger individuals (Franklin et al. 2001). PP has been shown an independent risk predictor for CHD, especially in subjects aged 60 and older (Franklin et al. 2001).

Systolic BP is increased if cardiac output increases (Pal and Pal 2005) or if early pulse wave reflections during systole affect left ventricular contraction, ejecting blood into the aorta and creating a pressure pulse (Benetos et al. 2003). Increasing arterial stiffness results in elevated SBP and decreased DBP (Benetos et al. 2003). As a result, PP will also increase with arterial aging. In addition, DBP levels can increase with increasing peripheral vascular resistance (Benetos et al. 2003). Therefore, depending on the mechanism of influence of air pollution on BP, long-term exposure to air pollution can theoretically result in increased SBP and PP and decreased DBP, or in increased DBP.

In the current study, I observed a similar direction of association – mostly positive – of air pollution with SBP, DBP, and PP. This finding is coherent with the current evidence. Some studies have reported a positive long-term association of air pollution with SBP only (e.g., Foraster et al. 2014), but most with both SBP and DBP (e.g., Chuang et al. 2011; Dong et al. 2013; Schwartz et al. 2012). Sørensen et al. (2012), who showed an inverse association of long-term exposure to NO_x with BP, reported similar results with SBP and DBP. A positive association of air pollution with both SBP and DBP could indirectly prove that two physiological pathways, namely, increasing peripheral vascular resistance and increasing arterial stiffness could be involved. This finding should be confirmed in further studies,

6.12. Association of road traffic noise with BP

The observational cohort allowed an analysis with air pollution and noise as simultaneous exposures in order to test for the independence of effects. In the results presented in this work, road traffic noise was associated with SBP and PP, independent of air pollution, but did not show associations with hypertension or BPLM intake. The association of $PM_{2.5}$ with BP was robust to adjustment for road traffic and tram noise. Therefore, the effects of noise and air pollution in the study population are likely independent of each other.

To date, there are only a few studies investigating noise and air pollution simultaneously. Most studies report a similar finding: the independence of air pollution and noise effects. For example, a positive association of road traffic noise with BP, independent of air pollution, was observed in a large Danish cohort (Sørensen et al. 2011); in the same cohort, the inverse association of NO_2 with BP was independent of road traffic noise (Sørensen et al. 2012). In a Dutch study, road traffic noise ≥ 55 dB was positively associated with hypertension in subjects aged 45 to 55 years, though not in the entire cohort; this association was independent of PM_{10} levels (de Kluizenaar et al. 2007). In a Swiss cohort, railway noise was positively associated with BP, independent of NO_2, whereas road traffic noise was associated with BP only in a subgroup of diabetic subjects (Dratva et al. 2012). In a part of the German KORA cohort (city of Augsburg), road traffic noise was positively associated with isolated systolic hypertension, whereas in the other part of the same cohort (greater Augsburg), noise was inversely related to hypertension; both findings were independent of $PM_{2.5}$ (Babisch et al. 2014). In the same cohort, $PM_{2.5}$ was positively linked to hypertension, independent of road traffic noise, although this relationship was not statistically significant (Babisch et al. 2014). The evidence of the association of road traffic noise as a single exposure with BP is inconsistent (Babisch 2010), although a positive association with hypertension has been reported in a recent meta-analysis of 24 studies (van Kempen and Babisch 2012).

It is possible that air pollution and noise affect different pathophysiologic pathways, or at least not completely overlapping ones. Whereas the adverse effect of air pollution on BP is likely dominated by an oxidative stress downstream cascade, road traffic noise is a stressor affecting the endocrine system and the ANS (Babisch et al. 2014).

6.13. Lessons learned from the analysis with different housekeeping genes

I found some variability in the expression of housekeeping genes in different exposure groups, especially noticeable for GAPDH. The activity levels of GAPDH (the product of this gene takes part in energy metabolism) were lower in groups with high concentrations of NO_2. GAPDH is a multifunctional protein that is involved in microtubule bundling modification,

membrane fusion, calcium flux modification, gene transcription, DNA repair and replication, nuclear RNA export, and cell death (Hara et al. 2006). It is highly speculative whether this could be a real and not a chance finding. However, at least one study has showed that GAPDH can react with peroxynitrite, a product of NO_2 (Mohr al. 1994). NO can also trigger posttranslational modification of GAPDH, which may mediate neuronal cell death (Hara et al. 2006).

It is recommended to check the expression of housekeeping genes in the study groups, which will allow detecting any inconsistencies. It is also necessary to present the crude expression of the genes that are studied, in addition to the one corrected for the housekeeping genes activity, and study the differences thoroughly. A careful consideration for the choice of the housekeeping gene for the measurement is advisable.

