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Effects of short-term exposure to fine and ultrafine particles from indoor sources on arterial stiffness

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DEDICATION

I dedicate my dissertation work to my family and many friends.

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Effects of short-term exposure to fine and ultrafine particles from indoor sources on arterial stiffness

Abstract

Objectives: Particulate air pollution is linked to adverse cardiovascular health effects. Up to date most studies have investigated ambient air pollution. The aim of the study was to investigate the effect of short-term exposure to indoor fine and ultrafine particles on arterial stiffness.

Methods: We analyzed the association of a 2 hour exposure to fine and ultrafine particle emissions from typical indoor sources (candle burning, toasting bread, frying sausages) with changes in arterial stiffness indices (Augmentation Index (AIx) and Augmentation Pressure (AP)) in 55 healthy volunteers in a randomized cross-over controlled exposure study. Particle emissions were quantified using 2 hour means of particle mass concentration (PMC), size-specific lung-deposited particle surface area concentration (PSC) and size-specific particle number concentration (PNC). AIx and AP were measured before, directly, 2, 4 and 24 hours after exposure. We performed multiple mixed linear regression analyses of different particle metrics and arterial stiffness indices adjusting for potential confounders.

Results: Overall, particle metrics showed different patterns among different sources. Measured PMC was highest during frying sausages with medians of 86.4 [µg/m³] and 75.2 [µg/m³] for PM_{10} and $PM_{2.5}$, respectively. Measured PSC and PNC were highest during candle burning with medians of 2,355.1 [µm²/cm³] and 2,070.3 x 10^3 [n/cm³], respectively. For candle burning**,** a PNC increment of 50,000 [n/cm³] was associated with an increase of 0.4 % (0.1; 0.6) in AIx directly after exposure. For toasting bread, exposure increases of 10 µg/m^3 in PM_{10} and $PM_{2.5}$ were associated with increases in AIx of 1.1% (95%-CI: 0.6; 1.6) 2 hours after exposure and 1.6% (95%-CI: 0.5; 2.7) directly after exposure, respectively. For frying sausages, a PSC increment of 1,000 [µm²/cm³] and a PNC increment of 50,000 [n/cm³] were associated with AIx increases of 2.1% (95%-CI: 0.5; 3.6) directly after exposure (PSC) and 1.0 (95%-CI: 0.3; 1.8) 24 hours after exposure (PNC), respectively.

Conclusions: Our study showed positive associations between short-term exposure to fine and ultrafine particle from indoor sources and arterial stiffness. The differences in chemical composition of source-specific particles might account for the differences observed for different particle size metrics and particle sources.

Effekte von kurzeitiger Exposition gegenüber feinen- und ultrafeinen Partikeln aus Innenraumquellen auf die arterielle Steifigkeit

Zusammenfassung

Zielsetzung: Partikuläre Luftverschmutzung ist mit unerwünschten kardiovaskulären Gesundheitseffekten verbunden. Ziel der Studie war es, die Wirkung einer kurzfristigen Exposition gegenüber feinen und ultrafeinen Partikeln aus typischen Innenraumquellen auf die arterielle Steifigkeit zu untersuchen.

Methodisches Vorgehen: Wir analysierten die Assoziation von Partikelemissionen aus Innenraum-Quellen (Kerzenverbrennung, Toasten, Braten) mit Änderungen der arteriellen Steifigkeit (Augmentationsindex (AIx) und Augmentationsdruck (AP)) bei 55 gesunden Probanden in einer randomisierten kontrollierten Cross-Over-Expositionsstudie. Die Partikelmassenkonzentration (PMC), die lungendeponierbare Partikeloberflächenkonzentration (PSC) und die größenspezifische Partikelzahlkonzentration (PNC) wurden während der 2-stündigen Exposition gemessen. AIx und AP wurden vor, unmittelbar nach sowie 2, 4 und 24 Stunden nach der Exposition gemessen. Multiple adjustierte gemischte lineare Regressionsanalysen verschiedener Partikel-Metriken und arterieller Steifigkeitsindizes wurden unter Berücksichtigung potenzieller Störfaktoren berechnet.

Ergebnisse: Die höchste PMC wurden beim Braten mit einem Median von 86.4 [µg/m³] und 75.2 [µg/m³] für PM_{10} bzw. $PM_{2.5}$ gemessen. Die höchste PSC und PNC wurden bei der Kerzenverbrennung mit einem Median von 2355.1 [µm²/cm³] bzw. 2070.3 x 103 [n/cm³] gemessen. Hinsichtlich des Abbrennens von Kerzen, war ein Inkrement von 50,000 [n/cm³] PNC mit einem Anstieg von 0.4 % (0.1; 0.6) im AIx unmittelbar nach der Exposition assoziiert. Hinsichtlich Toasten waren Inkremente von jeweils 10 $\mu g/m^3$ PM_{10} und $PM_{2.5}$ mit einem Anstieg von 1,1 % (95%-CI: 0.6; 1,6) des AIx 2 Stunden nach der Exposition (PM_{10}) bzw. 1,6 % (95%-CI: 0,5; 2,7) direkt nach der Exposition ($PM_{2.5}$) assoziiert. Hinsichtlich Braten, waren Inkremente von 1.000 [µm²/cm³] 50,000 [n/cm³] PNC mit einem Anstieg von 2,1% (95%-CI: 0,5; 3,6) des AIx unmittelbar nach der Exposition (PSC) bzw. 1,0% (95%-CI: 0,3; 1,8) des AIx 24 Stunden nach der Exposition (PNC) assoziiert.

Diskussion / Schlussfolgerung: Unsere Studie zeigte positive Assoziation zwischen einer kurzzeitigen Exposition gegenüber feinen und ultrafeinen Partikeln aus Innenraumquellen und der arteriellen Steifigkeit. Die Unterschiede in der chemischen Zusammensetzung der quellenspezifischen Partikel könnte ein Grund für die beobachteten Unterschiede für verschiedene Größenfraktionen und Partikelquellen sein.

List of Abbreviations

- % Percent
- °C Celsius
- µg Microgram
- µm Micrometer
- AHA American Heart Association
- AIx Augmentation Index
- AMS Aerosol Mass Spectrometry
- ANS Autonomic Nervous System
- AP Augmentation Pressure
- AS Arterial stiffness
- BMI Body-Mass-Index
- bpm Beats per minute
- Ca Calcium
- CI Confidence Interval
- cm Centimeter
- CO Carbon monoxide
- CV Cardiovascular
- CVD Cardiovascular Disease
- DBP Diastolic Blood Pressure
- EC Elemental Carbon
- ED Endothelial dysfunction
- EEA European Environment Agency
- EPIA Effects of ultrafine Particles from Indoor Activities
- ESCAPE European Study of Cohorts for Air Pollution Effects
- EU European Union
- Fe Iron
- FeNO Fraction of exhaled nitric oxide
- h Hour
- Hg Mercury
- HRV Heart rate variability
- IQR Interquartile range
- IUF Leibniz Research Institute for Environmental Medicine
- IUTA Institute of Energy and Environmental Technology
- K Potassium
- kg Kilogram
- LANUV State Agency for Nature, Environment and Consumer Protection
- m Meter
- m/s Meters per second
- m^2 Square Meters
- m^3 Cubic Meters
- mm Millimeter
- N Participant number
- NH_4 Ammonium
- nm Nanometer
- NO Nitric Oxide

- NO_2 Nitrogen dioxide
- NO_x Oxides of nitrogen
- NRW North Rhine Westphalia
- Ø Diameter
- O_3 Ozone
- OC Organic Carbon
- OR Odds Ratio
- PFT Pulmonary Function Test
- PM Particulate Matter
- $PM_{0.1}$ Particulate Matter with an aerodynamic Ø < 0.1 µm
- PM_1 Particulate Matter with an aerodynamic Ø < 1 µm
- PM_{10} Particulate Matter with an aerodynamic Ø < 10 µm
- $PM_{2,5}$ Particulate Matter with an aerodynamic Ø < 2,5 µm
- PMC Particle Mass Concentration
- PNC Particle Number Concentration
- PSC size-specific lung-deposited Particle Surface area Concentration
- PWA Pulse Wave Analysis
- PWV Pulse Wave Velocity
- RR Relative Risk
- SAS Statistical Analysis System
- SBP Systolic Blood Pressure
- SD Standard Deviation
- SO_2 Sulfur dioxide
- SO_4 Sulfate
- SOP Standard Operation Procedure
- Ti Titan
- TXRF Total Reflection X-ray Fluorescence
- UFP Ultrafine particles (particles with aerodynamic Ø < 100nm)
- WHO World Health Oganization
- Zn Zinc

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1. Introduction

1.1. Rationale - Background

Cardiovascular disease (CVD) is the number 1 cause of death globally, an estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths (World Health Organization (WHO) - CVD, 2016). Arterial stiffness is increasingly recognized as a risk factor for CVD, independent of conventional risk factors (Veerasamy et al. 2014). Arterial stiffness predates essential hypertension and has an impact on the coronary flow reserve even in nonsignificant coronary artery disease (Veerasamy et al. 2014). Furthermore, measurement of arterial elastic properties has been suggested as a tool for the assessment of subclinical target organ damage by the European Society of Hypertension and the European Society of Cardiology guidelines 2013 for the management of arterial hypertension (Mancia et al. 2013).

In addition to conventional cardiovascular risk factors (e.g. Hypertension and Diabetes), increasing evidence suggest that environmental exposures such as air pollution plays a role in the development of endothelial dysfunction and the progression of atherosclerosis (Brook et al. 2009) (Krishnan et al. 2012) (Künzli et al. 2010). Furthermore air pollution has been also associated with arterial stiffness (Mehta et al. 2014). Thus it is plausible to suggest that air pollution might play a role in arterial stiffness development. According to the Global Burden of Disease, Injuries, and Risk Factor study 2013, air pollution was responsible for 5·5 million deaths and 141.5 million disability-adjusted life-years (Christopher and Murray 2015). There is mounting epidemiological, biochemical and clinical evidence that demonstrate the effects of air pollution on cardiovascular health. (Brook et al. 2010) (Franklin, Brook, and Pope III 2015).

To date the majority of studies has focused on the investigation of the adverse health effects from ambient outdoor particles. Although about 90% of our time is spent indoors (Klepeis et al. 2001), much less evidence is available on the health effects of indoor particles. Many indoor activities such as burning processes, cooking activities and cleaning activities lead to increases in fine and especially ultrafine particles (UFP) (Fromme 2012). A recent review states that (UFP) defined as particles with aerodynamic diameters below 100nm contribute to about ~50% (ranging from 19% to 76% depending on resident activities) of residential particle exposure from indoor sources (Morawska et al. 2013). Although more research in this field is needed, initial data suggest that the high number concentration and high specific

surface area of UFPs are most likely the main characteristics responsible for greater toxicity and for adverse health effects (HEI Review Panel 2013) (Morawska et al. 2013).

Controlled human exposure studies have shown rapid adverse effects on arterial stiffness during short-term particulate matter (PM) exposure, with particle sources mainly being wood smoke, concentrated ambient particles and diesel exhaust (Unosson et al. 2013) (Brook et al. 2014) (Lundback et al. 2009). In addition, the use of short-term indoor air purifier interventions reducing the indoor $PM_{2.5}$ concentration was significantly associated with decreases in several circulating inflammatory biomarkers and improved endothelial function (Chen et al. 2015) (Allen et al. 2011).

The aim of this study is therefore to investigate whether exposure to fine and specifically ultrafine particles from common indoor activities such as burning candles (CB), frying sausages (FS) and toasting bread (TB) leads to changes in arterial stiffness in healthy volunteers. For this, we conduct a controlled cross-over chamber exposure study with healthy volunteers. We perform an in-depth characterization of emitted particles combining established exposure metrics i.e., size-specific particle mass concentration (PMC) with novel exposure metrics such as lung deposited surface area concentration (PSC) and size-specific particle number concentration (PNC) to detect a possible link between special characteristics of the emitted particles and arterial stiffness.

1.2. Air pollution

Sources and components of Air pollution

Air is life. We inhale at least 10,000 liters of air every day. Air pollution is contamination of the indoor or outdoor air by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere (WHO - Air pollution, 2016). According to European Environment Agency (EEA), the major sources of air pollution include industry, power generation, waste disposal, road transport, domestic sources and agriculture avoided (EEA - Sources of air pollution, 2016). Air pollutants can be classified in primary air pollutants, which are directly emitted into the atmosphere and secondary air pollutants, which are formed within the atmosphere itself. Air Pollutants of major public health concern include particulate matter, carbon monoxide (CO), ozone (O_3), oxides of nitrogen (NO_x) and sulfur dioxide (SO_2) (WHO - Air quality guidelines, 2005).

Measurement of particulate matter

Particulate matter is a complex mixture of extremely small particles and liquid droplets made up of acids, organic chemicals, metals, and soil or dust particles. According to its aerodynamic diameter, PM is generally classified into thoracic (Ø ≤ 10 μm; PM_{10}), fine (Ø ≤ 2.5 μm; $PM_{2.5}$), and ultrafine (Ø ≤ 0.1 μm; $PM_{0.1}$) particles. PM_{10} particles preferentially deposit in the upper airways, while the $PM_{2.5}$ and UFPs particles are much more likely to reach the smallest airways and alveoli (Nemmar et al. 2013). Larger particles (e.g. PM_{10}) make up the major portion of particle mass (Cassee et al. 2013), therefore measurements of PMC in [$\mu g/m^3$] are used from the EEA in air quality guide lines to define limit and target values for these particles (Guerreiro et al. 2015). Direct measurements of UFP mass are challenging because the mass concentration of particles in the UFP range is very low (HEI Review Panel 2013). One reasonable and common approach to measure UFPs is using PNC because 80% to 90% of PNC is in the ultrafine range in urban areas (Morawska et al. 1998)(Zhu et al. 2002). The greater surface area per mass compared with larger-sized particles of the same chemistry renders UFPs more active biologically (Oberdörster et al. 2005), thus assessment of PSC is another method for measuring UFPs (HEI Review Panel 2013). Based on this background, we used size-specific PM mass concentration for measurement of larger particles and particle number concentration or surface area concentration for the ultrafine fraction.

Air quality levels in Europe

From the EEA (European Environment Agency) 2015 air quality report, it is clear that application of air quality policies have delivered many improvements in reducing emissions for a number of pollutants (Guerreiro et al. 2015) However, the EU limits and target values for particulate matter (PM) continued to be exceeded in large parts of Europe in 2013. The EU daily limit value for PM_{10} (50 $\mu g/m^3$) was exceeded in 22 of the 28 EU Member States, and the target value for $PM_{2.5}$ (25 $\mu g/m^3$) was exceeded in 7 Member States (Guerreiro et al. 2015). Indoor PM levels are dependent on several factors including outdoor levels, infiltration, types of ventilation and filtration systems used, indoor sources, and personal activities of occupants (Chen and Zhao 2011) These factors can differ drastically between indoor building, thus, it is difficult to draw general conclusions for a uniform and widely applicable guideline for PM-Levels. However, WHO guidelines for indoor air quality do exist for selected pollutants commonly present in indoor air such as benzene, carbon monoxide, formaldehyde, naphthalene, nitrogen dioxide, polycyclic aromatic hydrocarbons (especially benzo[a]pyrene), radon, trichloroethylene and tetrachloroethylene (Penney et al. 2010).

Sources of particulate matter air pollution

The sources and composition of larger particles generally differ from those of smaller particles: coarse PM (particles 2.5–10 μm in diameter) consists in large part of insoluble earth crust- derived minerals, biological material (such as pollen, endotoxins, fungi, and bacteria), and sea salts (Adams et al. 2015). On the other hand, smaller particles such as UFPs are derived mainly from combustion processes that include the burning of wood and other forms of biomass, and the combustion of fossil fuels for transportation, energy production, home heating, and cooking (HEI Review Panel 2013). $PM_{2.5}$ (which includes the ultrafine fraction) includes combustion particles, which are often made up of a carbon core with attached hydrocarbons and metals, hydrocarbons, and secondary particles formed from oxides of sulfur and nitrogen (Adams et al. 2015)

Indoor sources of particulate matter

The mean time (h) adults spend daily at home is 15.7h/day in Germany, 15.6h/day in USA and 15.8h/day Canada (Brasche and Bischof 2005). Although in most societies today the larger portion of our time is spent indoors, only a small portion of studies found in my literature review investigate adverse health effects of small particles (e.g. PM) from indoor sources. However, initial studies indicate that specific indoor activities and combustion processes, like candle burning or open fireplaces, emit high amounts of fine and ultrafine particles (UFP), leading to human exposure levels that possibly cause pulmonary as well as systemic inflammation (Ghio et al. 2012) (Sørensen et al. 2005) (Ward and Noonan 2008). Afshari et al. (2005) have shown that candle burning, cooking food, cleaning activities and use of electric engines or furnaces emit high amounts of fine and ultrafine particles leading to considerable human exposure (Afshari, Matson, and Ekberg 2005). In a recent workshop on health risks of indoor PM, the main indoor sources of PM and UFP are listed have been categorized in combustion sources and non-combustion sources (Table 1). In addition it is important to highlight that outdoor PM penetration into the indoor environment can contribute substantially to indoor air (Chen and Zhao 2011). Furthermore, in a recent workshop on health risks of indoor PM, Brent Stephens addressed the need for more integration between epidemiologists and exposure scientists, building scientists, and indoor air scientists to explore all the different variables, which might have an impact on air exchange rates between outdoor and indoor air, such as window opening frequencies, building design characteristics and air conditioner usage (Butler, Madhavan, and Alper 2016). Although a detailed characterization of the many indoor sources is still a challenge, these studies provide some

information on the indoor sources, which might mediate some of the health related effects contributed to residential exposure.

Table1: **Indoor PM and UFP emission rates for combustion and non-combustion sources in homes** - Workshop on health risks of indoor PM (Butler et al. 2016)

UFP Emitting Device	Size Range (nm)	Emission Rate (#/min)	Reference
Flat iron with steam	20-1,000	6.0×10^{9}	Afshari et al. (2005)
Electric frying pan	10-400	$1.1\text{-}2.7\times10^{10}$	Buonnano et al. (2009)
3D printer w/PLA	10-100	$\sim2.0\times10^{10}$	Stephens et al. (2013)
Vacuum cleaner	20-1,000	3.5×10^{10}	Afshari et al. (2005)
Scented candles	20-1,000	8.8×10^{10}	Afshari et al. (2005)
Gas stove	20-1,000	1.3×10^{11}	Afshari et al. (2005)
3D printer w/ABS	10-100	$\sim1.9\times10^{11}$	Stephens et al. (2013)
Cigarette	20-1,000	3.8×10^{11}	Afshari et al. (2005)
Electric stove	20-1,000	6.8×10^{11}	Afshari et al. (2005)
Frying meat	20-1,000	8.3×10^{11}	Afshari et al. (2005)
Radiator	20-1,000	8.9×10^{11}	Afshari et al. (2005)
Desktop 3D printers	10-100	$\sim10^{8} - \sim10^{12}$	Azimi et al. (2016)
Laser printers	6-3,000	$4.3\times10^{9} - 3.3\times10^{12}$	He et al. (2010)
Cooking on a gas stove	10-400	$1.1\text{-}3.4\times10^{12}$	Buonnano et al. (2009)

NOTES: Highlighted items are combustion-related; all other items are non-combustion sources. PLA and ABS are thermoplastics used as 3D printer feedstock.