6.14. Strengths and limitations of the analysis

There are some limitations of the observational study. First, measurement of BP was performed once at baseline and at follow-up, which has implications for the analysis with both BP and hypertension. BP is highly variable parameter, and individual variation may conceal relatively small changes caused by air pollution. Defining hypertension based on a single measurement may also lead to misclassification in those individuals whose BPs get unexpectedly low or high at the time of measurement (for example, “white coat hypertension” or cold-induced hypertension). Few repeated measurements, preferably in different seasons, are desirable for the correct identification of hypertension. Second, a longitudinal analysis of the air pollution effect in the HNR cohort is challenging: the exposure concentrations decreased over the follow-up time whereas the outcome naturally increased with age. At the same time, the cross-sectional analysis precludes a causal interpretation of the complex interplay between environmental factors, related confounders and individual susceptibility. Third, no information on actual exposure dose (e.g., how many hours the participant spends at home each day) was included in the air pollution assessment. Finally, despite the significant effort made to produce unbiased estimations, there is always a chance for residual confounding due to some unmeasured factors in the observational study.

At the same time, the observational study had multiple strengths. A well-characterized, large population-based cohort was used. The measurements were performed by certified personnel using standardized protocols. All data were checked for plausibility. The outcome measurements were performed according to the WHO recommendations three times with each participant, and the mean of the second and the third measurements was used in the analysis, mini-

mizing the white coat effect. BPLM intake was assessed with WHO Anatomical Therapeutic Chemical classification categories, checking the working substance and the dose for each medication. Hypertension was defined according to the JNC7 definition (Chobanian et al. 2003). Self-reported hypertension and medication use were used as additional outcomes. The thorough and integrative exposure assessment employed and an assessment that accounted for different components of the air pollution mixture along with other residence-related environmental and social factors is the strength of this study. It was possible to investigate multiple environmental factors at once and their possible interactions. The availability of two time points in the HNR study allowed for the analysis to be repeated and for the baseline findings to be compared and confirmed at follow-up. Careful model specification and model checks enhanced the validity of the regression findings. As an additional strength for the data analysis, the effect of BPLM was investigated using multiple correction strategies.

The major limitation of the experimental study was the low number of animals (5 per group), which limits the power to identify the significant effects. To overcome this limitation, I aimed to increase the data quality using a standardized procedure for gene expression measurement. Using various analysis methods (correction with at least two housekeeping genes, sensitivity analyses, etc.), I attempted to test the robustness of the findings. Another limitation is that the results of an animal study may not be extrapolated to humans. However, only genes with identical functions in mice and men were chosen; therefore, this limitation is a minor one in this study. Finally, the experimental study is limited because only two exposures – diesel and NO_2 – were investigated. An additional difficulty was that, according to a predefined experimental design, exposure to diesel was only investigated in combination with NO_2; thus, it was not possible to assess the effect of DEE alone. Further studies with more exposure variants in groups could shed more light on the biologic pathways underlying the cardiovascular effects of air pollution.

7. SUMMARY AND OUTLOOK

The results of this study on the effect of long-term air pollution and its specific components on blood pressure and hypertension and on possible underlying pathophysiologic mechanisms can be summarized as follows:

→ Long-term exposure to ambient urban background $PM_{2.5}$ was associated with elevated BP in a population-based cohort, independent of relevant confounders and co-exposure to gaseous O_3, NO_2, NO, and CO in addition to other residential exposures (e.g., traffic volume, ambient noise, and neighborhood social deprivation).

→ The association of $PM_{2.5}$ with BP was linear (i.e., dose-dependent) without a threshold.

→ Subjects medicated with BPLM showed a higher increase in BP related to air pollution. It is necessary to account for BPLM intake in the analyses with population-based cohorts, where a large proportion of individuals are taking BPLM.

→ No statistically significant association of air pollution with hypertension was observed, which is possibly due to a relatively small effect size and insufficient power. Further studies, employing longitudinal analysis techniques, could provide more clear results.

→ An unexpected and thus far not investigated association between atmospheric ammonia and BP was identified. It is a goal of future studies to clarify whether this association is real and what the underlying biological pathways are.

→ In the experimental study I found some confirmations that long-term exposure to traffic-related air pollution affects the activities of the genes involved in the immune response, the oxidative stress response and xenobiotic metabolism; at the same time, the activity of these genes is relevant for vascular pathology and elevated BP.

Blood pressure is a major modifiable risk factor for morbidity and mortality in the developed world. The proportion of hypertensive subjects in the population continues to grow. Small but persistent elevation in blood pressure due to exposure to air pollution could, on a population level, result in a substantially higher burden of CVD. Reductions of air pollution concentrations will therefore result in a noticeable decrease of rates of cardiovascular events and mortality in the population.

The findings on air-pollution–induced chronic BP elevation in this study were based on relatively low air pollution concentrations, quite common for Germany and other European coun-

tries, and below the current regulatory standards. Therefore, results of this study provide supportive evidence that efforts aimed at reducing air pollution levels below the current regulatory standards will result in substantial population health benefits.

The findings of this study show, on one hand, a consistent association of fine particulate matter with elevated BP in a population-based cohort, and, on the other hand, supportive evidence that traffic-related air pollution can induce inflammation, oxidative stress and vascular reactions, which might favor hypertension in the long run. These findings support the hypothesis that air-pollution–induced increases in BP could be one possible biological pathway of how long-term PM exposure may promote atherosclerosis, a common underlying pathology for cardiovascular morbidity and mortality.

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APPENDIX

Table 38. Estimates for the covariates in the main model with SBP at baseline.

Variable (increment)	Change, mmHg (95%-CI)	p-value
Intercept	53.008 (40.382, 65.633)	0.000
$PM_{2.5}$ (365-day mean) (1 µg/m³)	0.471 (0.087, 0.855)	0.016
Time trend (centered) (100 days)	-0.367 (-0.603, -0.132)	0.002
Time trend squared (centered) (100 days²)	0.003 (0.002, 0.004)	0.000
$PM_{2.5}$ (7-day mean) (1 µg/m³)	0.083 (-0.009, 0.175)	0.076
Lnight (traffic) (5 dB)	0.327 (-0.008, 0.662)	0.056
Lden (tram) (5 dB)	0.002 (-0.039, 0.043)	0.928
Distance to road (1 km)	0.048 (-0.741, 0.837)	0.905
Age (1 year)	0.618 (0.517, 0.719)	0.000
Sex (male)	8.182 (6.405, 9.958)	0.000
BMI (centered) (1 kg/m²)	0.617 (0.459, 0.774)	0.000
BMI squared (centered) (1 (kg/m²)²)	-0.022 (-0.037, -0.007)	0.004
WHR	13.915 (4.411, 23.420)	0.004
LDL/HDL ratio	1.097 (0.438, 1.756)	0.001
HDL (mg/dL)	0.134 (0.086, 0.182)	0.000
Triglycerides (mg/dL)	0.024 (0.018, 0.030)	0.000
Smoking (current)	(reference)	
Smoking (former)	2.528 (0.913, 4.143)	0.002
Smoking (never)	2.946 (1.337, 4.555)	0.000
ETS	0.894 (-0.419, 2.206)	0.182
Physical activity (<1 time/week)	(reference)	
Physical activity (1 time/week)	-2.309 (-4.129, -0.489)	0.013
Physical activity (2-3 times/week)	-1.597 (-3.260, 0.066)	0.060
Physical activity (>3 times/week)	-2.463 (-3.857, -1.069)	0.001
Education (low)	(reference)	
Education (medium)	-0.053 (-1.877, 1.771)	0.954
Education (high)	-0.943 (-3.001, 1.115)	0.369
Economic activity (employed)	(reference)	
Economic activity (unemployed)	-1.095 (-3.450, 1.261)	0.362
Economic activity (homemaker)	1.409 (-0.550, 3.368)	0.159
Economic activity (retired)	0.721 (-1.011, 2.452)	0.415
N=4,584.		

Table 39. Estimates for the covariates in the main model with DBP at baseline.

Variable (increment)	**Change, mmHg (95%-CI)**	**p-value**
Intercept	59.785 (53.360, 66.209)	0.000
$PM_{2.5}$ (365-day mean) (1 µg/m³)	0.313 (0.105, 0.521)	0.003
Time trend (centered) (100 days)	-0.409 (-0.536, -0.281)	0.000
Time trend squared (centered) (100 days²)	0.001 (0.000, 0.001)	0.000
$PM_{2.5}$ (7-day mean) (1 µg/m³)	0.022 (-0.028, 0.071)	0.392
Lnight (traffic) (5 dB)	0.098 (-0.084, 0.279)	0.291
Lden (tram) (5 dB)	0.003 (-0.019, 0.026)	0.760
Distance to road (1 km)	-0.013 (-0.440, 0.414)	0.953
Age (centered) (1 year)	-0.104 (-0.159, -0.049)	0.000
Age squared (centered) (1 year²)	-0.011 (-0.016, -0.006)	0.000
Sex (male)	4.189 (3.227, 5.150)	0.000
BMI (centered) (1 kg/m²)	0.400 (0.315, 0.486)	0.000
BMI squared (centered) (1 (kg/m²)²)	-0.020 (-0.028, -0.012)	0.000
WHR	7.157 (2.003, 12.311)	0.007
LDL/HDL ratio	0.682 (0.322, 1.042)	0.000
HDL (mg/dL)	0.080 (0.054, 0.106)	0.000
Triglycerides (centered) (mg/dL)	0.017 (0.013, 0.022)	0.000
Triglycerides squared (centered) ((mg/dL)²)	0.000 (0.000, 0.000)	0.037
Smoking (current)	(reference)	
Smoking (former)	1.499 (0.625, 2.372)	0.001
Smoking (never)	2.165 (1.294, 3.037)	0.000
ETS	0.474 (-0.235, 1.184)	0.190
Physical activity (<1 time/week)	(reference)	
Physical activity (1 time/week)	-0.555 (-1.540, 0.429)	0.269
Physical activity (2-3 times/week)	-0.587 (-1.488, 0.313)	0.201
Physical activity (>3 times/week)	-0.892 (-1.647, -0.138)	0.020
Education (low)	(reference)	
Education (medium)	0.146 (-0.842, 1.134)	0.772
Education (high)	-0.200 (-1.315, 0.914)	0.725
Economic activity (employed)	(reference)	
Economic activity (unemployed)	-0.352 (-1.631, 0.926)	0.589
Economic activity (homemaker)	0.712 (-0.352, 1.776)	0.190
Economic activity (retired)	0.324 (-0.613, 1.261)	0.498
N=4,584.		