PM, particulate matter; UFP, ultra-fine particle.

Biological pathways

Although the exact mechanisms whereby PM is capable of triggering diverse health effects on the cardiovascular system is still unclear, the findings of multiple experiments suggest 3 general pathways; (Figure 1)

(1) a "spill- over"(release) of proinflammatory or oxidative stress mediators generated in the lungs into the systemic circulation

(2) the instigation of autonomic nervous system imbalance

(3) the penetration of certain particles or components directly into cardiovascular tissues.(Brook et al. 2010) (Franklin et al. 2015).

The First, inhalation of particulate air pollution could mediate the generation and release of proinflammatory mediators (e.g. cytokines) or vasoactive molecules (e.g. Endothelin, NO) within the lungs alveolae, with a subsequent systemic inflammation resulting in detrimental vascular effects (Brook et al. 2010). Second, pollutants may cause autonomic nervous system

(ANS) imbalance favoring sympathetic over parasympathetic tone, and this effect may be prompted by stimulation of one or more of the following receptors; pulmonary sensory receptors, baroreceptors, and carotid body chemoreceptors (Widdicombe and Lee 2001) (Perez, Hazari, and Farraj 2015). Third, particles and/or soluble components (e.g., metals) may translocate across the alveolar membrane into the bloodstream and directly influence the vascular endothelium (Peters et al. 2006) (Furuyama et al. 2009). Furthermore the relevance of each pathway in mediating health effects may vary depending upon time course of exposure, pollutant characteristics, susceptibility of the individuals, and likely overlap some degree in their adverse actions (Brook et al. 2010).

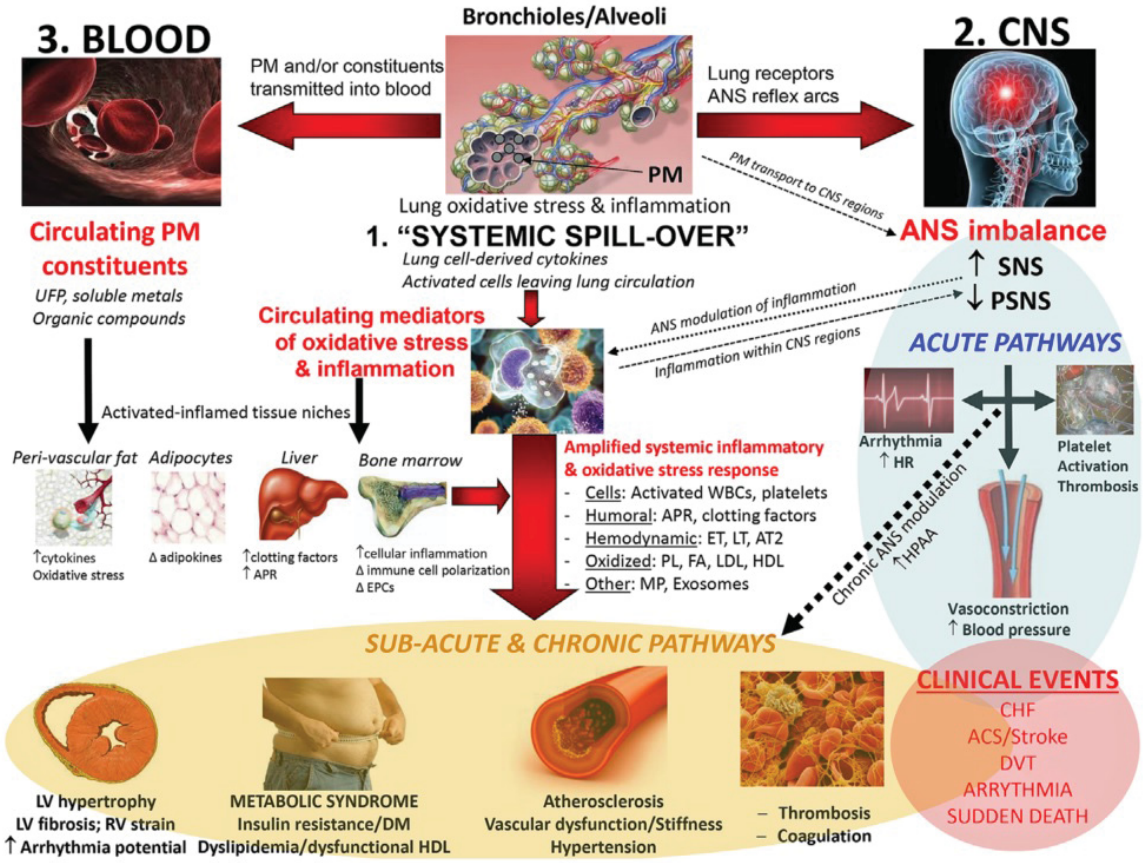

Figure 1: **Potential biological mechanism linking PM with cardiovascular disease and its sequelae**. ANS, autonomic nervous system; APR, acute phase response; AT2, angiotensin-2; DM, diabetes mellitus; CHF, congestive heart failure; CNS, central nervous system; DVT , deep venous thrombosis; EPCs, endothelial progenitor cells; ET, endothelins; FA, fatty acids; HDL, high- density lipoproteins; HR, heartrate; LDL, low-density lipoproteins; LT, leukotrienes; LV, left ventricle; PL, phospholipids; PSNS, parasympathetic nervous system; RV, right ventricle; SNS, sympathetic nervous system; UFP, ultrafine particles; WBCs, white blood cells. (Franklin et al. 2015)

In addition to the three biological pathways for PM, animal experiments have shown possible translocation of UFPs directly into the brain via the olfactory nerve (Oberdörster et al. 2004),

therefore a recent review on UFPs presented a slightly modified pathway figure for UFPs (Figure 2).

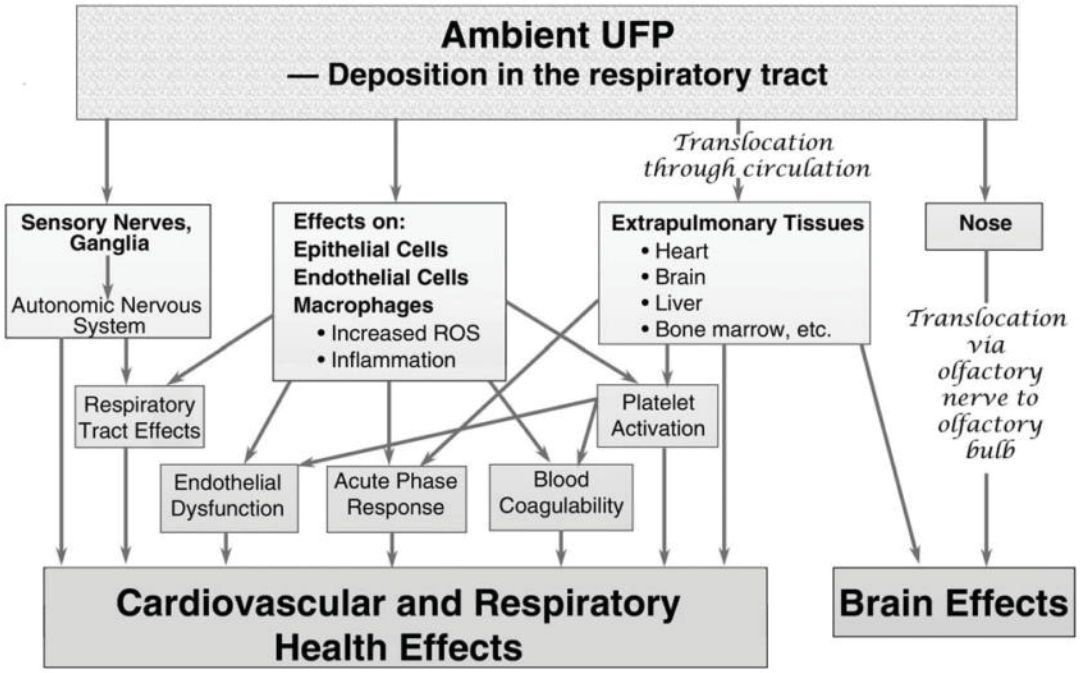

Figure 2**: Hypothesized pathways via which inhalation of UFPs may lead to effects on cardiovascular and respiratory systems and on the brain.** ROS, reactive oxygen species ; UFP, ultrafine particles (HEI Review Panel 2013)

1.3. Arterial stiffness and pulse wave analysis

Background (The arterial system: function and anatomy)

The hemodynamic function of the systemic arterial system is to deliver blood at high pressure and in a continuous stream to peripheral vascular beds. It can be separated into three anatomic regions with distinct functions: (1) The large elastic arteries (e.g. aorta, carotid) serve predominately as a cushioning reservoir or '*Windkessel*' that stores blood during ventricular ejection (systole) and expels it to the tissue during diastole, so that the capillaries receive a steady blood flow through the whole cardiac cycle. (2) The muscular arteries (e.g. radial, popliteal) actively modify wave propagation by changing smooth muscle tone and diameter with little change in mean arterial blood pressure, thus determining the extent to which and timing at which the reflected wave arrives back at the heart. (3) The arterioles, by changing their caliber, alter peripheral resistance and, therefore, aid in the maintenance of mean arterial

blood pressure, and the delivery of a steady or continuous flow of blood to the organs and tissues according to their need (Nichols, et al., 2011, p.77).

Arterial Stiffness - definition, pathology and mortality

Arterial stiffness (AS) is a term employed to define the arteries' capacity to expand and contract during the cardiac cycle. Arteries become stiffer with age due to alterations in their morphology and the composition of the major structural proteins, elastin and collagen (Greenwald et al. , 2007) (Avolio, Jones, and Tafazzoli-Shadpour 1998). The elastic properties of the arterial wall are mainly responsible for the dampening of the arterial pressure wave in the large arteries (e.g. aorta, carotid) (London and Pannier 2010). Stiffness of elastic arteries like the aorta is an independent predictor of all-cause mortality and has been linked to increased mortality, especially CV mortality, in patients with uncomplicated hypertension (Laurent et al. 2001), diabetes mellitus type 2 (Cruickshank et al. 2002), end-stage renal disease (Shoji et al. 2001), older individuals (Steppan et al. 2011) and even the general population (Hansen et al. 2006).

Arterial stiffness and its clinical assessment

Three types of arterial stiffness can be considered: local, regional or segmental, and systemic (Bortel et al. 2002). All available non-invasive methods focus on the quantification of a number of surrogate parameters that are related to the degree of arterial stiffness (Parati and Bernardi 2006). The three types of AS:

1. Local AS can be measured using an arterial ultrasound (Kullo et al.,2007) or magnetic resonance imaging (MRI) (Cavalcante et al.,2011). This method requires expensive equipment (especially in the case of MRI) and a high level of technical expertise and is often impractical within the clinical or epidemiological setting.

2. Pulse wave velocity (PWV), measured in [m/s] is used to measure regional AS. Measuring the PWV along the aortic and aorto-iliac pathway (i.e. carotid-femoral wave speed) is the most clinically relevant, due to the large amount of epidemiological evidence for its predictive value for CV events (Laurent et al. 2006).

3. Pulse wave analysis (PWA) has emerged as a non- invasive and widely used technique to investigate systemic arterial stiffness (Stoner, Lambrick, et al. 2012). In addition, the simplicity, validity, reliably and great clinical and epidemiological potential were the main advantages of PWA that were recently summarized (Stoner, Young, and Fryer 2012). Furthermore Augmentation Index (AIx) calculated by central PWA of the

aorta has been shown to be an independent predictor of future clinical CV events and all-cause mortality (Vlachopoulos et al. 2010).

This paragraph presented the different types of AS and their clinical significance. The outcomes presented in my thesis were measured by using PWA and therefore this method will be explained in detail.

Pulse wave reflection/Analysis

With each left ventricle contraction a pressure wave travels down the arterial tree (Forward pressure wave). This pressure wave is reflected in the periphery wherever there is a discontinuity in the arterial system (e.g. branching points, areas of alteration in arterial stiffness) and returns as a backward wave (backward pressure wave) against the direction of blood flow and toward the heart (Nichols et al., 2011 p.197) The reflected wave is fast (order of m/s), thus the pressure wave recorded at any site of the arterial tree is the sum of the forward and backward pressure wave (Figure 3) (Murgo et al. 1981) (Vlachopoulos and O'rourke 2000).

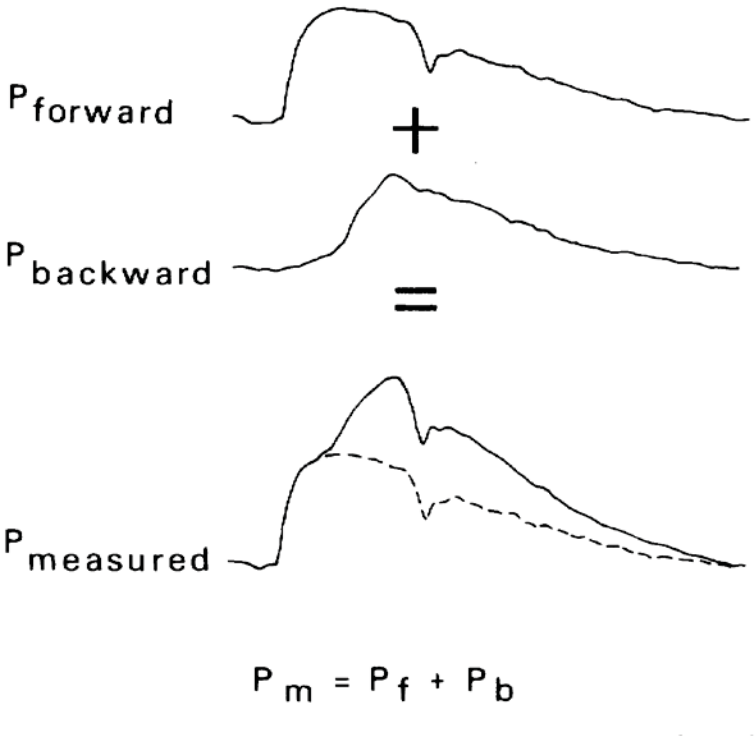

Figure 3: **Ascending aortic pressure wave (P measured) broken up into forward-traveling (P forward) and backward-traveling (P backward) waves under control conditions.** (Murgo et al. 1981)

In healthy individuals, when the large elastic arteries (e.g. aorta) are compliant (e.g. in a young adult), the forward propagated wave traveling from the heart is responsible for peak

systolic blood pressure (SBP) (Gkaliagkousi and Douma 2009). Under these circumstances, the forward traveling wave is relatively slow and leads to a proportionately slow reflected wave that tends to arrive back from the periphery in diastole, thereby augmenting diastolic blood pressure (DBP) (Figure 4) and preserving coronary perfusion (Gkaliagkousi and Douma 2009). In the case of stiff arteries (i.e. velocity of traveling wave is increased), the reflected wave arrives back at the central arteries earlier, causing augmentation of the systolic pressure and a consequent decrease in diastolic pressure. High central systolic pressures hasten the development of left ventricular hypertrophy, whereas low diastolic pressures reduce coronary artery perfusion (Mackenzie, Wilkinson, and Cockcroft 2002).

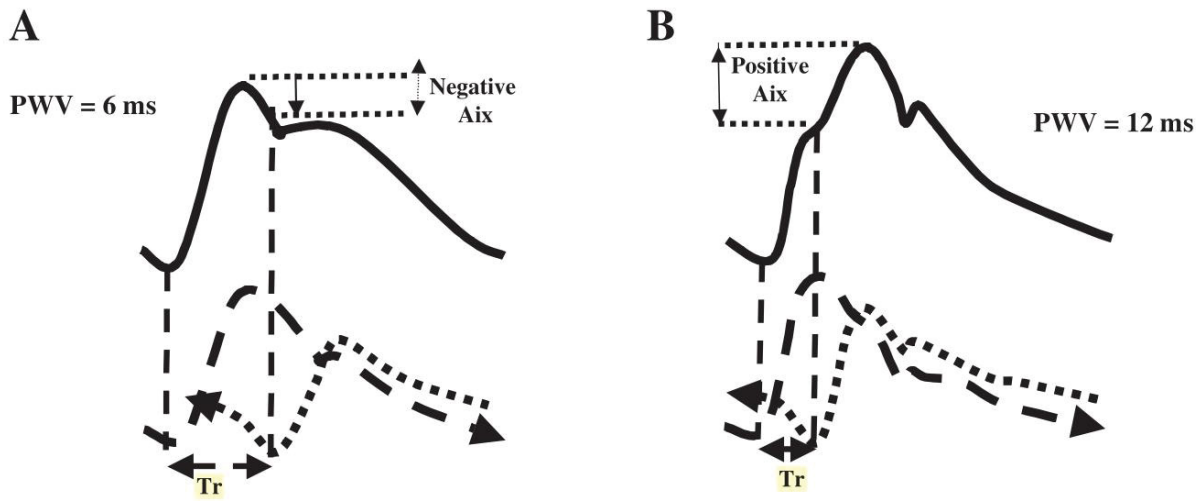

Figure 4: (A, B) **Influence of PWV on aortic pulse pressure shape and travelling time of pressure wave.** (A) Low PWV reflected wave impacts during late systole and diastole (long Tr), and AIx is negative. (B) High PWV reflected wave impacts during systole (shorter Tr), and AIx is positive.
AIx, Augmentation index ; Tr, travelling time of pressure wave from the aorta to reflecting sites and back (milliseconds); PWV, pulse wave velocity.(London and Pannier 2010)

How is the central (aortic) pulse wave synthesized?

Early recordings of ascending aortic pressure and flow waves, and pulse wave analysis (PWA), were obtained in the cardiac catheterization laboratory using high-fidelity multisensor (pressure and velocity) catheters (Nichols et al. 2011). Nowadays the non-invasive applantation tonometry is used to synthesize a reproducible and accurate representation of the aortic pressure waveform (O'Rourke and Seward 2006). Tonometry means "measuring of pressure," whereas applanation means "to flatten. A superficial artery (e.g. radial artery) is flattened (but not occluded) by placing a hand- held tonometer (pressure sensor) over the artery against solid structures such as bone. A mathematical equation using a fast Fourier transformation has resulted in a Food and Drug Administration approved algorithm that permits derivation and calculation of central pressure indices (e.g. AIx) from a peripheral brachial blood pressure and concomitant recording superficial pulse wave with applantation

tonometry (Chen et al. 1997). In our study we used the SphygomoCor® system from "AtCor" Medical, which utilizes the described mathematical formula and produces a synthesized central pulse wave with various central heamodynamic indices including AIx and AP.