Table 40. Estimates for the covariates in the main model with PP at baseline.

Variable (increment)	**Change, mmHg (95%-CI)**	**p-value**
Intercept	-9.277 (-18.061, -0.494)	0.038
$PM_{2.5}$ (365-day mean) (1 µg/m³)	0.172 (-0.096, 0.439)	0.208
Time trend (centered) (100 days)	0.039 (-0.125, 0.203)	0.638
Time trend squared (centered) (100 days²)	0.002 (0.002, 0.003)	0.000
$PM_{2.5}$ (7-day mean) (1 µg/m³)	0.060 (-0.004, 0.124)	0.065
Lnight (traffic) (5 dB)	0.231 (-0.002, 0.465)	0.052
Lden (tram) (5 dB)	-0.003 (-0.031, 0.026)	0.850
Distance to road (1 km)	0.050 (-0.499, 0.599)	0.859
Age (1 year)	0.724 (0.653, 0.794)	0.000
Sex (male)	4.026 (2.790, 5.261)	0.000
BMI (centered) (1 kg/m²)	0.211 (0.101, 0.321)	0.000
BMI squared (centered) (1 (kg/m²)²)	-0.002 (-0.012, 0.009)	0.745
WHR	5.893 (-0.719, 12.506)	0.081
LDL/HDL ratio	0.357 (-0.102, 0.815)	0.128
HDL (mg/dL)	0.053 (0.020, 0.087)	0.002
Triglycerides (mg/dL)	0.010 (0.006, 0.014)	0.000
Smoking (current)	(reference)	
Smoking (former)	0.987 (-0.137, 2.111)	0.085
Smoking (never)	0.711 (-0.409, 1.830)	0.214
ETS	0.398 (-0.515, 1.311)	0.393
Physical activity (<1 time/week)	(reference)	
Physical activity (1 time/week)	-1.759 (-3.025, -0.493)	0.007
Physical activity (2-3 times/week)	-1.041 (-2.198, 0.116)	0.078
Physical activity (>3 times/week)	-1.559 (-2.529, -0.589)	0.002
Education (low)	(reference)	
Education (medium)	-0.300 (-1.569, 0.970)	0.644
Education (high)	-0.835 (-2.267, 0.597)	0.253
Economic activity (employed)	(reference)	
Economic activity (unemployed)	-0.997 (-2.636, 0.642)	0.233
Economic activity (homemaker)	0.457 (-0.906, 1.820)	0.511
Economic activity (retired)	0.365 (-0.840, 1.569)	0.553
N=4,584.		

Table 41. Estimates for the covariates in the main model with prevalent hypertension at baseline.

Variable (increment)	**OR (95%-CI)**	**p-value**
Intercept	0.003 (0.001, 0.012)	0.000
$PM_{2.5}$ (365-day mean) (1 µg/m³)	1.000 (0.955, 1.046)	0.994
Time trend (centered) (100 days)	0.989 (0.962, 1.018)	0.454
Time trend squared (centered) (100 days²)	1.000 (1.000, 1.000)	0.009
$PM_{2.5}$ (7-day mean) (1 µg/m³)	1.006 (0.995, 1.017)	0.312
Lnight (traffic) (5 dB)	1.021 (0.981, 1.063)	0.307
Lden (tram) (5 dB)	0.999 (0.994, 1.004)	0.749
Distance to road (1 km)	0.978 (0.890, 1.074)	0.637
Age (1 year)	1.072 (1.059, 1.085)	0.000
Sex (male)	1.249 (1.012, 1.541)	0.038
BMI (centered) (1 kg/m²)	1.096 (1.076, 1.117)	0.000
BMI squared (centered) (1 (kg/m²)²)	1.001 (0.999, 1.004)	0.318
WHR	5.830 (1.872, 18.157)	0.002
LDL/HDL ratio	0.946 (0.869, 1.030)	0.203
HDL (mg/dL)	0.999 (0.993, 1.005)	0.699
Triglycerides (mg/dL)	1.003 (1.002, 1.004)	0.000
Smoking (current)	(reference)	
Smoking (former)	1.378 (1.139, 1.668)	0.001
Smoking (never)	1.252 (1.036, 1.512)	0.020
ETS	1.108 (0.949, 1.295)	0.195
Physical activity (<1 time/week)	(reference)	
Physical activity (1 time/week)	0.886 (0.715, 1.098)	0.268
Physical activity (2-3 times/week)	0.788 (0.649, 0.957)	0.016
Physical activity (>3 times/week)	0.750 (0.636, 0.884)	0.001
Education (low)	(reference)	
Education (medium)	0.850 (0.682, 1.060)	0.149
Education (high)	0.854 (0.667, 1.095)	0.213
Economic activity (employed)	(reference)	
Economic activity (unemployed)	1.024 (0.779, 1.345)	0.866
Economic activity (homemaker)	1.083 (0.861, 1.362)	0.497
Economic activity (retired)	1.167 (0.953, 1.430)	0.136
N=4,584.		