Augmentation pressure and Augmentation index

The additional increase of the amplitude of the central blood pressure (i.e. central pulse pressure) due to the reflected wave is called augmented pressure (AP). The percentage of this augmentation in relation to the central pulse pressure is defined as central Augmentation index (AIx) (Figure 5).

$$AIx(\%) = \frac{AP}{PP} * 100\%$$

In healthy elastic arteries, AIx is related mainly to the magnitude of the reflected wave from small-artery tone/structure wave rather than to its velocity, whereas in stiff arteries the relationship between PWV and AIx is stronger (Kelly et al. 2001). In the Anglo-Cardiff Collaborative Trial (ACCT) performed in 4,001 healthy individuals, it was shown that both AIx and PWV increase with age but AIx increases more in young individuals and PWV increases more in older ones, suggesting that the former is a more sensitive marker of arterial stiffness in young and the latter is more sensitive in older subjects (McEniery et al. 2005)

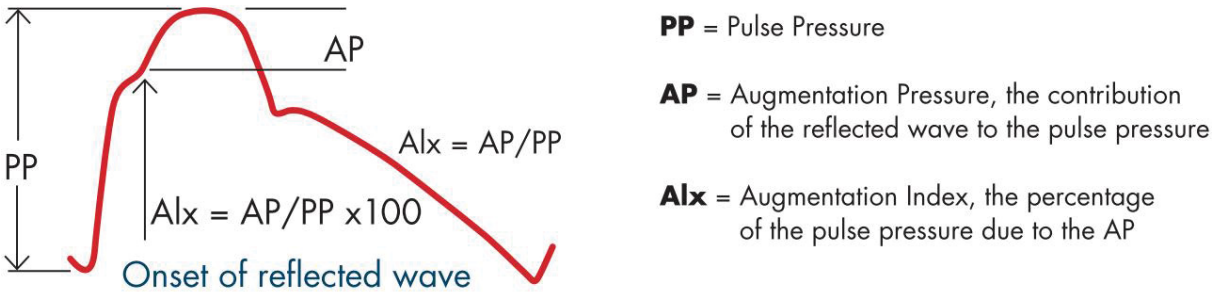

Figure 5: **Idealized central aortic pressure waveform**. Left ventricle ejection initiates the pressure wave. The return of the reflected wave before the conclusion of systole generates a deflection in the systolic contour upstroke (marked with the arrow) and produces an augmentation in the pressure profile. The total excursion of the pressure wave (PP) is the central pulse pressure. AP indicates augmentation pressure; AIx, augmentation index (Townsend, Black, et al. 2015).

Augmentation pressure and Augmentation index – Clinical significance

Early studies have shown that both central haemodynamic indexes (AIx and AP) have strong predictive value of cardiovascular disease (Nürnberger et al. 2002) (Weber et al. 2004). Their measurement, especially AIx, have been shown to be an independent predictors of future clinical CV events and all-cause mortality (Vlachopoulos et al. 2010). Furthermore a recent review concludes that central hemodynamic monitoring (e.g. AIx) has great potential to

provide a new diagnostic and therapeutic basis for preventing systemic target organ damage by offering personalized therapy suitable for the arterial properties in each individual patient with hypertension (Hashimoto 2014). In addition a very recent American Society of Hypertension position paper on central blood pressure waveforms stated that the clarification of both the value of measuring central pressure waveforms or measures of arterial stiffness, education of practitioners about interpreting central pressure data, and the best methodology to obtain pressure waveforms in clinical practice remains a need for broader consensus among investigators and policy makers (Townsend, Rosendorff, et al. 2015). In summary, although further research in needed to reach a wide consensus, the studies mentioned above demonstrates the big potential role of central pressure waveform indices (e.g. AIx) in clinical and research settings by adding valuable information to the risk assessment and management of patients, especially those with hypertension.

1.4. Air pollution and health

History of Air pollution and Health

During the past century several major incidents showed how acute changes in air quality can have devastating impact on human health. One of the first documented air pollution catastrophes was the fog in December 1930 in the Meuse valley in Belgium claiming 60 deaths in 3 days.(Nemery, Hoet, and Nemmar 2001) A land mark in air pollution epidemiology was the infamous London smog in December 1952 with an estimated death toll in excess of 12,000 people after reassessment in 2001 (Bell and Davis 2001). A more recent event was the 5 day smog episode in January 1985 in the Rhine-Ruhr area, increasing daily mortality by 8% (Wichmann 2004). These incidents triggered worldwide legislative activities, i.e. the establishment of air quality guide lines and regulatory acts in order to limit the adverse health effects of air pollutants.

Air pollution: still a major environmental risk to health?!

The major health effects of air pollution can be divided into short-term effects and long term effects. Although air quality policies in have delivered many improvements in reducing emissions, considerable impacts on human health and on the environment persist (Guerreiro et al. 2015). In 2013 air pollution, from indoor and outdoor sources, caused 5.5 million deaths in 2013 and was the 4th highest ranking risk for death in the world, being listed above other commonly recognized factors, such as low physical activity, a high-sodium diet, high

cholesterol, and drug use (Forouzanfar et al., 2015). Long-term epidemiological studies such as the European Study of Cohorts for Air Pollution Effects (ESCAPE) (Beelen et al. 2014), the American Cancer Society's Cancer Prevention Study II (CPS-II) (Jerrett et al. 2009) and the Rome Longitudinal Study (Cesaroni et al. 2013) provide evidence for relatively large health effects of chronic, long-term exposure air pollution to PM (e.g. $PM_{2.5}$), NO2 and ozone. The persistence of such health effects has many reasons. In 2014, 92% of the world population was living in places where the WHO air quality guidelines levels were not met (WHO – Ambient air quality, 2016). An analysis of results from the ESCAPE-Study showed that long-term exposure to particulate air pollution even within concentration ranges well below the present European annual mean limit value of 25 µg/m³ for $PM_{2.5}$ (European Commission air quality standards) were associated with natural-cause mortality (Beelen et al. 2014). These studies and others emphasize the ongoing impact of air pollution on our health in general and especially on the cardiovascular system.

1.5 Linking PM to cardiovascular disease outcomes

Particulate matter and the development of cardiovascular disease (long term)

Air pollution is not only associated with CVD, the overall evidence summarized in a scientific statement from the American Heart Association (AHA) suggests that particulate air pollution leads to CVD and increased CV mortality, especially for individuals who are already at risk for CVD or its complications (Brook et al. 2010). Recent epidemiologic evidences supporting this causality continues to grow (Franklin et al. 2015). Associations between particulate matter air pollution and a higher morbidity and mortality specifically of cardiovascular diseases are well documented in a substantial number of studies (Brook et al. 2010; Hoek et al. 2013). In a recent meta-analytic review of the literature of long-term air pollution exposure and mortality, a pooled estimate from the reviewed studies indicate that a 10-µg/m^3 increment increase in $PM_{2.5}$ is associated with increases of 6% (95% CI: 4%-8%) for all-cause mortality and 11% (95% CI: 5%-16%) for cardiovascular mortality (Hoek et al. 2013). In addition to cardiovascular mortality, in a sub analysis of the multicenter combined meta-analysis of up to 22 European cohorts (ESCAPE Project) with 11 cohorts substantial and statistically significant associations between fine PM and the incidence of acute coronary events, even at pollution levels below current European limits, were reported (Cesaroni et al. 2014). For a $PM_{2.5}$ under the current annual European limit value of 25 µg/m^3, a 5 µg/m^3 increase in estimated annual mean $PM_{2.5}$ was associated with a 18% increased risk of coronary events (hazard ratio 1.18, 95% confidence interval 1.01 to 1.39) and similar findings were seen for

PM_{10} under the current annual European limit value of 40 μg/m^3 (Cesaroni et al. 2014). A recent analysis of the ESCAPE Study has shown that long-term concentrations of $PM_{2.5}$ from traffic-specific particulate matter was positively associated with the with prevalence (OR 1.41 [1.10, 1.80]) and incidence of hypertension (RR 1.38 [1.03, 1.85]) (Fuks et al. 2016). In another study, Hoffmann et al. (2007,2009) showed long term residential exposure to traffic (e.g. $PM_{2.5}$) was also found to be positively associated with coronary atherosclerosis and peripheral atherosclerosis in the Heinz Nixdorf Recall cohort study with 4814 subjects (Hoffmann et al. 2007) (Hoffmann et al. 2009). Furthermore the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) with 6795 Subjects presents evidence that long term increased concentration of $PM_{2.5}$ is associated with the progression of coronary calcification, consistent with an acceleration of atherosclerosis (Kaufman et al. 2016). These studies demonstrate the ongoing accumulation of evidence, revealing the extent to which long term PM exposure effects the development of atherosclerosis and CVD.

Particulate matter and hypertension (Short-term)

Raised blood pressure (i.e. Hypertension) is closely linked to arterial stiffness (Payne, Wilkinson, and Webb 2010) and is considered to be one of the key risk factors for CVD (Kjeldsen et al. 2014). Controlled human exposure studies and observational data have shown rapid adverse effects on BP during short-term PM exposure (Brook and Rajagopalan 2009)(Urch et al. 2005). Brook et al. 2009 has shown that high blood pressure could mediate the effects of ambient air pollution on CV mortality (Brook et al. 2009). Moreover short-term increases in BP have been linked to PM-related triggering of cardiovascular events (Robert D. Brook et al., 2010). Furthermore the findings reviewed in a very recent updated review on air pollution and blood pressure presented an unequivocal relationship between particulate pollutants and BP with large public health repercussions (Giorgini et al. 2016). In addition evidence shown in the same review led to concluding that hypertensive consequences of $PM_{2.5}$ exposure represent one of the most important biological mechanisms explaining the role of particulate pollution as a modifiable factor contributing to CV morbidity and mortality (Giorgini et al. 2016). Although, many studies have strong evidence suggesting a causal relationship between particulate matter and hypertension, further research is important to definitively clarify the full nature of this issue.

Particulate matter and endothelial dysfunction (Short-term)

Another mechanism that has been proposed for acute changes in arterial stiffness is endothelial dysfunction (ED) (Tanaka and Safar 2005). ED is a systemic disorder and a critical element in the pathogenesis of atherosclerotic disease and its complications (Bonetti,

Lerman, and Lerman 2003). Flow-mediated dilation (FMD), the most common noninvasive method for measuring ED (Flammer et al. 2012), was significantly lower after a 2 hour exposure to concentrated ambient PM in an exposure study with 44 healthy volunteers (Tong et al. 2015). Another cohort of 93 elderly nonsmoking adults suggest that short-term exposures to traffic-related air pollutants contribute to microvascular dysfunction (Zhang et al. 2016). In addition, short-term secondhand smoke exposure, an important source of indoor particulate matter exposure in non-smokers, leads to a measurable disturbance of endothelial function (Bonetti et al. 2011). All these studies and others show a positive association between short-term PM exposure and ED, thus contributing to changes in AS.

1.6 Linking PM to arterial stiffness

Particulate matter and systemic arterial stiffness

Some studies have shown positive correlations between short-term exposures to air pollution, mainly from outdoor sources, and correlates of systemic arterial stiffness (AIx, AP). In a longitudinal analysis with 370 participant of the Veterans Affairs Normative Aging Study, 2007–2011, positive associations could be observed between short-term increases in ambient particulate pollutants and augmentation indices (AIx and AP) by using radial artery applanation tonometry for pulse wave analysis (Mehta et al. 2014). Based on the air quality data collected at a central monitoring station, an IQR(interquartile range increase) increase in short-term $PM_{2.5}$ exposure (3.6 $\mu g/m^3$) and PNC exposure (7,874 n/cm^3) was found to be associated with a 0.8 [%] (95% - CI: 0.2, 1.4) and a 2.2% (95% CI: 0.9, 3.5) increase in the AIx, respectively (Mehta et al. 2014). In a panel study with 26 male welders monitored for 24 hours on a welding day (6 hours of exposure), a 2.6-$\mu g/m^3$ IQR increase in $PM_{2.5}$ was associated with a 1.0% increase (95% CI: −0.3, 2.4) in AIx immediately post exposure compared with pre exposure and during exposure (Fang et al. 2008). In a randomized double blinded crossover human exposure study with 12 healthy volunteers, 10 min after a 60 min exposure with diluted diesel exhaust (approximately 350 $\mu g/m^3$) increases of 2.5[mmHg] (p=) and 7,8[%] (p=) in AP and Aix were observed compared to filtered air, respectfully. (Lundback et al. 2009). In another chamber exposure study, healthy male subjects breathing second hand smoke from 15 cigarettes in an unventilated room for 1 hour experienced a significant increase in aortic arterial stiffness (Mahmud and Feely 2004).In this study, a significant rise in the AIx, from -1.7 [%] ± 5.2 at baseline to 14 [%] ± 4.8 at the end of 60 min in males ($P < 0.001$) (Mahmud and Feely 2004). In yet another randomized double blinded

crossover human exposure study, 14 healthy volunteers were exposed to diluted wood smoke (average PM_1 was 314±38 μg/m^3) or filtered air for three hours during intermittent exercise (Unosson et al. 2013). The later study showed significant increases in central arterial stiffness, measured as AIx, AP and PWV, 10 min after the exposure as compared to filtered air ($p < 0.01$ for all) although there was no effect on blood pressure (Unosson et al. 2013). These studies demonstrate significant association between different metrics of particulate air pollution and correlates of systemic arterial stiffness, especially AIx. But the major amount of studies has shown such associations for outdoor sources, except for smoking and models of exposure to biomass combustion, and the effect of indoor sources on AIx and AP remain unexplored.

Indoor particles and cardiovascular health

Some studies have studied the impact of PM from indoor sources on health related effects. In a large population-based cohort with a total of 63,257 of middle-aged and elderly Chinese adults in Singapore, researchers have found that long-term exposure to incense burning at home on a daily basis for > 20 years was associated with increased risk of cardio vascular mortality (Pan et al. 2014). Next to long-term associations, short-term exposures to air pollution can also influence cardiovascular outcomes. In a randomized crossover intervention study, 45 healthy adults living in a wood smoke impacted community were exposed to consecutive 7-day periods of filtered and non-filtered air and the impact of particle exposures (i.e. $PM_{2.5}$) on endothelial function and systemic inflammation (both predictors of cardiovascular morbidity) was examined (Allen et al. 2011). Air filtration was associated with improved endothelial function and decreased concentrations of inflammatory biomarkers (Allen et al. 2011). This supports the theory that cardiovascular effects of particulate matter may be mediated through systemic inflammation and impaired endothelial function and this study shows that these effects may be favorably influenced by a reduction of particle concentrations (Allen et al. 2011). A short-term cross-sectional study investigated the relationship between exposure to airborne indoor and outdoor particulate matter (PM) and cardiovascular and respiratory health in a population-based sample of 58 residences in Copenhagen, Denmark (Karottki et al. 2014). Higher PNC -levels, which were mainly driven by candle burning, were associated with lower lung function, and with higher HbA1c and leukocyte counts. In the same study indoor $PM_{2.5}$ levels were positively associated with CRP (Karottki et al. 2014). In our previous publication on respiratory effects of fine and ultrafine particles from indoor sources, we found suggestive evidence for associations of short-term exposure to common indoor sources with decreases in lung function (Soppa et al. 2014).

These recent studies demonstrate some associations between long and short exposure to indoor sources and health-related changes and in on study cardio vascular mortality rates, but further investigation in this field would be both advantageous and beneficial.

1.7 Hypothesis and specific research questions

The main hypothesis is that a short-term exposure to fine and ultrafine particles from indoor sources (e.g. $PM_{2.5}$) leads to changes in central arterial pulse wave indices (AP and AIx). My specific research questions are:

1. To which extent does a 2 h short-term exposure to fine and ultrafine particles from indoor activities lead to increases in indices of arterial stiffness (AIx and AP) in a controlled exposure setting?
2. How does short-term exposure to fine and ultrafine particles from candles burning, toasting bread and frying sausages differ in their effect on indices of arterial stiffness (AIx and AP) in a controlled exposure setting?

1.8 Specific aims of the thesis

To investigate the research questions mentioned above, the specific aims of my thesis included:

1. To take part in the planning process of the EPIA-Study, a sham-controlled cross-over exposure study, by writing standardized operating procedure for the clinical assessments.
2. To conduct screening examinations on potential participants for the EPIA-Study according to SOPs.
3. To conduct field work in the framework of the EPIA-Study by preforming repeated clinical tests such as lung-function tests and pulse wave analysis on 55 participants of the EPIA-Study.
4. To conduct the statistical analysis of the association between different particles metrics (i.e. PMC, PSC and PNC) and my outcomes (i.e. AIx and AP).

2. Participants and Methods

2.1. EPIA-Study-Overview:

Design and aims

The study was performed on behalf of the German federal environment agency as cooperation between the IUF (Leibniz Research Institute for Environmental Medicine) and the IUTA (Institute of Energy and Environmental Technology).

The aims of this study were:

1- identifying potentially relevant indoor sources of ultrafine particles.

2- Characterizing the particulate emissions of these sources.

3- Investigating underlying biological pathways and short-term health-related effects.

The human exposure study was a cross-over sham-controlled exposure study with healthy volunteers and was conducted between October 2012 and June 2013. All subjects gave written, informed consent prior to participation. The study was performed with the approval of the ethics committee of the Heinrich-Heine-University of Düsseldorf (reference no. 3830, 06.07.2012), in accordance with the declaration of Helsinki. After thorough pre study screening process, which included a screening telephone interview, an in-depth personal interview for in- and exclusion criteria, questionnaires for risk factors and some clinical examinations, suitable participants were recruited. To investigate possible health related effects of short-term exposure to ultrafine particles from indoor sources, several in vitro and in vivo tests on subject's samples and subjects were performed before, during and after the different exposures. Participants were examined by trained personnel. Examinations included questionnaires for subjective feeling and recent changes in health-related variables and several clinical examinations. The following clinical examinations were conducted repeatedly at different time points during the exposure days: Blood pressure, PWA, PWV, HRV (Heart rate variability), FeNO (Fraction of exhaled nitric oxide), PFT (Pulmonary function test), Neurocognitive test, Nasal lavage and a Blood test.

2.2 Study population:

Recruitment of participants

Participants were recruited from the community of the cities Duisburg, Essen, Mülheim and Düsseldorf in Germany, via public postings, flyers and advertisements.

From 154 potential volunteers who were interested to participate in the EPIA-study, a total of

55 adult healthy men and women met all inclusion criteria and were successfully recruited for the study (Figure 6). Potential participants who did not fulfill all recruitment criteria were excluded during the screening phase. During the field phase of the study 18 participants have dropped out of the study do to different reasons. One Participant had stopped taking his medical treatment for hypertension without informing us which came later to our attention through very high BP values. Another participant had an epileptic seizure during an exposure day. Due to both underlying medical condition, which were primary exclusion criteria, the data of both participants were removed from the study. The remaining 16 drop-out cases were mainly related to time constraints of the participants.

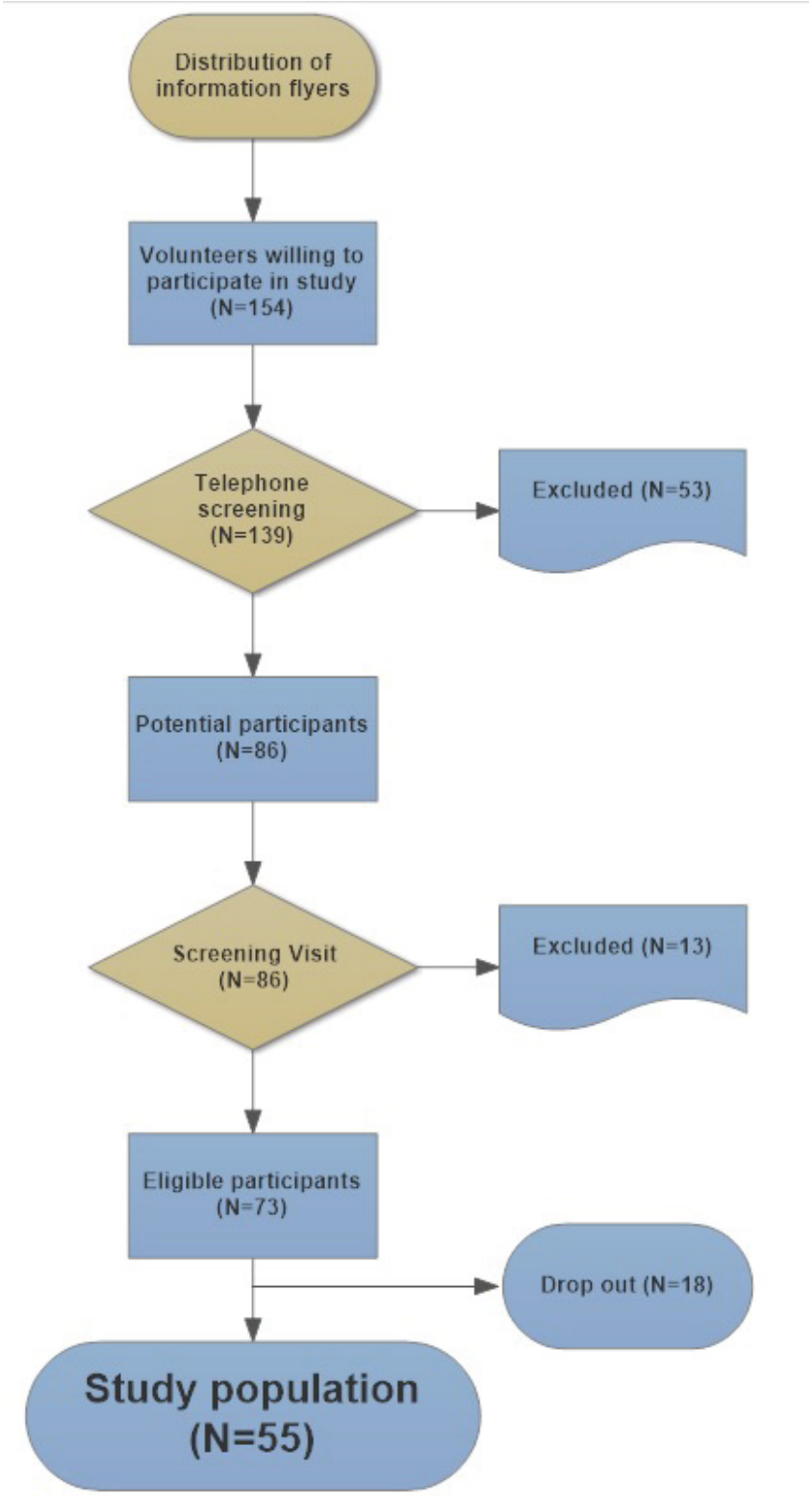

Figure 6: **Recruitment process of study participants**. N: Number of participants.

Pre-study screening

Interested Participants were screened thoroughly for health concerns and risk factors, which could prevent them from becoming eligible participants. Screening process started with a

telephone interview to find out if the person met basic eligibility requirements for the study. Eligible persons became an appointment for the screening visit. The screening visit included a physical assessment (body height and weight, resting BP and heart rate), a screening questionnaire to check for inclusion and exclusion criteria and a general questionnaire to gather information about social economic status, lifestyle, medications and medical history (The screening questionnaire and general questionnaire are both found in appendices). Main inclusion criteria were: Age between 18-79, speaking and understanding German and being a non-smoker or ex-smoker for at least 10 years. Main exclusion criteria were as follows: Existing or planned pregnancy (within the next 6 months), planned surgery (within the next 6 months), occupational exposure to smoke or air pollutants (e.g. welder, chemical laboratory technicians), pre-terminal diseases, diagnosed inflammatory diseases, cardiovascular events within last 3 months (e.g. heart attack, pulmonary embolism, stroke), uncontrolled arterial hypertension, type 1 and 2 diabetes mellitus, a history of chronic respiratory diseases, chronic infectious diseases and neurologic or mental disease (psychosis, claustrophobia).

Furthermore during the screening clinical assessments (PWA, PWV, FeNO-Test, PFT, neurocognitive test and a nasal lavage) were done in order to train subjects in some of the tests, especially tests where subject cooperation and compliance is needed e.g. PFT. This facilitated the course of tests during the exposure days. Included participants received printed documents, which served as memory aid throughout the study. These documents consisted of a study pamphlet (general information about the study), instruction sheet (information on how to behave one day before each exposure) (Memory aid documents are included in the appendices!). The participants were instructed to maintain their habitual diet, physical activity levels, lifestyle factors and body weight throughout the study. Participants taking oral contraceptives or antihypertensive or thyroid drugs were instructed to continue taking their medication without changes. All participants were instructed to abstain from alcohol and extreme physical exercise for 24h before the exposures and from caffeine drinks for at least 4 h before beginning of each exposure (Soppa et al. 2014).

2.3 Exposure

Exposure chamber

The "Research Division of Environment and Sustainability" headed by PD Dr.Kuhlbusch at IUTA-Duisburg was responsible for creating the exposure environment and monitoring, measuring and analyzing all data regarding particle emission. For the exposure scenarios a

specialized exposure chamber was constructed with an area of approx. 16 m^2 and a volume of approx. 48 m^3 (Figure 7). The air conditioning system worked in a circulating mode, hence not causing additional air exchange. Air flow rate was approximately 250 m^3/h. During exposure, temperature and relative humidity was controlled and temperature maintained at 24 °C. The walls were made from powder-coated sheet metal, windows from glass; the roof was covered with antistatic polyethylene film (109 –1010 ohms). Particle monitors, operated during the exposure periods at a time resolution of minutes and showed a relatively constant steady-state particle concentration. The sampling ports were installed close to the chairs where the participants remained during exposure, distances ranged between 0.5 and 1.5 m. The vents (air conditioner working in a circulating mode) ensured good dispersion within the chamber. Tests made by handheld monitors (DISCmini, Matter Aerosol, Wohlen, Switzerland) at various locations around the participant's seating area revealed particle number variations within the instrument uncertainties (Soppa et al. 2014)**.**

Figure 7: **Exposure chamber with participants during an exposure scenario.**

Exposure scenarios

The following 3 indoor sources, which were shown to have high and reproducable particle emission rates in perliminary examinations, were used in human exposure scenarios: Frying sausages (FS), candle burning (CB) and toasting bread (TB). The health-related effects of the chosen sources were investigated on two different levels; a middle level (Level 1) exposure and a high level (Level 2) exposure (Table 2).

Table 2**: Exposure description**

Exposure	Exposure-Description (2h per participant)
Room Air (RA)	No emitting Source (Sham object: Air ventilator)
Candle burning Level 1 (CB1)	Burning 20 white Christmas-tree candles continuously
Candle burning Level 2 (CB2)	Burning 40 white Christmas-tree candles continuously
Toasting bread Level 1 (TB1)	Toasting 2 pieces of sliced bread with a two-slot toaster continuously
Toasting bread Level 2 (TB2)	Toasting 4 pieces of sliced bread with 2 two-slot toasters continuously
Frying sausages Level 1 (FS1)	Frying 3 sausages in a frying pan with short active emission breaks for cooldown
Frying sausages Level 2 (FS2)	Frying 6 sausages in two frying pans with short active emission breaks for cooldown

Although only 3 different sources were actually tested, particiants were told that 4 different exposure scenarios were tested. For the sham exposure (RA) an air ventilator was placed in the chamber, which simulated the "4th" exposure source. Blinding the subjects for all other exposures was not possible due to the smell and sight of frying saugeses, burning candles and toasting bread (Figure 8) During CB, 20 white Christmas-tree candles (level 1, CB1) or 40 candles (level 2, CB2) were placed in candleholders, which were positioned apporx.15cm from one another and attached to a wooden board covered with aluminum foil. Throughout the exposure the wooden board was placed on a table with a height of approx.1, 2 meters and the candles were immediately replaced shortly before burning out. For the exposure toasting bread (TB), bread was toasted with one (level 1, TB1) or two (level 2, TB2) identical time controlled 2-slice-toasters of the same model ("Executive Edition TT 61103" from Siemens). Each cycle of toasting bread lasted up to three minutes. To ensure constant emission and in order to prevent the toasters from overheating, the toasting process was done alternately in three toasters of the same type. Frying 3 sausages per pan (FS) was executed in one (level 1,

FS1) or two (level 2, FS2) Teflon®-coated pans (Tefal-H11506) without additional fat, the pans were placed on an electric hotplate (Severin-KP1057). To avoid extensive heat accumulation and burning of residues the frying was paused after three cycles (0.5 h of continues frying) for about 10min. During this break the pans were cleaned and were left to cool down (Soppa et al. 2014).

Figure 8: **The activated exposure sources candles burning, toasting bread and frying sausages.**

Exposure monitoring – Particle metrics

Particle concentration in the exposure chamber was monitored continuously during each session to ensure consistent particle pollution levels for all participants and each exposure scenario and to calculate the individual 2 h mean particle exposure for each participant and each exposure (Figure 9). Fluctuation of concentrations due to for example opening of the door was included in the personalized mean exposure. The concentration of particels with a diameter of 5,6 - 540nm was measured once per second using a Fast Mobility Paricle Sizer (FMPS, Model 3091, TSI Inc., Shoreview, MN, USA). This device sorts and counts particels accordings to there specific electric mobility. Working according to the same principale was the SMPS (Scanning Mobility Particle Sizer, Model 3936 with electrostatic classifier Model 3080, TSI Inc.), which was used to measure concentration of particels with a diameter of 6,5 - 750nm every 2-4minutes. The concentration of larger particels with a daimeter of 0,6 – 20 µm was recorded once every 2-4 minutes using an Aerodynamic Particle Spectrometer (APS, Model 3321, TSI Inc.). This device sorted particles according to their specific aerodynamic properties. The surface area concentration of respirable Nanoparticles (LSDA-Lung-Deposited Surface Area) with a diameter of 20-1000nm, which can enter pulmonary alveoli,

was measured in µm2/cm^3 once every 10 seconds using a Nanoparticle Surface Area Monitor (NSAM, Model 3550, TSI Inc.). Furthermore, size-specific mass concentrations of PM_1, $PM_{2.5}$ and PM_{10} were calculated from particle size and number concentrations assuming spherical particles and a particle density of 1 g/cm^3 for all exposure scenarios (Soppa et al. 2014)**.** In addition to the vast particle measurements in the chamber, one or two subjects per day wore a portable measurement device (DiSCMini - Diffusion Size Classifier from Matter Aerosol, Switzerland) during the entire the experiment day.

Exposure monitoring – Particle chemistry

A High-Resolution Time-of-Flight Aerosol Mass Spectrometry (HR-ToF-AMS, Aerodyne Research) enabled a continuous (once per second) acquisition of complete mass spectra of individual particles with aerodynamic diameter of 60 - 600nm, and enabled the resolution of distinct chemical species based on mass defect. For the ultrafine particles a NAS (Nanometer Aerosol Sampler, Model 3089, TSI Inc) was used to create samples of the aerosols by electrostatic charging them onto a substrate. This substrate with a uniform particle deposition was removed and examined using a Total Reflection X-ray Fluorescence (TXRF, EAG Laboratories) to characterize the particle-bound contents in the ultrafine fraction by performing a trace element analysis. In addition an organic carbon and elemental carbon (OC-EC) aerosol analysis was conducted with the Lab OC-EC Aerosol Analyzer (Sunset Laboratory Inc., USA).

Figure 9: **An overview of the exposure monitoring instruments**.

2.4 Field work

General conduction

Participants were exposed on separate occasions at the same time of day at least two weeks apart to eliminate any carryover effect. On a study day four participants were scheduled to arrive at the study center in the morning at 30 minute intervals between each other in order to minimize long waiting periods. After a short evaluation of his or her current health condition, he or she would start with directly with the first series of pre-exposure medical examinations (T0). If a participant had a current infection or had taken anti-inflammatory drugs recently, the exposure was rescheduled. Preceding participant's exposure, the indoor source would be activated to reach predetermined particulate concentration in order to create comparable exposure conditions. After the pre exposure medical examinations the participants entered the exposure chamber for 2 hours, after 1 hour exposure the BP and Pulse measurements (T1) were performed by a trained staff member who was present inside the chamber throughout the session. During the exposure participants were sitting quietly in the chamber reading or working on a laptop. They did not exercise during exposure sessions so breathing patterns corresponded to usual quiet indoor activities. Immediately after leaving the chamber the post exposure medical examinations (T3) were conducted. Afterwards Participants received a lunch break for about 70 minutes. In this time the participants were not allowed to leave the study center to avoid unwanted exposure to ambient air. In the afternoon the post 2 examinations (T3) and post 4 examinations (T4) took place 2 hours and 4 hours after exposure accordingly. On the next day exactly 24 hours after the exposure participants came in again briefly for the sixth and last post exposure medical examination (T5) (Table 3).

Table 3: **Time schedule for all examinations**

	Pre Expo (T0)	During Expo (T1)	Post Expo (T2)	2h post Expo (T3)	4h post Expo (T4)	24h post Expo (T5)
General questionnaire	Once					
Current health questionnaire	X					
Subjective feeling questionnaire	X	X	X	X	X	X
Blood pressure / Pulse	X	X	X	X	X	X
PWA	**X**		**X**	**X**	**X**	**X**
PWV / HRV	X		X			X
FeNO-Test	X			X		X
Pulmonary function tests	X				X	X
Neurocognitive test	X				X	X
Nasal lavage	X			X		X
Blood test	X			X		X

Expo: Exposure, h: hours, PWA: Pulse wave analysis, PWV: Pulse wave velocity, HRV: Heart rate variability, FeNO: Fraction of exhaled nitric oxide

During the preparation phase of the EPIA-Study extensive standard operating procedures were compiled for all clinical assessments. The step by step instructions promoted quality, comparability and credibility of the data through consistent implementation of the medical assessments. All of the measurements were conducted by trained investigators.
Out of the many assessed health outcomes in the EPIA-Study, I will present in my thesis the central arterial pulse wave indices (i.e. augmentation index (Aix) and augmentation pressure (AP)). Both central arterial pulse wave indices can be used as an indirect surrogate for systemic arterial stiffness (Laurent et al. 2006).

PWA – equipment and setting

A non-invasive tonometry-based approach with the SphygmoCor® System (CPV; AtCor Medical, Sydney, Australia) (Figure 10) was used as a clinical tool for the assessment of central blood pressure and arterial stiffness in the EPIA-Study. The SphygmoCor® Pulse

wave analysis (PWA) option provided a derived ascending aortic blood pressure waveform and a range of central arterial indices (AtCor Medical Pty. Ltd. Sydney Australia 2010). PWA measurements were performed under standardized conditions in a quiet examination room with room temp of 22 22 °C +/- 1 °C according to the study protocol which was built on the recommendations of the research applications manual from AtCor Medical Pty. Ltd., 2010 and information provided by an AtCor training course.

Figure 10: **SphygmoCor with accessories** (AtCor Medical Pty. Ltd. Sydney Australia 2010).

PWA –Procedure:

In the EPIA study pulse wave analysis (PWA) was measured at five different time points (Table 2). Each PWA-Session was performed at least 2 minutes after the brachial blood pressure measurement session to enable the radial artery to refill with blood.

Thus participants were in a relaxed seated position for at least 15 minutes prior to starting the PWA. After placing the hand of subject in a stable dorsiflex position using a small pillow and locating the radial artery pulse with the finger index to insure best results, the pressure

transducer (tonometer) was applied gently over the strongest pulse point.
Once a strong and reproducible waveform was obtained, the tonometer was kept in this position, and subsequent waveforms were saved for analysis. In order to obtain high quality waveforms, the recommended quality control parameters from the AtCor research applications manual (AtCor Medical Pty. Ltd. Sydney Australia 2010) were directly controlled and unacceptable measurements were repeated. The operator index was used as the main quality control parameter and it is calculated from indices of the average pulse wave, pulse height variation, diastolic variation and shape deviation (Table4). Each measurement with an operator index under 80%, which is considered acceptable, was immediately repeated (AtCor Medical Pty. Ltd. Sydney Australia 2010). At the end of each measurement session a wide range of central arterial indices were obtained. In the results section of my thesis the statistical analysis of the following parameters are shown: AIx, AP, both parameters adjusted for heart rate 75 [bpm].

Table 4: **Bedside quality control parameters for operator index**

Quality Control requirements	**Reason it might be poor.**
Average pulse height ≥ 80	Signal too weak
Pulse height variation ≤5%	Variable amounts of pressure have been applied by the operator during the study.
Diastolic variation ≤ 5%	Tremor from patient or the operator
Shape deviation≤ 4	Not enough similarity between succsecive Pulse waves
Operator index >80%	Overall combination of above.

2.5 Statistical analysis plan

Exposures of interest (Independent variables)

- Continuous Variables:
  Personal 2-h average exposure of particle mass (PMC in [µg/m³]: PM_{10}, $PM_{2.5}$, PM_1), particle number concentration (PNC in [#/cm³] <100 nm) and particle surface concentration (PSC – LDSA in [µm²/cm³]) were the main exposures of interest.

- Categorical variables:
  The type of indoor source (CB, FS, and TB).

Outcomes of interest (Dependent variables)

The main continuous PWA-Variables, AIx in [%] and AP in [mmHg], both corrected for a heart rate of 75 [bpm], were the main outcomes evaluated in my thesis. In order to secure a qualitative and representative sample of PWA-Measurements, only measurements that had fulfilled all quality control criteria were analyzed. The following quality controlled criteria from research applications manual (AtCor Medical Pty. Ltd. Sydney Australia 2010).were applied (Table 5).

Table 5: **Quality control criteria for PWA-Measurements**.

Quality Control Criteria PWA	
Operator- Index	≥ 80
Augmentation index	< 50%
Inconclusive	= no
OR	
Operator- Index	≥ 80
Augmentation index	< 50%
Inconclusive	= yes
central T1	80 ms < x < 150 ms

PWA: Pulse wave analysis,

Covariates of interest (used in the full model)

- Covariates gathered from the screening appointment:
  Age in years (continuous) was calculated as the difference in years between the screening date and the date of birth.
  Sex (categorical: male or female) was assessed as self-reported in the general questionnaire.
  Height in [cm] (continuous) was measured using an ultrasonic height measuring unit (Model: MZ10020, ADE GmbH&Co) according to standard operating procedure.
  Weight (continuous) was measured by a standard electronic weight scale.

- Covariates gathered on exposure days:
  Travel time to the study center in (continuous) and **mean of transportation** (categorical: public transport or car) were assessed as self-reported in the pre exposure evaluation questionnaire.

Personal 2-h average **temperature** in degrees Celsius (continuous) and **humidity** in percent (continuous) in the chamber.

- Additional covariates:
  Ambient $PM_{2.5}$ concentrations, averaged over the last five days before the study day, in [$\mu g/m^3$] (continuous), which are recorded by the State Agency for Nature, Environment and Consumer Protection in North Rhine Westphalia (LANUV) at the nearest monitoring station to the home of the participants, were collected.

In addition, information on level of school education, smoking status and medical history (e.g. history of metabolic disease) was also gathered. Almost all of the participants were highly educated and healthy; therefore due to the similarities of our study population these covariates were not included in the full model.

Statistical analysis

Initially, descriptive statistics of the variables were calculated. The mean, the standard deviation, the median, Q1, Q3 and the range were calculated for quantitative variables. For categorical variables the number and percent are shown. In addition, Spearman's rank correlations between the different exposure metrics (i.e. PMC, PNC and PSC) were calculated.