Table 42. Estimates for the covariates in the main model with BPLM use at baseline.

Variable (increment)	**OR (95%-CI)**	**p-value**
Intercept	0.013 (0.003, 0.063)	0.000
$PM_{2.5}$ (365-day mean) (1 µg/m³)	0.982 (0.937, 1.028)	0.434
Time trend (centered) (100 days)	1.031 (1.003, 1.061)	0.032
Time trend squared (centered) (100 days²)	1.000 (1.000, 1.000)	0.612
$PM_{2.5}$ (7-day mean) (1 µg/m³)	1.001 (0.990, 1.012)	0.866
Lnight (traffic) (5 dB)	1.007 (0.968, 1.049)	0.719
Lden (tram) (5 dB)	0.999 (0.994, 1.004)	0.759
Distance to road (1 km)	0.952 (0.866, 1.048)	0.316
Age (1 year)	1.065 (1.052, 1.078)	0.000
Sex (male)	0.700 (0.564, 0.868)	0.001
BMI (centered) (1 kg/m²)	1.075 (1.054, 1.096)	0.000
BMI squared (centered) (1 (kg/m²)²)	1.002 (0.999, 1.004)	0.146
WHR	7.261 (2.294, 22.983)	0.001
LDL/HDL ratio	0.789 (0.720, 0.866)	0.000
HDL (mg/dL)	0.980 (0.974, 0.986)	0.000
Triglycerides (mg/dL)	1.001 (1.001, 1.002)	0.000
Smoking (current)	(reference)	
Smoking (former)	1.336 (1.095, 1.631)	0.004
Smoking (never)	1.281 (1.050, 1.563)	0.015
ETS	1.095 (0.933, 1.284)	0.266
Physical activity (<1 time/week)	(reference)	
Physical activity (1 time/week)	0.866 (0.696, 1.078)	0.198
Physical activity (2-3 times/week)	0.841 (0.688, 1.028)	0.091
Physical activity (>3 times/week)	0.755 (0.637, 0.895)	0.001
Education (low)	(reference)	
Education (medium)	0.911 (0.736, 1.128)	0.393
Education (high)	1.030 (0.807, 1.313)	0.814
Economic activity (employed)	(reference)	
Economic activity (unemployed)	1.023 (0.759, 1.378)	0.882
Economic activity (homemaker)	1.161 (0.912, 1.477)	0.226
Economic activity (retired)	1.204 (0.980, 1.478)	0.076
N=4,584.		

Table 43. The distribution of exposure, outcome and most characteristics in the baseline analysis subset and among participants excluded from the analyses at baseline.