To analyze exposure source-related changes in central arterial indices, we performed multiple mixed linear regression analyses with a random participant intercept, including an indicator for each exposure source (room air as reference) and the intra-individual difference of central arterial indices at time point t_n compared to values before the exposure (t_0) as the dependent variable. Each time point was analyzed in a separate regression model.

$$(\Delta AIx \text{ or } \Delta AP)_{t\text{-}t0} = \beta_0 + \beta_1 * I_{CB} + \beta_2 * I_T + \beta_3 * I_{FS} + \overrightarrow{\beta_X} * \mathbf{X} \qquad \text{(model 1)}$$

β: Coefficient

I_{CB}, I_T, I_{FS}: Indicator variables for exposure source

X: matrix of other independent variables

t: Index for the time point

Covariates in the full model included age, height, weight, sex, mean temperature and humidity in the chamber, travel time before exposure, mode of transportation and ambient $PM_{2.5}$

concentration, averaged over the last five days before the study day.
In a second analysis step, we examined the time-dependent impact of personal mean exposure on changes in central arterial indices by including indicator variables for time point and interaction terms for time point*exposure metric. Because of possible source-specific effects of the three particle sources on central arterial indices, we conducted separate models for each exposure scenario (CB, TB and FS).

$(\Delta AIx \text{ or } \Delta AP)_{t\text{-}t0} = \beta_0 + \beta_1 * Exposure + \beta_{2t} * I_t + \beta_{3t} * Exposure * I_t + \overrightarrow{\beta_X} * \mathbf{X}$ (model 2)

I_t: Indicator variable for time of measurement.

X: matrix of other independent variables

t: time points, here 2-5

For the comparison of source-specific effects we calculated the changes in central arterial indices per fixed increment of the particle metric (PM_{10}, $PM_{2.5}$, PM_1: 10 µg/m^3, PNC: 50,000 particles/cm^3, PSC: 1,000 $\mu m^2/cm^3$). For the comparison of the effect of different particle metrics within one source, we calculated the changes in central arterial indices per interquartile range (IQR) of the exposure.

Statistical analyses were performed using SAS version 9.2 (SAS/STAT Software, SAS Institute, Inc., Cary, NC, USA) and RStudio (R 3.2.5, Development Core Team, Vienna, Austria).

<u>Comment - Participant numbers</u>

A total of 55 Participant were included in the study but not all 55 subjects participated in all exposure scenarios and therefore the number of participants exposed to the sources differs in the following results.

3. Results

3.1. Description:

Study population

The study population comprised 55 healthy adults residing in the state of NRW, Germany between October 2012 and June 2013. 28 females and 27 males between 20 and 79 years old with an average age of 32.5 years ± 16, 3 were exposed to different indoor sources and went through a series of medical examinations (Table 6). Additional personal exposure characteristic from ambient air such travel time to the study center and means of transport are seen in Table 7.

Table 6: **Personal characteristics of the 55 participants at their first visit at the EPIA Study (September 2012 - April 2013)**

Personal characteristic	**Measure**
Age [years], mean (SD)	32.5 (16.3)
Born in Germany, N (%)	35 (65)
Male, N (%)	27 (49)
Weight [kg], mean (SD)	71.7 (13.3)
Height [cm], mean (SD)	173.9 (9.4)
BMI [kg/m2], mean (SD)	23.6 (3.2)
Level of School Education, N (%)	
Low	4 (7,3)
Medium	5 (9.1)
High	42 (76.3)
Missing	4 (7.3)
Employed, N (%)	26(49)
Smoking status, N (%)	
Ex-smoker	3 (5.4)
Non-smoker	51 (92.7)
Missing	1 (1.9)
Controlled hypertension, N (%)	2 (3.7)
Baseline blood pressure	
Systolic[mmHg], mean (SD)	116.0 (13.8)
Diastolic[mmHg], mean (SD)	74.8 (9.7)
Baseline central arterial indices	
Augmentation pressure[mmHg], mean (SD)	2.4 (4.2)
Augmentation Index[%], mean (SD)	8.1 (13.1)
History of allergy, N (%)	17 (33)
History of meatabolic disorder, N (%)	2 (3.7)
History of cancer, N (%)	1 (1.9)

BMI: body mass index, SD: standard deviation, N: number of participants

Table7: **Personal exposure characteristics (N= 256) collected during the EPIA study for 55 participants (October 2012 – June 2013)**

Exposure characteristic	**Measure**
Travel time, hours, mean (SD)	1.1 (0.6)
Means of transportation, N (%)	
Public Transport	144 (56.3)
Car	102 (39.8)
Missing	10 (3.9)
Ambient $PM_{2.5}$ concentrations, , averaged over the last five days before the study day in [µg/m³], mean (SD)	17.3 (8.4)

SD: Standard deviation, N: number of participants

Meteorological variables

During exposure, temperature and relative humidity of the exposure chamber was controlled; temperature was maintained at approximately 24 °C (Table 8) and low humidity levels were maintained fewer than 40% (Table 9).

Table 8: **Temperature [°C] in chamber during the exposure scenarios for N participants**

Outcomes	**N**	**Min.**	**25%**	**Median**	**Mean±SD**	**75%**	**Max.**	**Missing**
Room Air	44	21.7	23.8	24.2	24.2±0.9	24.9	25.5	2
Candle burning	44	22.2	23.3	24.2	24.3±1.3	24.9	27.7	0
Toasting bread	41	21.0	23.2	23.6	23.7±1.1	24.4	25.8	4
Frying sausages	46	21.4	22.8	23.2	23.1±0.8	23.5	25.1	0

N: number of participants, Min: ninimum, Max: Maximum

Table9: **Humidity [%] in chamber during the exposure scenarios for N participants**

Outcomes	**N**	**Min.**	**25%**	**Median**	**Mean±SD**	**75%**	**Max.**	**Missing**
Room Air	44	19.4	25.9	31.9	30.3±5.0	33.5	38.2	2
Candle burning	44	22.5	29.8	39.8	36.2±7.4	41.9	46.8	0
Toasting bread	41	24.0	33.1	38.5	37.7±7.3	42.8	52.9	4
Frying sausages	46	19.9	30.1	37.3	35.9±8.9	43.1	49.9	0

N: number of participants, Min: minimum, Max: maximum

Exposures

Table 10 and Table 11 present a description of the 2 hour means of different particle metrics calculated for each exposure scenario and each participant. During the sham exposure room air, mean particle mass concentration (PMC) were 6.3 ± 5.8 [µg/m³], 4.8 ± 5.4 [µg/m³] and 3.3 ± 3.5 [µg/m³] for PM_{10}, $PM_{2.5}$and PM_1 respectively. During indoor activities exposures (CB, FS, and TB) mean PMCs increased, reaching values between 51.4±28.3 [µg/m³] for PM_1 during TB and 147.9±127.5 [µg/m³] for PM_{10} during FS. During the sham exposure room air, mean particle surface concentration (PSC) and particle number concentrations (PNC) for ultrafine particles (Diameter<100 nm) were 22.9 ± 18.0 [µm²/cm³] and 3,391.7 ± 2,142.2 [#/cm³], respectively. During indoor activities exposures mean PNCs and PSC of ultrafine particles increased, reaching values up to 2,551.1 ± 794.4 [µm²/cm³] and 2,070,348.4 ± 400,621.4 [#/cm³] during CB for PSC and PNC, respectively. Furthermore PMC (PM_{10}, $PM_{2.5}$ and PM_1), PNC and PSC calculated for level 2, were at least twice as high as in level 1 during FS, TB and CB (Figure 11).

Table 10: Description of the induvial 2-hour exposure means of the different particle metrics in RA and CB for N participants

Source/Metric	N	Min.	25%	Median	Mean±SD	75%	Max.	IQR	Missing
Room Air(RA):									
PM_{10} [µg/m³]	34	2.4	3.7	4.3	6.3±5.8	5.4	30.1	1.8	12
$PM_{2.5}$ [µg/m³]	34	1.5	2.5	3.1	4.8±5.4	3.6	26.8	1.1	12
PM_1 [µg/m³]	34	0.9	1.3	2.2	3.3±3.5	2.6	16.8	1.3	12
PSC [µm²/cm³]	46	7.5	13.8	17.5	22.9±18	20.3	75.6	6.9	0
*PNC [#/cm³]	46	1.3	1.8	3.0	3.4±2.1	4.1	9.9	2366.7	0
Candle burning(CB):									
PM_{10} [µg/m³]	33	30.9	48.1	58.2	63.5±20.8	79.5	99.7	31.1	11
$PM_{2.5}$ [µg/m³]	33	29.7	45.3	56.0	60.4±20.0	78.1	95.0	32.8	11
PM_1 [µg/m³]	33	28.1	43.0	52.2	56.5±19.7	71.1	94.1	33.9	11
PSC [µm²/cm³]	44	1211.6	2186.0	2355.1	2551.1±794.4	2539.0	4675.9	1387.8	0
*PNC [#/cm³]	44	1144.7	1778.0	1994.6	2070.3±400.6	2376.0	2876.1	793089.6	0

N: number of participants, PM_{10}: Particulate matter Ø < 10 µm, $PM_{2,5}$: Particulate matter Ø < 2,5 µm, PM_1: Particulate matter Ø < 1 µm, PSC: size-specific lung-deposited particle surface area concentration, PNC: particle number concentration (Ø< 100 nm), * PNC per 1000 particles/cm^3

Table 11: Description of the induvial 2 hour exposure means of the different particle metrics in TB and FS for N participants

Source/Metric	N	Min.	25%	Median	Mean±SD	75%	Max.	IQR	Missing
Toasting bread(TB):									
PM_{10} [µg/m³]	37	19.9	36.6	43.6	68.3±65.1	93.5	396.5	53.5	8
$PM_{2.5}$ [µg/m³]	37	18.6	34.7	40.0	57.3±33.4	88.7	144.5	51.0	8
PM_1 [µg/m³]	37	17.1	32.9	38.4	51.4±28.3	81.2	103.9	45.0	8
PSC [µm²/cm³]	45	1203.0	1682.0	1985.7	2286.4±1004.0	2417.0	4636.2	1835.4	0
*PNC [#/cm³]	44	669.6	847.2	984.5	1073.4±329.9	1196.0	1842.2	610334.5	1
Frying sausages(FS):									
PM_{10} [µg/m³]	40	26.1	47.8	86.4	147.9±127.5	221.3	547.4	244.7	6
$PM_{2.5}$ [µg/m³]	40	24.5	41.3	75.2	116.1±86.5	186.0	298.5	188.4	6
PM_1 [µg/m³]	40	21.8	35.3	64.9	99.0±72.5	154.7	252.3	161.8	6
PSC [µm²/cm³]	46	612.9	928.5	1270.7	1773.8±1099.8	2321.0	4422.2	2593.0	0
*PNC [#/cm³]	46	199.9	285.3	336.3	375.4±139.8	411.1	749.9	285746.6	0

N: number of participants, PM_{10}: Particulate matter Ø < 10 µm, $PM_{2.5}$: Particulate matter Ø < 2,5 µm, PM_1: Particulate matter Ø < 1 µm, PSC: size-specific lung-deposited particle surface area concentration, PNC: particle number concentration (Ø< 100 nm), * PNC per 1000 particles/cm^3

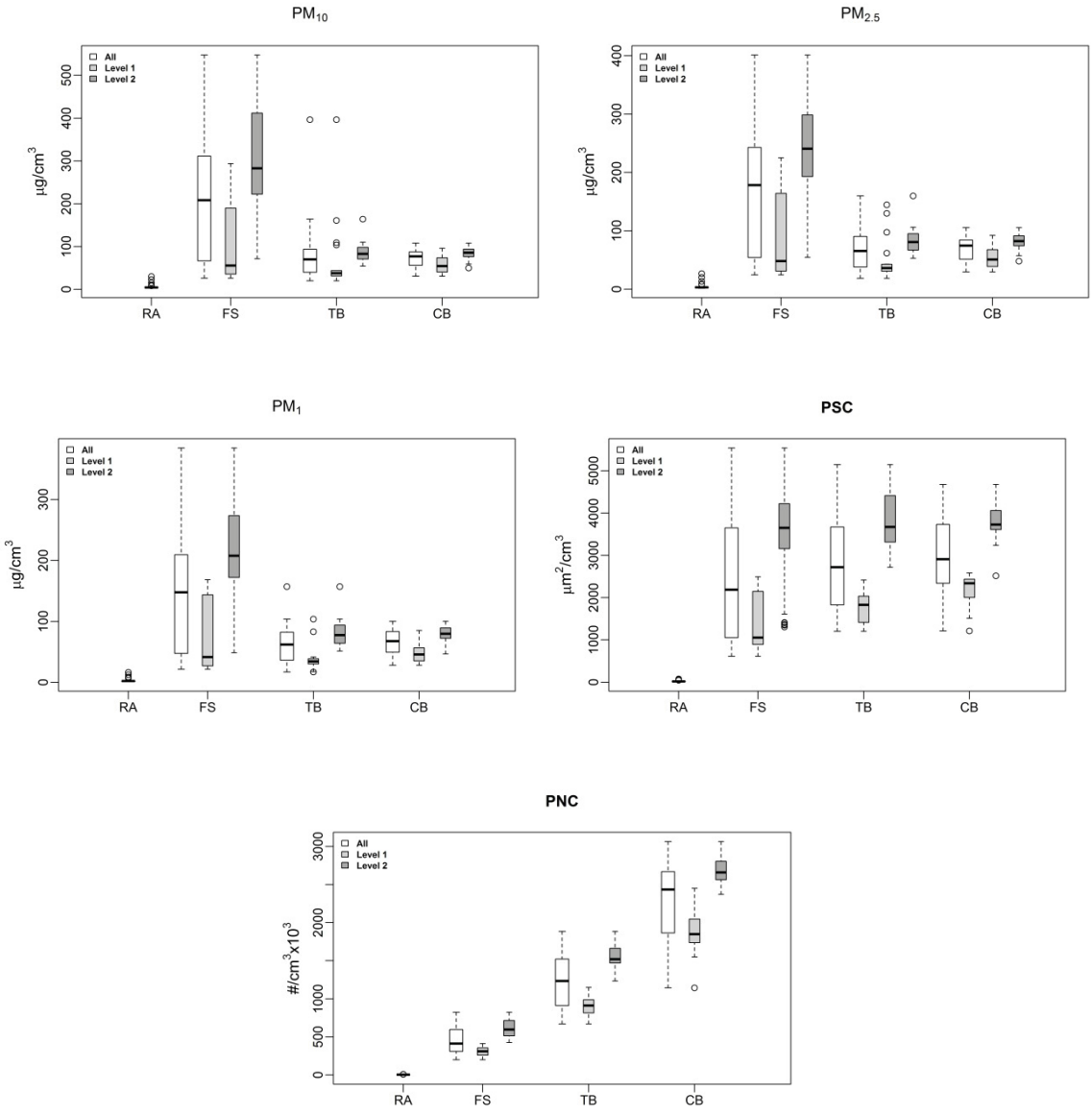

Figure 11: **Description of personal 2 hour means of different particle metrics**: PM_{10}: Particulate matter Ø < 10 µm, $PM_{2,5}$: Particulate matter Ø < 2,5 µm, PM_1: Particulate matter Ø < 1 µm, PSC: size-specific lung-deposited particle surface area concentration, PNC: particle number concentration (Ø< 100 nm) during Room air (RA), Candle burning (CB), Toasting bread (TB) and Frying sausages (FS) for level 1 and level 2.

Main exposures - correlations

The following Spearman's rank correlation coefficients describe the relationship between the different exposure metrics for each exposure scenario. Although all the coefficients are positive throughout the different exposure scenarios, several differences are seen. Larger particles (PM>1 **µm**) were highly correlated with each other. On the other hand smaller particels (PNC<100 nm) were highly correlated with PSC (Table 12). In addition during FS, a higher correlation between PMC and UFP number concentration is seen compared to CB and TB. This demonstrates the high contribution of UFP to the particle mass concentrations.

Table 12 **Spearman's rank correlation coefficients for different exposure metrics in each exposure scenario**.

Candle burning (N=33)					
	PM_{10}	$PM_{2.5}$	PM_1	PNC	PSC
PM_{10}	1	1	0.98	0.39	0.77
$PM_{2.5}$	1	1	0.99	0.43	0.79
PM_1	0.98	0.99	1	0.52	0.85
PNC	0.39	0.43	0.52	1	0.81
PSC	0.77	0.79	0.85	0.81	1
Toasting bread (N=37)					
	PM_{10}	$PM_{2.5}$	PM_1	PNC	PSC
PM_{10}	1	0.82	0.49	0.32	0.38
$PM_{2.5}$	0.82	1	0.90	0.68	0.77
PM_1	0.49	0.90	1	0.82	0.91
PNC	0.32	0.68	0.82	1	0.96
PSC	0.38	0.77	0.91	0.96	1
Frying sausages (N=40)					
	PM_{10}	$PM_{2.5}$	PM_1	PNC	PSC
PM_{10}	1	0.96	0.93	0.71	0.87
$PM_{2.5}$	0.96	1	0.99	0.80	0.94
PM_1	0.93	0.99	1	0.83	0.96
PNC	0.71	0.80	0.83	1	0.92
PSC	0.87	0.94	0.96	0.92	1

N: number of participants, PM_{10}: Particulate matter Ø < 10 µm, $PM_{2,5}$: Particulate matter Ø < 2,5 µm, PM_1: Particulate matter Ø < 1 µm, PSC: size-specific lung-deposited particle surface area concentration, PNC: particle number concentration (Ø< 100 nm)

Particle chemistry

During CB, AMS measurements showed that the particle-bound content was dominated by organic compounds (43%) and nitrate (54%). the latter came from the oxidation of Ammonium salts, which is used in candle wick impregnation. The remaining 3% consisted of small amounts of Ammonium (NH_4), sulfate (SO_4) and Chloride (Cl). Furthermore filter samples showed total carbon average concentrations of 90 [$\mu m/m^3$]. 9% of the total carbon was elementary carbon (i.e. Soot) and the rest was organic. With TXRF, Potassium (K), assumed to be from candle wick, was clearly detected and a small amounts of Calcium (Ca), Iron (Fe), Zinc (Zn) and Titan (Ti).

During TB, AMS measurements showed that the particle-bound content was dominated by organic compounds (98.5%). In addition very small amounts (1.5%) of nitrate (NO_3), (NH_4), (SO_4) and (Cl) were observed. Furthermore filter samples showed total carbon average concentrations of 480 [$\mu m/m^3$]. Less than 1% the total carbon mass was elementary carbon,

50-60% of the total carbon mass was organic and the rest could not be evaluated. It was suggested be inside water bound particles. With TXRF, (Ca), (Cl), (Fe) and a small amount of (Zn) were clearly detected. (Fe) assumed was assumed to from heating filament emissions. **During FS**, AMS measurements showed that the particle-bound content was also dominated by organic compounds (99%). In addition very small amounts (1%) of nitrate (NO_3), (NH_4), (SO4) and (Cl) were observed. Furthermore filter samples showed total carbon average concentrations of 380 [$\mu m/m^3$]. Less than 1% the total carbon mass was elementary carbon, 60% of the total carbon mass was organic and the rest could not be evaluated. With TXRF, (K), (Cl), and Sodium (Na) were clearly detected.

Central arterial indices

The number of participants differs between exposures because not all volunteers participated in all exposure scenarios and sporadically pulse wave analysis could not be determined at the assigned time point due to practical reasons (Table 13). Central arterial indices from 1123 pulse wave analysis were included in my analysis and their description for each exposure scenario is shown in Table 14 and Figure 12.

Table 13: **Total number of participants included for each exposure scenario and measurement time**

	Pre Expo	Post Expo	2h post Expo	4h post Expo	24h post Expo	Total Participants
Room Air	39	37	42	38	41	46
Candle burning						
Level 1 (CB1)	32	34	32	34	30	35
Level 2 (CB2)	35	31	34	33	32	37
Toasting bread						
Level 1 (TB1)	29	29	32	31	27	34
Level 2 (TB2)	31	31	32	29	33	34
Frying sausages						
Level 1 (FS1)	27	30	31	32	31	35
Level 2 (FS2)	31	25	30	30	28	34

.

Table 14**: Description of main outcomes of all 1123 PWA-Measurments from all 55 participants**

Outcomes	**Minimum**	**25% percentile**	**Median**	**Mean±SD**	**75% percentile**	**Maximum**
AP[mmHg]	-9	-1	1	2.1±4.5	5	22
AIx[%]	-29	-5	6	6.5±14.1	18	46

AP: Augmentation Pressure, AIx: Augmentation Index, SD: Standard Deviation.

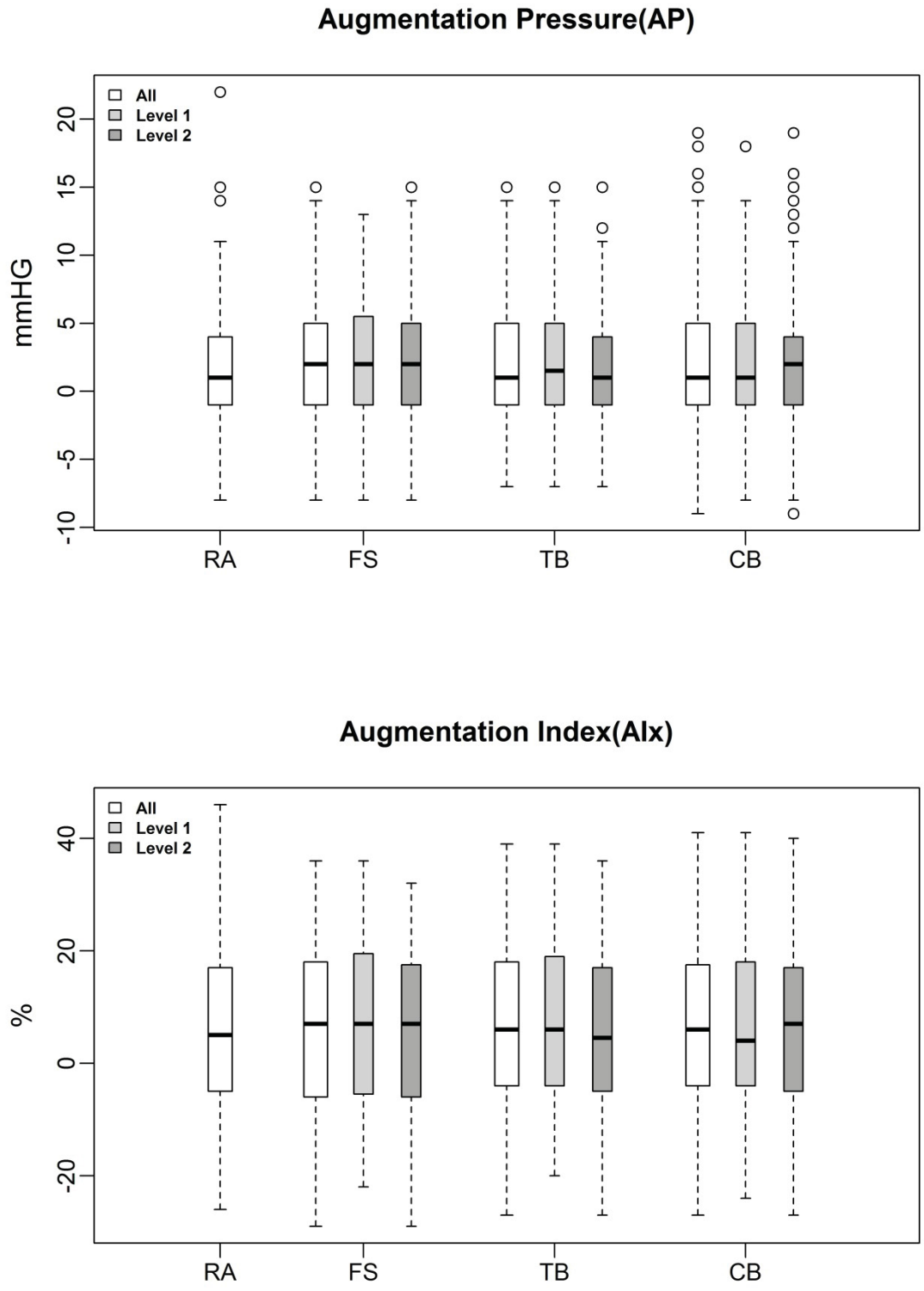

Figure12: **Distribution of all included central arterial indices (AP & AIx)** with median, 25% and 75% percentile during Room air(RA), Candle burning (CB), Toasting bread (TB) and Frying sausages (FS) for level 1 and level 2.

3.2. Associations between exposure sources and arterial stiffness

Model I

Compared to room air, CB was associated with an increase in AP and AIx directly after exposure, controlling for age, height, sex, mean temperature and humidity in the chamber, travel time before exposure, mode of transportation and ambient $PM_{2.5}$ concentration, averaged over the last five days before the study day. A significant increase of 1.8 mmHg (95%-CI: 0.2; 3.5) and 6.3% (95%-CI: 1.1; 11.5) is seen for AP and AIx, respectively. TB and FS exposure were not associated with clear changes in PWA indices, even though a slight increase in point estimates directly after exposure also occurred (Figure 13). Similar effects were seen in the crude model before the adjustment for the co variables mentioned above (Appendices).

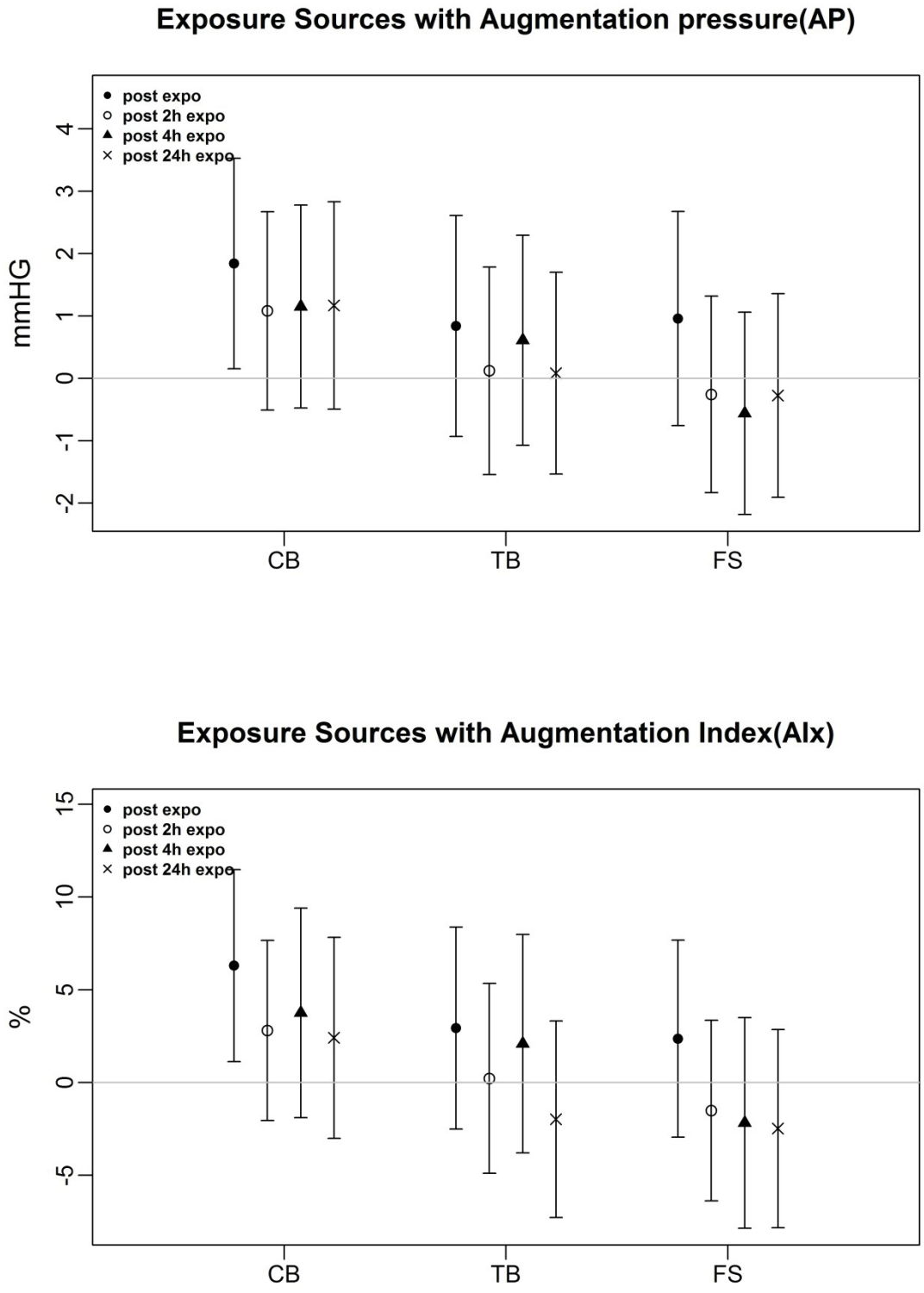

Figure 13: **Mean effect estimates and 95% Confidence Intervals (CI) for changes in augmentation pressure (AP) and augmentation index (AIx) depending on exposure scenario (candle burning CB, toasting bread TB and frying sausages FS) in the fully adjusted model**

3.3. Associations between exposure metrics and arterial stiffness

Model II – Main model - METRIC

Figure 14 till Figure 18 illustrates the changes in the fully adjusted model for augmentation index (AIx) shown for each exposure metric in each exposure scenario (CB, TB, FS). Similar effects for augmentation pressure (AP) and in the crude model have been observed (Appendices).

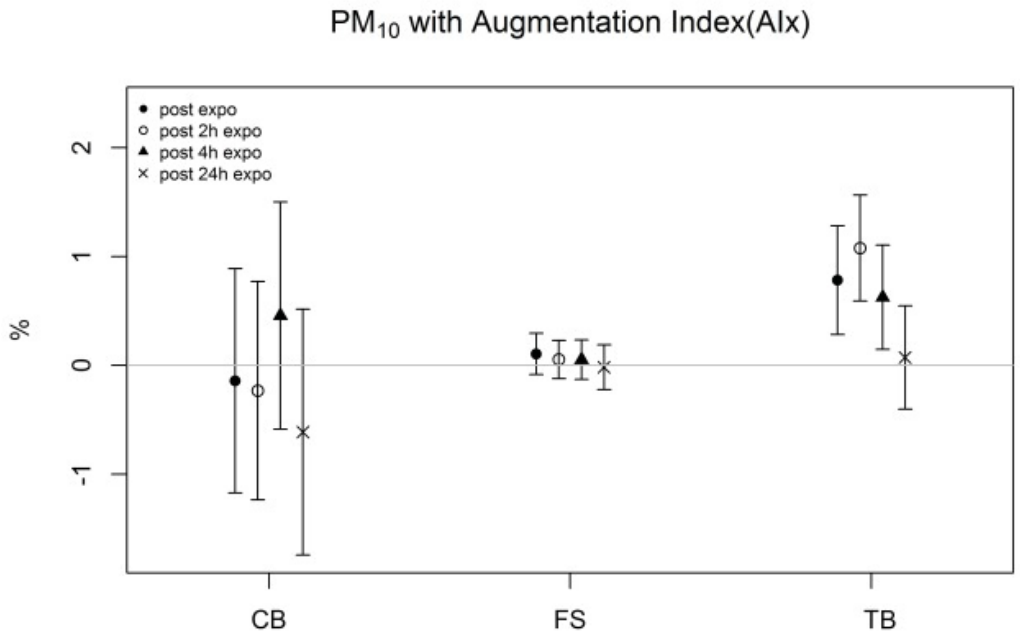

Figure 14**: Mean effect estimates and 95% confidence intervals (CI) for changes in AIx associated with an increase of 10 µg/m^3 PM_{10} during candle burning (CB), frying sausages (FS) and toasting bread (TB) in the fully adjusted model**

After the 2 hour exposure with TB, a 10 µg/m^3 PM_{10} was associated with a 0.8 % (95%-CI: 0.3; 1.3) , a 1.1 % (95%-CI: 0.6; 1.6) and a 0.6 % (95%-CI: 0.2; 1.1) increase in AIx directly after, 2h after and 4 h after the exposure, respectively. After24 h, no association was visible. PM_{10} from other sources was not associated with changes in AIx.

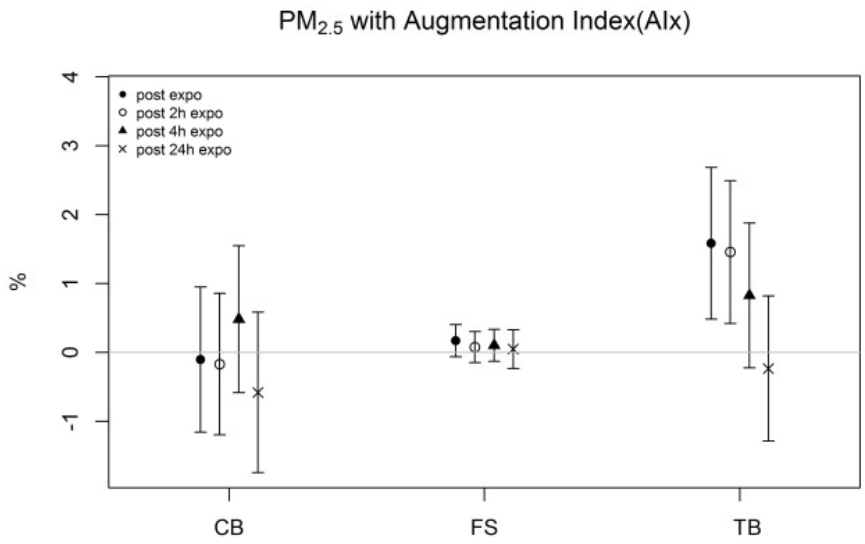

Figure 15**: Mean effect estimates and 95% confidence intervals (CI) for changes in AIx associated with an increase of 10 µg/m^3 $PM_{2.5}$ during candle burning (CB), frying sausages (FS) and toasting bread (TB) in the fully adjusted model**

Similarly, only 10 µg/m^3 $PM_{2.5}$ from TB was associated with a 1.6 % (95%-CI: 0.5; 2.7) and a 1.5 (95%-CI: 0.4; 2.5) increase in AIx directly after and 2h after, respectively. After 4 h, the association was attenuated and vanished after 24 h. $PM_{2.5}$ from other sources was not associated with changes in AIx.

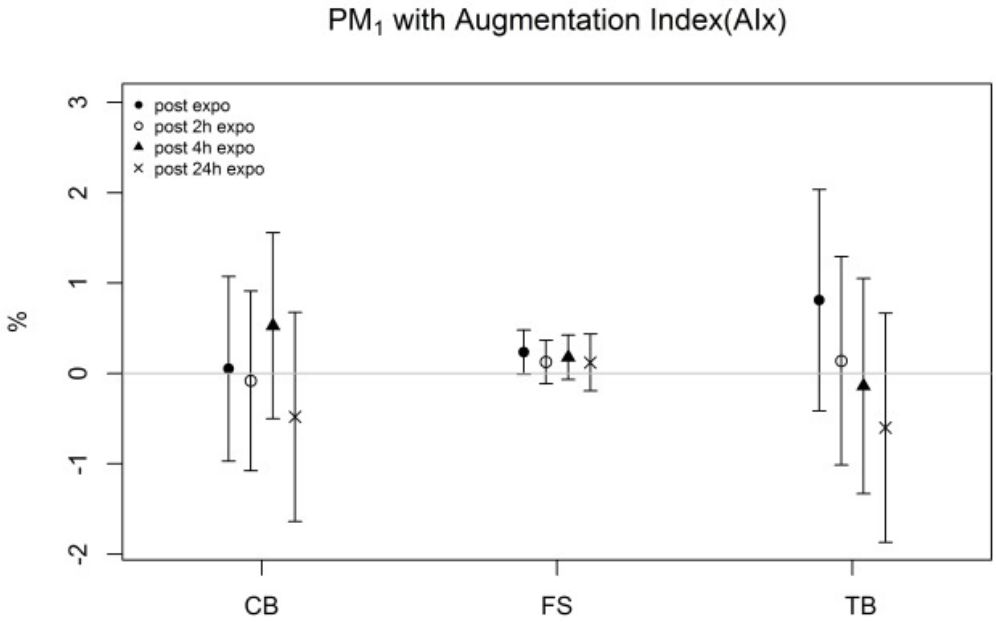

Figure 16: **Mean effect estimates and 95% confidence intervals (CI) for changes in AIx associated with an increase of 10 µg/m^3 PM_1 during candle burning (CB), frying sausages (FS) and toasting bread (TB) in the fully adjusted model**

Although some increases are seen, no clear associations between PM_1 and AIx were observed in all three sources.

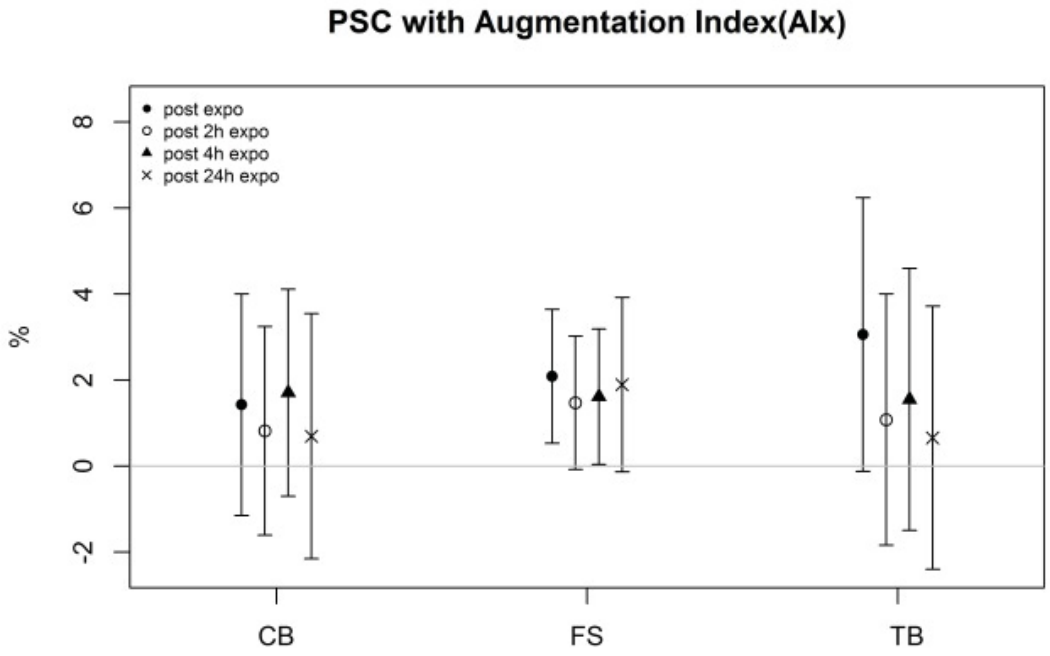

Figure 17: **Mean effect estimates and 95% confidence intervals (CI) for changes in AIx associated with an increase of 1,000 µm^2/cm^3 PSC during candle burning (CB), frying sausages (FS) and toasting bread (TB) in the fully adjusted model**

After the 2 hour exposure with FS, a 1,000 µm^2/cm^3 in PSC was associated with a 2.1% (95%-CI: 0.5; 3.6) and a 1.6% (95%-CI: 0.04; 3.2) increase in AIx directly after and 4 h after the exposure, respectively. In the post 2h measurement and after 24 h, no association was visible. PSC from other sources was not associated with changes in AIx.