Variable (unit), statistics	Analysis subset (N=4,584)		Excluded (N=230)	
	Description	N missing	Description	N missing
$PM_{2.5}$ (µg/m³), Mean ± SD	16.7 ± 1.6	0	16.4 ± 1.6	5
PM_{10} (µg/m³), Mean ± SD	20.7 ± 2.6	0	20.5 ± 2.6	5
PMcoarse (µg/m³), Mean ± SD	4.0 ± 1.6	0	4.2 ± 1.8	5
PM_1 (µg/m³), Mean ± SD	11.6 ± 1.3	0	11.4 ± 1.3	5
PN (×10^4/L), Mean ± SD	8.8 ± 1.9	0	8.7 ± 1.9	5
O_3 (µg/m³), Mean ± SD	35.4 ± 1.6	0	35.4 ± 1.6	5
NO_2 (µg/m³), Mean ± SD	40.1 ± 4.2	0	39.5 ± 4.3	5
NO (µg/m³), Mean ± SD	12.7 ± 4.5	0	12.2 ± 4.2	5
SO_2 (µg/m³), Mean ± SD	8.7 ± 1.1	0	8.6 ± 1.1	5
CO (µg/m³), Mean ± SD	0.3 ± 0.1	0	0.3 ± 0.1	5
NH_3 (µg/m³), Mean ± SD	2.6 ± 0.4	0	2.5 ± 0.3	5
SBP (mmHg), Mean ± SD	133.2 ± 20.8	0	132.8 ± 21.6	15
DBP (mmHg), Mean ± SD	81.4 ± 10.9	0	81.5 ± 11.0	14
PP (mmHg), Mean ± SD	51.7 ± 14.7	0	51.4 ± 15.6	15
Hypertension, n (%)	2611 (57.0%)	0	151 (65.7%)	31
BPLM use, n (%)	1628 (35.5%)	0	87 (37.8%)	16
Self-reported hypertension, n (%)	1955 (42.7%)	10	100 (43.7%)	1
Self-reported BPLM use, n (%)	1432 (31.2%)	1	65 (28.5%)	2
Age (years), Mean ± SD	59.6 ± 7.8	0	59.9 ± 8.1	0
Sex (male), n (%)	2274 (49.6%)	0	121 (52.6%)	0
CHD, n (%)	296 (6.5%)	0	46 (20.0%)	15
T2DM, n (%)	623 (13.6%)	0	29 (12.6%)	0
BMI (kg/m²), Mean ± SD	27.9 ± 4.6	0	28.1 ± 5.5	29
LDL:HDL ratio, Mean ± SD	2.7 ± 1.1	0	2.8 ± 1.1	38
Smoking, n (%)		0		10
Current	1064 (23.2%)		74 (32.2%)	
Former	1585 (34.6%)		87 (37.8%)	
Never	1935 (42.2%)		89 (38.7%)	
Pack-years, Mean ± SD	16.1 ± 24.6	112	17.8 ± 25.8	19
ETS exposure, n (%)	1658 (36.2%)	0	104 (45.2%)	17
Alcohol (drinks/week), Mean ± SD	5.3 ± 10.2	107	5.1 ± 10.7	16
No sport, n (%)	2100 (45.8%)	0	127 (55.2%)	1

Variable (unit), statistics	Analysis subset (N=4,584)		Excluded (N=230)	
	Description	**N missing**	**Description**	**N missing**
Education, n (%)		0		16
<10 years	517 (11.3%)		46 (20.0%)	
11–17 years	2558 (55.8%)		134 (58.3%)	
>=18 years	1509 (32.9%)		82 (35.7%)	
Economic activity, n (%)		0		15
Employed	1847 (40.3%)		96 (41.7%)	
Unemployed	290 (6.3%)		30 (13.0%)	
Homemakers	642 (14.0%)		36 (15.7%)	
Retired	1805 (39.4%)		113 (49.1%)	

Table 44. The distribution of exposure, outcome and most characteristics in the follow-up analysis subset and among participants excluded from the analyses at follow-up.

Variable (unit), statistics	Analysis subset (N=3,240)		Excluded (N=232)	
	Description	N missing	Description	N missing
$PM_{2.5}$ (µg/m³), Mean ± SD	15.6 ± 1.4	0	15.7 ± 1.3	0
PM_{10} (µg/m³), Mean ± SD	19.8 ± 3.1	0	20.2 ± 3.3	0
PMcoarse (µg/m³), Mean ± SD	4.2 ± 2.4	0	4.5 ± 2.7	0
PM_1 (µg/m³), Mean ± SD	11.0 ± 1.1	0	11.1 ± 1.1	24
PN (×10^4/L), Mean ± SD	7.7 ± 1.7	0	7.7 ± 1.5	24
O_3 (µg/m³), Mean ± SD	37.6 ± 2.0	0	37.4 ± 2.0	24
NO_2 (µg/m³), Mean ± SD	38.6 ± 4.7	0	39.3 ± 4.8	24
NO (µg/m³), Mean ± SD	7.7 ± 2.2	0	8.1 ± 2.3	24
SO_2 (µg/m³), Mean ± SD	7.4 ± 1.1	0	7.6 ± 1.1	24
CO (µg/m³), Mean ± SD	0.3 ± 0.0	0	0.3 ± 0.0	24
NH_3 (µg/m³), Mean ± SD	2.8 ± 0.3	0	2.8 ± 0.3	24
SBP (mmHg), Mean ± SD	134.6 ± 19.8	0	132.6 ± 19.5	11
DBP (mmHg), Mean ± SD	79.2 ± 10.5	0	77.3 ± 10.3	11
PP (mmHg), Mean ± SD	55.4 ± 14.7	0	55.3 ± 14.8	11
Hypertension, n (%)	2157 (66.6%)	0	158 (68.1%)	11
BPLM use, n (%)	1595 (49.2%)	0	117 (50.4%)	0
Self-reported hypertension, n (%)	1434 (45.4%)	80	107 (48.0%)	9
Self-reported BPLM use, n (%)	1391 (43.3%)	25	102 (44.5%)	3
Age (years), Mean ± SD	64.6 ± 7.6	0	66.3 ± 7.8	0
Sex (male), n (%)	1630 (50.3%)	0	101 (43.5%)	0
CHD, n (%)	263 (8.1%)	0	38 (16.4%)	7
T2DM, n (%)	614 (19.0%)	0	49 (21.1%)	0
BMI (kg/m²), Mean ± SD	28.3 ± 4.8	0	29.1 ± 5.9	11
LDL:HDL ratio, Mean ± SD	2.3 ± 0.8	0	2.3 ± 0.9	25
Smoking, n (%)		0		7
Current	552 (17.0%)		35 (15.1%)	
Former	1301 (40.2%)		88 (37.9%)	
Never	1387 (42.8%)		123 (53.0%)	
ETS exposure, n (%)	799 (24.7%)	0	49 (21.1%)	8
Alcohol (drinks/week), Mean ± SD	7.1 ± 11.2	0	5.6 ± 10.5	61
No sport, n (%)	1379 (42.6%)	0	123 (53.0%)	6
Education, n (%)		0		4