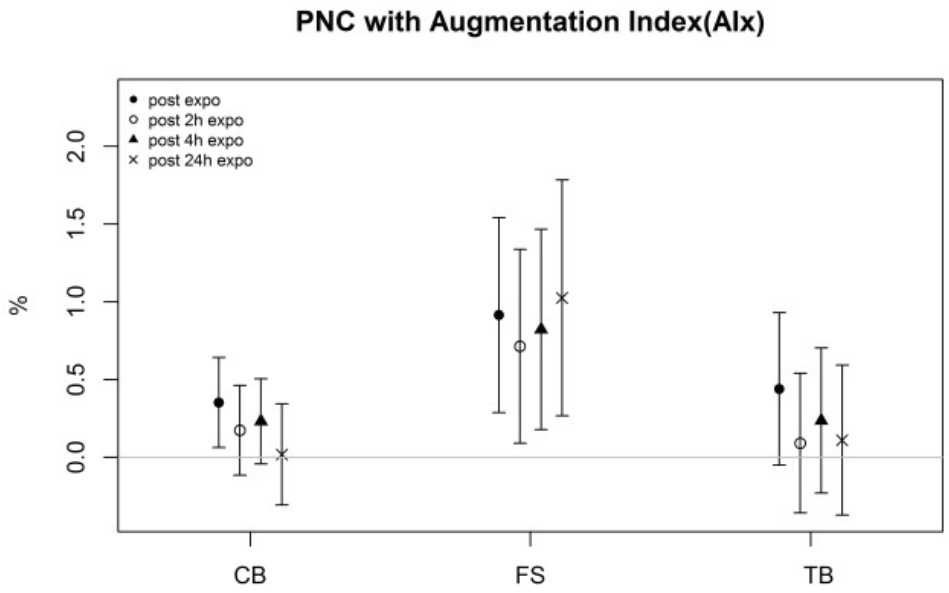

Figure 18: **Mean effect estimates and 95% confidence intervals (CI) for changes in AIx associated with an increase of 50,000 particles/cm^3 PNC during candle burning (CB), frying sausages (FS) and toasting bread (TB) in the fully adjusted model**

Furthermore the 2 hour exposure with FS, a 50,000 particles/cm^3 in PNC was associated with increases in AIx at all measured time points after the exposure. Directly after the exposure an increase of 0.9% (95%-CI: 0.3; 1.5) in AIx was observed. After a lower value of 0.7 % (95%-CI: 0.1; 1.3) at the 2 hour measurement was seen, AIx continued to increase reaching its highest measured value of 1.0 (95%-CI: 0.3; 1.8) after 24 h. In addition during CB, PNC was associated with a 0.4 % (0.1; 0.6) increase in AIx directly after the exposure. PNC from TB was not associated with changes in AIx.

Model II – Main model - IQR

Figure 19 till Figure 21 illustrates the changes in the fully adjusted model for augmentation index (AIx) when comparing different particle metrics using the IQR as exposure unit in each exposure scenario (CB, TB, FS). Similar effects for augmentation pressure (AP) and in the crude model have been observed (Appendices).

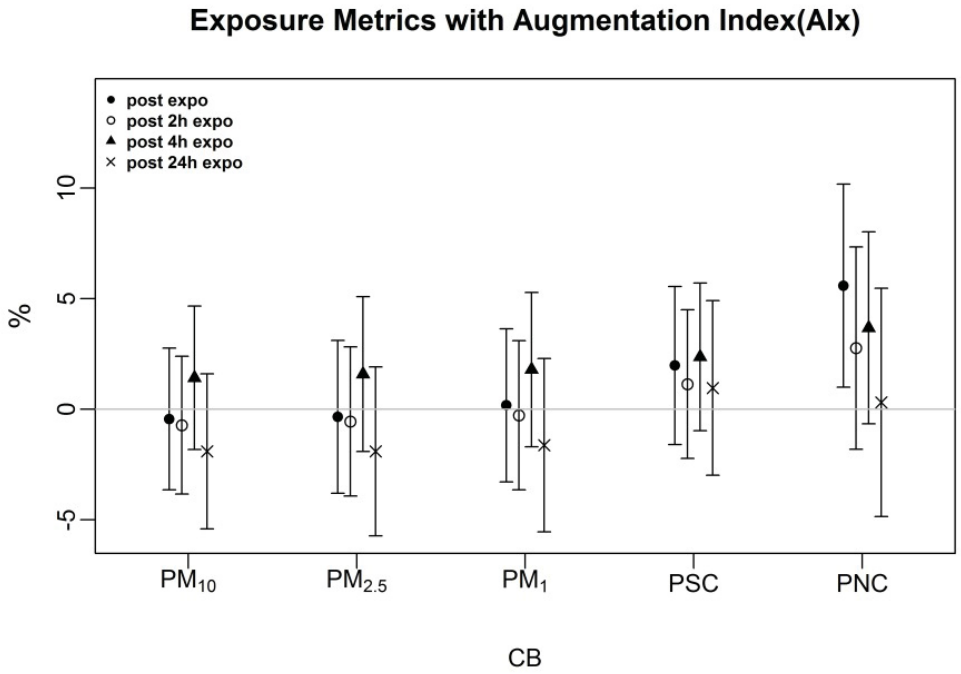

Figure 19**: Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AIx during candle burning (CB) in the fully adjusted model**

After the 2 hour exposure with CB, a significant association was found between PNC and AIx. PNC was associated with a 5.6 % (95%-CI: 1.0; 10.2) increase in AIx per IQR of 793,089.6 directly the exposure. The other metrics were not clearly associated with increases in AIx.

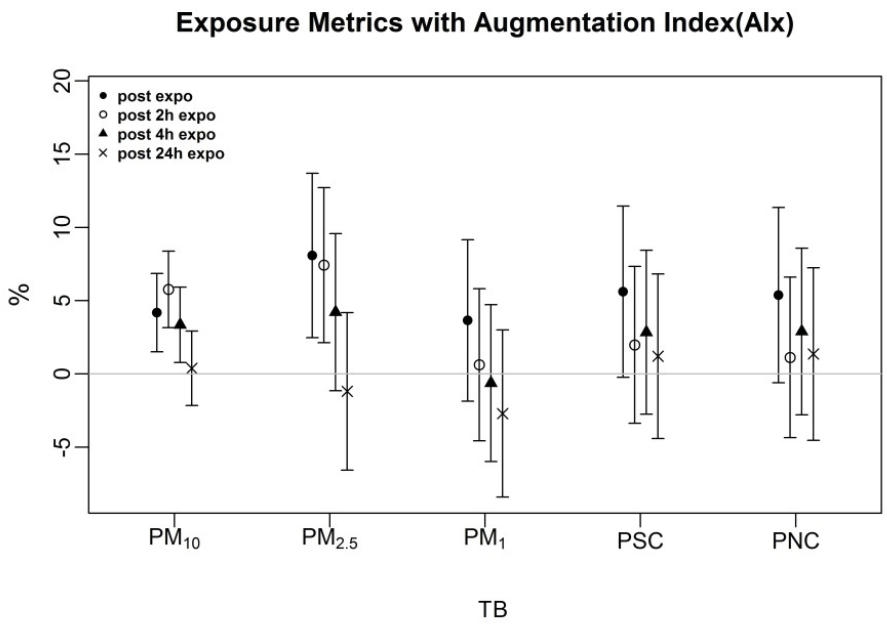

Figure 20**: Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AIx during toasting bread (TB) in the fully adjusted model**

After the 2 hour exposure with TB, significant associations were found between PM_{10} and $PM_{2.5}$ and AIx. PM_{10} was associated with a 4.2 % (95%-CI: 1.5; 6.9), 5.8 % (95%-CI: 3.2; 8.4) and 3.4 % (95%-CI: 0.8; 5.9) increase in AIx per IQR of 53.5 directly after, 2h after and 4h after the exposure, respectively. Similarly, $PM_{2.5}$, was associated with an 8.1 % (95%-CI: 2.5; 13.7) and 7.4 % (95%-CI: 2.1; 12.7) increase in AIx per IQR of 45 directly after and 2h after the exposure, respectively. After an initial increase in AIx directly after and 2h after TB in association with PM_{10} and $PM_{2.5}$, the association were attenuated and vanished after 24h. Other metrics were not associated with increases in AIx.

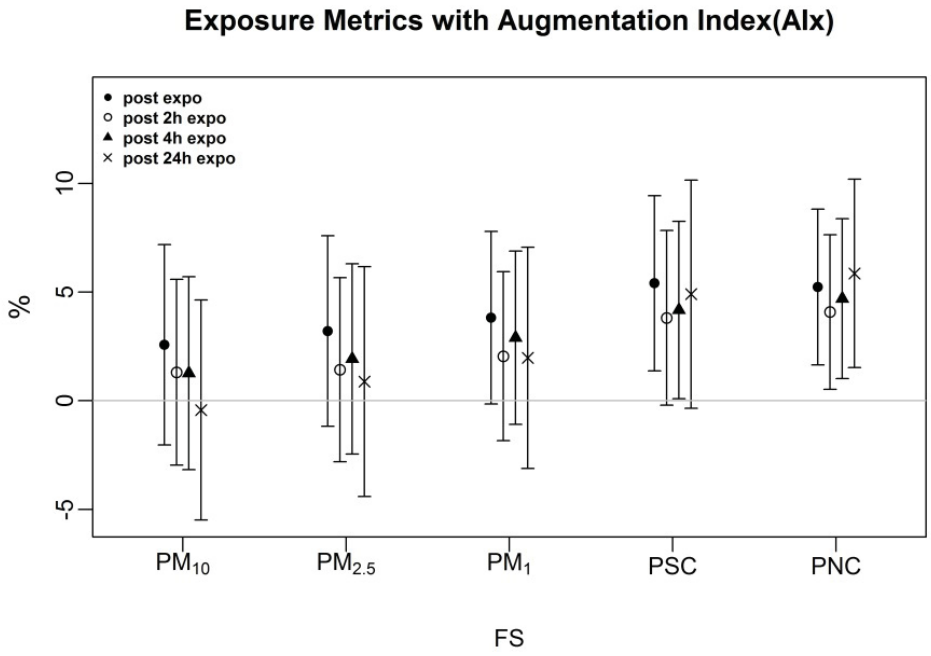

Figure 21: **Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AIx during frying sausages (FS) in the fully adjusted model**

After the 2 hour exposure with FS, significant associations were found between PSC and PNC and AIx. PSC was associated with a 5.4 % (95%-CI: 1.4; 9.4) and 4.2 % (95%-CI: 0.1; 8.3) increase in AIx per IQR of 2593 directly after and 4h after the exposure, respectively. PNC, was associated with a 5.2 % (95%-CI: 1.7; 8.8) , 4.1 % (95%-CI: 0.5; 7.6), 4.7 % (95%-CI: 1.0; 8.4) and 5.9 % (95%-CI: 1.5; 10.2) increase in AIx per IQR of 285,746.6 directly after, 2h after, 4h after and 24 after the exposure, respectively. Other metrics were not associated with increases in AIx.

4. Discussion and conclusions

Answer to hypothesis/research questions

The findings from this study suggest that short-term exposure to fine and ultrafine particles from indoor sources leads to acute changes in arterial stiffness. Indeed, the main 2 research questions could be answered; after a 2-hour exposure to indoor sources (CB, TB and FS) AIx and AP increased and the changes in these central arterial indices differ in their magnitude, duration and association to specific particle metrics.

Prior findings

Only a few studies have investigated the association of particle exposure with AS in a controlled exposure setting. Previous human exposure studies on air pollutants have produced somewhat inconsistent results in terms of central arterial indices. On one hand Unosson et al. (2013) demonstrated in a randomized, double-blinded crossover study with 14 healthy non-smoking subjects that a 3 hour exposure to diluted wood smoke (an average PM_1 of 314±38 $\mu g/m^3$) was associated with higher AIx and AP immediately after the exposure ($p < 0.001$ and $p = 0.004$, respectively) as compared to filtered air (Unosson et al. 2013). On the other hand in another double-blind randomized cross-over study with 16 healthy male fire fighters, a 1 hour exposure to wood smoke with mean PM_1 of 1,115 ± 151 [$\mu g/m^3$] had no effect on AP, AIx or pulse wave velocity ($p > 0.05$ for all) compared to filtered air (Hunter et al. 2014). Although both studies investigated diluted wood smoke in similar controlled exposure settings, the results of the first study with lower mean PM_1 for 3 hours has shown increases in AIx and the results of the latter study with 3 times higher PM_1 for 1 hour has shown no significant increases. These inconsistent findings might be due to differences in the methods, especially duration of exposure, and the complexity of the relationships between PM and AS.

Congruent with our findings, increases in AIx and AP have been seen in some human exposure studies with particle rich sources. In a randomized double-blinded crossover study with 12 healthy volunteers, the authors exposed participants to a 1 h of diluted Diesel exhaust with a particle mass (330 ± 12 $\mu g/m^3$) and an estimated particle number of (1.26 ± 0.01 × 106/cm^3). AP and AIx, measured by applanation tonometry of the radial artery, were measured 10min, 20min and 30min after the exposure (Lundback et al., 2009). A significant increase of 2.5 [mmHg] in AP and of 7.8[%] in AIx was shown in the first measurement, 10min after diesel exhaust exposure, compared to filtered air ($p = 0.01$ and $p = 0.02$

respectively). These increases normalized over the following 20 minutes. In our study a rapid increase in AIx of 8.1 % (95%-CI: 2.5; 13.7) with similar attenuation phenomena over a longer period of time (24h) was observed with the exposure TB, best seen in associations with the $PM_{2.5}$. In year 2008, Argacha et al. conducted a similar study, however they applied the exposure environmental tobacco for 1 hour and measured AIx at 8 times with 10min intervals starting with exposure begin. Mean $PM_{2.5}$ of the exposure was 300±19 [$\mu g/m^3$]. Significant increases in AIx were both seen during ($p=0.01$) and after ($p<0.01$) the exposure to tobacco smoke compared with normal air. The post exposure measurements showed a steep increase in AIx could predict further increases or at least an ongoing effect after exposure termination air (Argacha et al. 2008). Similar findings were observed in the exposure FS, especially in associations with the ultrafine metric PNC. These studies and others demonstrate the impact of exposure to various PM-rich sources on the arterial wall but due to the different nature of the sources and time measurements comparability to our results is limited.

Time course – Rapid effect / Reflex arches

According to our findings, the increases in arterial stiffness indices could be divided into acute increases and subacute increases. The rapid increases in arterial stiffness after short-term exposure to indoor sources in our human controlled study is in line with previous studies. In a recent review on the cardiovascular effects of particulate air pollution exposure (Langrish et al. 2012), short-term exposures caused increases in AS, blood pressure, myocardial ischemia and induced cardiac arrhythmias (Figure 22) (Langrish et al. 2012). Another recent review of 22 studies on selected CV effects of UFPs has shown similar results (Weichenthal 2012). This review presented significant associations between UFPs and short-term changes in components of the so-called ‘‘cardiac death triangle’’: the autonomic nervous system (measured by HRV), myocardial substrate (QT-interval in an Electrocardiograph), and myocardial vulnerability (QT-variance, T-wave alternans in an Electrocardiograph) (Zareba, Nomura, and Couderc 2001).Since these are very rapidly occurring effects on the CV system, it has been suggested, that these effects are probably driven by a particle-induced activation of the autonomic nervous system (Langrish et al. 2012). Furthermore these rapid changes are supported by the fact that human airways are lined with receptors and nerve endings that after stimulation by inhaled PM may be capable of altering reflex ANS pathways leading to a blunting of CV parasympathetic tone and a relative favoring of sympathetic activity (Widdicombe and Lee 2001). The rapid increases in AIx directly after the 2 hour exposure to TB and CB with subsequent decreases in the following measurements support the previous evidence and therefore might be the result of activation of these reflex arches.

Time course –Subacute and persistent effects - inflammation

Looking at the results from after exposure to FS, we observed not only increases directly after the exposure, but an ongoing rise in AIx beginning 2 hours after the exposure and was still seen at our last measurement 24 hours after the exposure (Figure 21). A pure elicitation of this response by autonomous nervous system imbalance seems improbable due to the very short exposure duration in our study. But this finding aligns with the inflammation pathway (Franklin et al. 2015), in which the proinflammatory mediators are generated and released as a result of particle exposure. Although only a small fraction of the inhaled mass may cross into the circulation, this represents a huge number of particles that may have a direct impact on the vascular endothelium (Langrish et al. 2012). Furthermore controlled exposures to concentrated ambient particulate matter and dilute diesel exhaust showed local inflammation within the lungs along with changes in the local antioxidant response (Langrish et al. 2012). One study conducted on rat alveolar macrophages, suggests that exposure to fine particles, particularly indoor-generated particles, have a larger inflammatory response than exposure to outdoor PM (Long et al. 2001). This findings is supported in our study, the ongoing increase in AIx was clearly seen in association with PNC. Furthermore recent studies both conducted on subpopulations of the Health2006 cohort, which consists of a randomly sample of people aged 18–69 years and living in the south-west part of the Copenhagen area in Denmark in 2006 (Olsen et al. 2014), showed mixed evidence. Olsen et al. 2014 presented in a cross-sectional study higher association for inflammation parameters (e.g. leukocyte count) after exposure to UFPs from outdoor sources compared with indoor sources (Olsen et al. 2014). Jantzen et al.(2016) demonstrated different associations in a subpopulation of the same cohort after exposure to fine and ultrafine particles from indoor sources; positive associations were shown for some measured inflammation outcomes (e.g. levels of endothelial progenitor cells) and negative associations were shown for others (e.g. basal capacity for reactive oxygen species production) (Jantzen et al. 2016). Although final results of inflammation mediators in our study to support our results are still a matter of ongoing evaluation, these findings after FS are in alignment with the plausible pathway mentioned.