Variable (unit), statistics	Analysis subset (N=3,240)		Excluded (N=232)	
	Description	**N missing**	**Description**	**N missing**
<10 years	289 (8.9%)		55 (23.7%)	
11–17 years	1817 (56.1%)		122 (52.6%)	
>=18 years	1134 (35.0%)		63 (27.2%)	

Table 45. Stratified description of study population at baseline by BPLM intake.

Variable	**No BPLM intake (N=2,956)**	**BPLM intake (N=1,628)**	**p (T-test,χ^2-test)**
$PM_{2.5}$ [µg/m³], Mean ± SD	16.6 ± 1.6	16.7 ± 1.6	0.243
PM_{10} [µg/m³], Mean ± SD	20.7 ± 2.6	20.7 ± 2.7	0.467
PM_{coarse} [µg/m³], Mean ± SD	4.0 ± 1.6	4.0 ± 1.6	0.994
PM_1 [µg/m³], Mean ± SD	11.6 ± 1.3	11.6 ± 1.3	0.138
PN [×10^4/L], Mean ± SD	8.8 ± 1.9	8.9 ± 1.9	0.202
O_3 [µg/m³], Mean ± SD	35.4 ± 1.6	35.5 ± 1.6	0.554
NO_2 [µg/m³], Mean ± SD	40.1 ± 4.2	40.3 ± 4.1	0.069
NO [µg/m³], Mean ± SD	12.6 ± 4.5	12.8 ± 4.5	0.130
SO_2 [µg/m³], Mean ± SD	8.7 ± 1.1	8.8 ± 1.1	0.033
CO [µg/m³], Mean ± SD	0.3 ± 0.1	0.3 ± 0.1	0.226
NH_3 [µg/m³], Mean ± SD	2.6 ± 0.4	2.6 ± 0.4	0.530
L_{den} (traffic) [dB], Mean ± SD	53.8 ± 9.6	54.2 ± 9.7	0.253
L_{den} (tram) [dB], Mean ± SD	12.1 ± 13.6	12.4 ± 13.6	0.433
Proximity to road [km], Mean ± SD	920.1 ± 781.9	868.3 ± 780.7	0.032
Area-level unemployment rate [%], Mean ± SD	12.4 ± 3.4	12.7 ± 3.5	0.003
SBP [mmHg], Mean ± SD	130.6 ± 20.3	137.8 ± 21.0	0.000
DBP [mmHg], Mean ± SD	81.1 ± 10.7	82.1 ± 11.1	0.002
PP [mmHg], Mean ± SD	49.5 ± 13.8	55.7 ± 15.6	0.000
Hypertension , %	33.3%	100.0%	0.000
Age [years]	58.1 ± 7.5	62.4 ± 7.5	0.000
Sex [male], %	48.8%	51.1%	0.140
CHD, %	1.5%	15.4%	0.000
T2DM, %	8.5%	22.9%	0.000
BMI [kg/m²]	27.1 ± 4.1	29.4 ± 5.0	0.000
Current smoker, %	26.1%	18.0%	0.000
Former smoker, %	33.1%	37.3%	
Never smoker, %	40.8%	44.7%	
ETS exposure, %	38.5%	31.9%	0.000
No sport, %	42.6%	51.7%	0.000
Education <10 years, %	9.6%	14.3%	0.000

N=4,584.

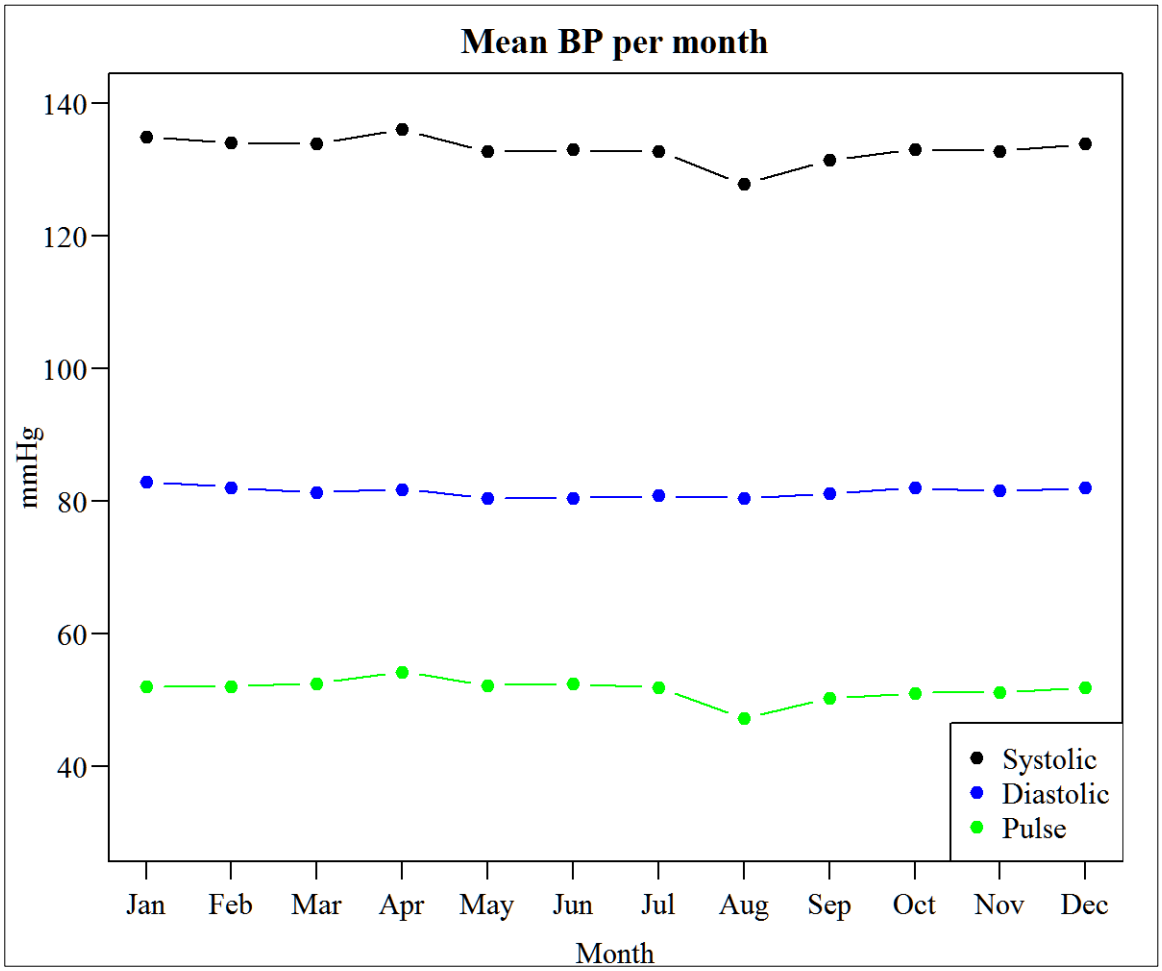

Figure 28. The mean BP values at baseline by the month of measurement.

Legend: N=4,584.

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PUBLICATIONS AND PRESENTATIONS

First authorship

Fuks KB, Weinmayr G, Foraster M, Dratva J, Hampel R, Houthuijs D, et al. 2014. Arterial Blood Pressure and Long-Term Exposure to Traffic-Related Air Pollution: An Analysis in the European Study of Cohorts for Air Pollution Effects (ESCAPE). Environ. Health Perspect.; doi:10.1289/ehp.1307725.

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Co-authorship

Künzli N, Perez L, Klot S von, Baldassarre D, Bauer M, Basagana X, et al. 2011. Investigating air pollution and atherosclerosis in humans: concepts and outlook. Prog. Cardiovasc. Dis. 53:334–43; doi:10.1016/j.pcad.2010.12.006.

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Posters

Fuks K, Riedel N, Dragano N, Viehmann A, Roggenbuck U, Nonnemacher M, et al. 2011. Residential history, duration of environmental and neighborhood socioeconomic exposures and mental and cardiovascular health (Poster). Yearly Conference of the International Society of Environmental Epidemiology. Barcelona, Spain. Published in: Envir Health Perspect 2011. Available: http://ehp.niehs.nih.gov/isee/PDF/isee11Abstract00760.pdf.

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LEBENSLAUF

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

ERKLÄRUNGEN

Erklärung:

Hiermit erkläre ich, gem. § 6 Abs. 2, f der Promotionsordnung der Math.-Nat. Fakultäten zur Erlangung der Dr. rer. nat., dass ich das Arbeitsgebiet, dem das Thema „ASSOCIATION OF LONG-TERM EXPOSURE TO AIR POLLUTION WITH ARTERIAL BLOOD PRESSURE AND HYPERTENSION“ zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von Kateryna Fuks befürworte.

Essen, den ……. Prof. Dr. Daniel Hoffmann ……..

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