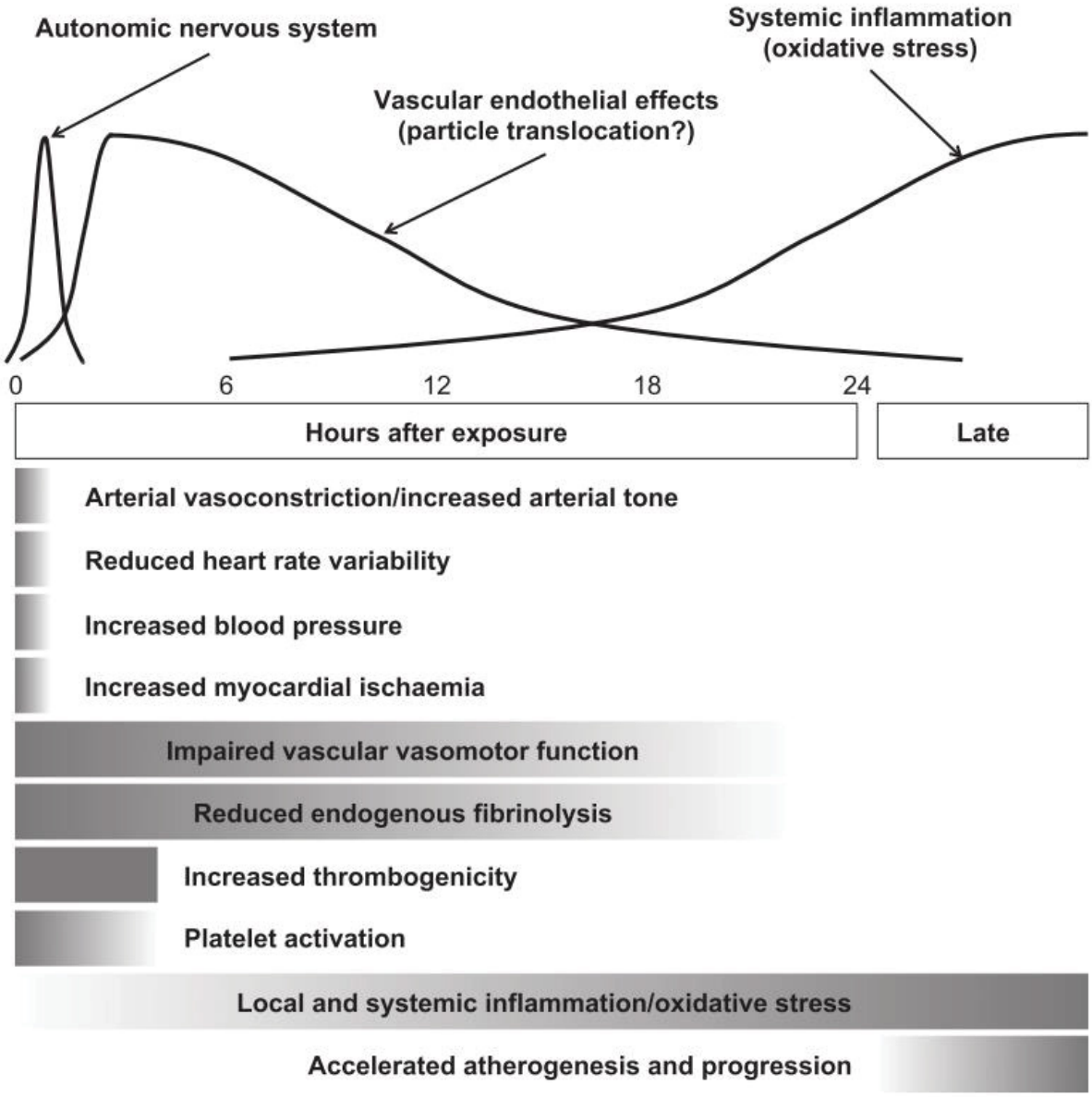

Figure 22. **Acute vascular, thrombotic and inflammatory effects of exposure to particulate air pollution and proposed underlying mechanisms** (Langrish et al. 2012).

Why did we choose pulse wave analysis to examine preclinical changes in arterial stiffness?

In our study, we chose to investigate arterial stiffness as assessed by PWA. There are many advantages in this choice. PWA has emerged as a noninvasive, valid, reliable, accurate, and widely used technique to investigate central blood pressures and arterial stiffness (Weber et al. 2010) (Papaioannou et al. 2007) (Papaioannou et al. 2016).

Moreover both examined central haemodynamic indexes were chosen due to their strong predictive value of cardiovascular disease (Nürnberger et al. 2002) (Weber et al. 2004) and their measurement, especially central AIx, have been shown to be an independent predictors of future clinical CV events and all-cause mortality (Vlachopoulos et al. 2010). In this review

of 11 longitudinal studies with 5648 subjects and a mean follow-up of 45 months, a 10% absolute increase of central augmentation index (AIx) was associated with a risk-factor-adjusted pooled relative risk (RR) of 1.318 (95% CI 1.093–1.588) for total CV events and a RR of 1.384 (95% CI 1.192–1.606) for all-cause mortality (Vlachopoulos et al. 2010). Furthermore a recent review on the association between central hemodynamics (e.g. AIx) and hypertensive organ damage in different organs concludes that central hemodynamic monitoring has great potential to provide a new diagnostic and therapeutic basis for preventing systemic target organ damage by and offering personalized therapy suitable for the arterial properties in each individual patient with hypertension (Hashimoto 2014). In the addition to the implementation of PWA in long term studies, the measurement of AS with this method has been used to assess short-term functional effects on arterial properties. In a review of 27 placebo-controlled trial using noninvasive measurements of arterial function to assess the efficacy of antihypertensive drugs in reducing arterial stiffness and wave reflections, it has been suggested that short-term changes in arterial stiffness and wave reflection could be clinically useful in monitoring the effect of antihypertensive treatment and other cardiovascular therapeutic approaches (Liu et al. 2013). Increases in AIx after short-term exposure to wood smoke, diesel exhaust and metal rich PM have been previously discussed (Unosson et al. 2013) (Lundback et al. 2009) (Fang et al. 2008). Taken together, the numerous advantages, wide spread use and strong predictive value led us to choose AIx as our main outcome in evaluating short-term physiological changes in the arterial wall.

Effects of different particle metrics separately

Comparing the effect of different particle metrics separately for each source, we see statistically significant ($p< 0.05$) positive association for PMC, PNC and PSC. During TB, associations between PM_{10} and $PM_{2.5}$ and AIx could be clearly identified directly after, 2 h and 4 h after the exposure. As mentioned previously, controlled human exposure to $PM_{2.5}$ from diesel exhaust and passive smoking have demonstrated similar increases in AIx (Lundback et al. 2009) (Argacha et al. 2008). The similar association supports the idea that PMC itself regardless of their source can induce changes in AIx. But the difference in the methods makes comparability to our results limited. In our study strongest increases in AIx were seen during FS and CB in association with ultrafine metrics (PNC and PSC). Increasing PNC, measured during all three sources, led to increases in arterial stiffness indices. The most stringent associations for all time points could be observed for PNC measured during FS. PSC seemed to follow the pattern of PNC, but less strong. These findings are in line with evidence shown in a review of 22 studies, which shows that UFP exposures have a measureable impact

on several physiological measures (e.g. endothelial vasomotor function) (Weichenthal 2012). Furthermore results of both animal and human studies provide evidence for respiratory and cardiovascular effects associated with exposure to UFPs, with the exception of some effects on the brain the findings are largely similar to those observed for exposures to fine particles (HEI Review Panel 2013). Taken together, different exposure metrics measured for each exposure scenario may a possible explanation for the different changes seen in AIx in the post exposure assessments. A supporting example from our results is seen during CB, the source with the highest mean PNC, significant increases in AIx were seen in association with PNC in both models. But during FS, the source with highest PMCs, a different picture is seen. Although some increases in AIx are seen in association with PMC, significant increases in AIx are seen in association with PNC and PSC. The findings in CB may be explained by the physical properties but the results in other exposures could not be completely explained by metrics alone.

What makes UFPs so special? (Specific characteristics for ultra-fine particles)

Although ,as mentioned before, UFPs don't add much to the PMC, their smaller aerodynamic size, larger numbers and higher surface concentration makes their interaction with living organisms different from larger particles. As an example, an extensive recent review on the health effects of ambient UFPs summarizes that in animal studies inhaled UFPs, but not fine or coarse particles, can translocate across the lung epithelium into the circulatory system and then be transported throughout the body where they have the potential to affect directly the cardiovascular system and other organs (HEI Review Panel 2013). Furthermore the association between PSC and increases in AIx in our study might be explained by results found in animal und in vitro studies which showed that one of the main causes of the greater inflammatory response of UFPs might be the large surface area of such particles (Donaldson et al. 2001) (Brown et al. 2007) (Renwick et al. 2004). The greater surface area per mass compared with larger-sized particles of the same chemistry renders UFPs more active biologically, thus UFPs have a higher potential for inflammation (Oberdörster et al. 2005). Another interesting point raised by the present study is that the haemodynamic effects associated with PNC ,which persistently showed significant increases after FS, is in line with previous results, showing that changes in PNC are correlated with increases in subclinical inflammatory markers of CVD risk, blood markers of inflammation and acute airway inflammation (Lane et al. 2016) (Fuller et al. 2015) (Strak et al. 2012). Summing up, the special characteristics of UFPs, especially through their ability to translocate into the circulatory system and higher contribution to PNC and PSC, gives them a special role in

mediating many physiological cardiovascular effects. Our associations between PNC and PSC and changes in AIx support previous evidence showing the impact of UFPs on several subclinical measures of CVD.

The role of chemical composition

Interestingly, further analysis of exposure characteristics showed that PSC levels in our study were very similar across the different exposure scenarios, but statically significant increases of AIx in association with PSC were only seen for FS. The specific organic compounds (e.g. organic carbon species) from FS, which made 99% of the particle bound content measured by AMS, might have led to the PSC associated increases in AIx. These finding supports the accumulative evidence, that the health effects linked to exposure to particulate matter are not only dependent on the physical properties of PM (such as PSC) but also the chemical and biological compositions play an important role (Morakinyo et al. 2016). A review on ambient PM properties have previously shown that the chemical characteristics are important for the adverse health effects, findings for metals were most convincing and were stated to be important for the development of both pulmonary and cardiovascular disease (Schwarze et al. 2006). Looking at indoor air, in a recent workshop on health risks of indoor PM, Charles Weschler stated that indoor PM includes thousands of organic species and the complexity is staggering, "We need to know more about the actual molecular nature of the chemicals present in indoor PM, both in terms of the transition metal complexes and the organic species". (Butler et al. 2016). The results of the chemical characterization show very small amounts of elemental carbon and some inorganic components consisting of trace metals (e.g. Iron), and ionic species (e.g. Nitrates). We have hypnotized that some of these chemical constituents, including iron being a transient metal with high oxidation potential, may have stimulated some inflammation, which led to the ongoing increases in AIx seen 24 hours after the exposure to FS. Findings from toxicological studies reported that organic compounds and transition metals present in ambient $PM_{2.5}$ may be significant due to their ability to stimulate inflammation with subsequent respiratory and cardiovascular effects (Morakinyo et al. 2016). Furthermore an observational follow-up study investigating the effect of metal-rich airborne particles from welding fumes on AIx, showed significant associations between some metals (e.g. Nickel) and increased AIx (Wong et al. 2015). Based on these studies and our findings, we assumed that not only physical characteristics but also the biochemical composition of the particles might have played a role in mediating certain effects. But our basic results were not able to certify such effects.

Relevance of outdoor and indoor particle exposure

The association between ambient particulate matter air pollution and a higher morbidity and mortality specifically of cardiovascular diseases is well documented in a significant number of studies (Brook et al. 2010; Hoek et al. 2013). Furthermore the scientific evidence is consistent with a causal relationship between $PM_{2.5}$ exposure and cardiovascular morbidity and mortality (Brook et al. 2010). People spend approximately 90 % of their time indoors (Klepeis et al. 2001) Thus, for many people, the risks to health may be greater due to exposure to air pollution indoors than outdoors. Due to the heterogeneity of the composition of indoor air from outdoor air and diverse indoor particle emitting sources (e.g. burning candles) and activities (e.g. sweeping), very complex indoor particle exposure patters exist (Fromme 2012). Recently it has been shown that the levels of indoor PM have the potential to exceed outdoor PM levels (Chen & Zhao 2011) and up to 30% of the burden of disease from particulate matter exposure can be attributed to indoor-generated particles (Morawska et al. 2013), thus studying the health related effects of PM exposure from indoor sources on the cardiovascular system is of rising importance. For example, a study of 63,257 Singapore Chinese over 5 years showed that long-term exposure to incense burning in the home environment was associated with an increased risk of cardiovascular mortality (Pan et al. 2014). In our study number concentrations of indoor sources were dominated by the contribution of UFPs, 2 hour mean exposure values reached up to 2.8×10^6 [#/cm³] during candles burning. This result is in line with a recent review, that shows all three sources (CB, TB and FS) among other sources contribute primarily to an increase in particle number concentration of ultrafine particles (Morawska et al. 2013). Moreover, experimental studies in full scale chambers characterizing indoor sources of fine and ultrafine sources demonstrated similar increases in PNCs for frying meat, toasting bread and candle burning (Dennekamp et al. 2001) (Afshari et al. 2005). According to Abt et al. (2000) some indoor sources, such as frying, can contribute to both the smaller (i.e. UFP) and larger particle concentrations (contributing to $PM_{2.5}$ and PM_{10}) (Abt et al. 2000). Very similar findings were found in our study as well, the highest levels of $PM_{2.5}$ and PM_{10} was measured during FS.

Indoor Air – Organic Materials

Another interesting point found in our study, the proportion of elemental and organic carbon is similar between particles emitted from the three sources. The aerosol mass spectrometry (AMS) showed that organic carbon compounds represent the greatest fraction for toasting

bread (50-60%), candle burning (40%) as well as for frying sausages (60%). Similar findings have been demonstrated in the sub analysis of exposures from 173 homes out of the Relationship of Indoor, Outdoor and Personal Air (RIOPA) Study (Weisel et al. 2005), which showed that organic material, especially organic carbon, appears to be the predominant species in indoor-generated $PM_{2.5}$ (Polidori et al. 2006). The sub analysis modeling suggested that at least 41%, but more likely 71% to 76% of organic compounds found indoors was emitted or formed indoors, rather than being transported inside from outdoors (Polidori et al. 2006). The toxic effect of organic constituents of ambient fine particles on cardiovascular outcomes have been demonstrated in a recent experimental study with mice (Keebaugh et al. 2015). Keebaugh et al. (2015) showed the exposure to concentrated PM after removal of organic constituents by thermal denuding led to no adverse effects (e.g. HRV and biomarkers of oxidative stress) compared to mice exposed to concentrated PM, thus concluding that removal of organic constituents from ambient particles results in significant reduction of toxic cardiovascular effects of air pollution exposure (Keebaugh et al. 2015). While in real-life situations, most of the organic compounds found in indoor air stems from outdoor sources, in our study the organic compounds were primarily generated by the active indoor sources. Therefore, comparability is limited. Nonetheless due to the high concentration of organic compounds in our results and results of the studies mentioned above, it is vital to further investigate the role of distinct organic constituents as well as distinct types of organic carbon compounds and their role in mediating the toxic effects of PM and UFPs.

Could the Smell/Odor produced by the indoor sources have caused changes in AIx?

During the exposure sessions participants have reported the odor produced from the activated source were somewhat intense or unpleasant, especially during the 2 hour exposure to FS. Some studies have examined the effect of odors on our emotions and ANS. Odorants have been shown able to induce basic emotions and evoke ANS responses (Bensafi et al. 2002) (Alaoui-Ismaïli et al. 1997). In another study an increased autonomic arousal was demonstrated in response to unpleasant odorants (Vernet-Maury et al. 1999). In addition a review of 134 publications that report experimental investigations of emotional effects on peripheral physiological responding in healthy individuals suggests considerable ANS response specificity in emotion (Kreibig 2010). The increases in AIx in our study are seen in correlation with different particle metrics throughout the different source. Thus making it more plausible, that the particles characteristics (e.g. number of particles) or chemical composition were responsible for changes in AIx.

Future studies

Globally, people are spending an increasing amount of time indoors, thus making further research on indoor air and its health related effects in our societies essential. As shown in this thesis, human studies have found links between indoor PM and increased systemic inflammation, blood vessel dysfunction, and increased blood pressure. Due to high variability between indoor environments and the lack of good models for predicting indoor PM levels, this field presents an important epidemiologic research challenge. More research is needed to better characterize the relative toxicity of PM and UFP generated indoors. In addition further research is needed to investigate the relationship between long-term exposure to indoor sources and actual clinical outcomes such as heart attack and stroke. The proposed research aspects can lead to expansion of the existing indoor quality guidelines in Europe and the world to include particulate air pollution in the future.

Strengths and limitations

Our study has several limitations. First of all, blinding the participants to the sources was not possible, due to real time operating of the sources in the chamber to mimic general indoor activities, which led to build up aromas and sounds from the source. Due to sick leave, premature study discontinuation or other personal reasons not all participants received all exposure scenarios. To create controlled and standardized exposure scenarios which are as uniform as possible across study participants, there were some differences to real-life exposures in a common household. The candles for example were replaced before burning down, decreasing soot emissions, and the sausages were fried in a pan without fat. Another limitation is that during the exposure of the participants we did not conduct a detailed biochemical characterization of the exposures. Another point was the questionnaires that were filled out by the participants, which led to some missing data regarding personal information. The strengths of our study are a detailed exposure characterization, including continuous measurements of number size distribution, size-specific mass concentrations and surface concentrations, enabling us to assess personal mean exposures for established and novel particle metrics. We achieved a high exposure contrast between the sham exposure and the experimental exposures for all particle metrics, which increased our power to detect even small, clinically not relevant effects. Another strength of the study is that we additionally controlled for ambient air pollution.

Conclusions

Our study showed positive associations between short-term exposure to fine and ultrafine particle from indoor sources and arterial stiffness. While our results showed an association of short-term exposure to fine and ultrafine particles from all three sources, the magnitude of these effects differ and were not always consistent. Significant increases in AIx were shown in association for PM_{10} and $PM_{2.5}$ during TB. On the other hand significant increases in AIx in association with PNC and PSC were seen during CB and FS. In addition to aerodynamic size of the particles, the differences in chemical composition of source-specific particles might account for these the differences observed for different particle size metrics and particle sources. Accordingly a detailed biochemical characterization of indoor sources and their role in mediating different physiological changes should be further studied, which could shed more light on the biological pathways involving various health effects and to verify their clinical significance.

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Appendix 3: Screening questionnaire

Name: _____
ID : |___|___|___| Datum: |___|___|.|___|___|.|___|___|___|___|

1	Geburtsdatum	|___|___|.|___|___|.|___|___|___|___| t t . m m . j j j j
2	Wohnort	_____
3	Nationalität	_____
4	Verstehen und sprechen Sie fließend Deutsch?	Ja ☐1 Nein ☐2 (PAK)
5	Üben Sie zur Zeit eine berufliche Tätigkeit aus? Wenn ja, welche? _____ _____	Ja ☐1 Nein ☐2
6	Sind Sie zurzeit schwanger?	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
7	Planen Sie in den nächsten 6 Monaten eine Schwangerschaft?	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
8	Rauchen Sie?	Ja ☐1 (PAK) Nein ☐2 (Ex-Raucher) (PAK) Nein ☐3 (noch nie geraucht)
9	Sind Sie täglichem Passivrauch ausgesetzt?	Ja ☐1 (PAK) Nein ☐2
10	Sind Sie beruflich erhöhtem Feinstaub ausgesetzt (wie z.B. Schüttgut, Schleifen)? Wenn ja, wann und wie lange? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
11	Waren Sie früher beruflich erhöhtem Feinstaub ausgesetzt (wie z.B. Schüttgut)? Wenn ja, wann und wie lange? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
12	Sind Sie in Ihrer Freizeit erhöhtem Feinstaub ausgesetzt? Wenn ja, wann und wie lange? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
13	Leiden Sie an einer Krebserkrankung? Wenn ja, an welcher? _____ _____	Ja ☐1 Nein ☐2 Weiß nicht ☐3
13a	Ist die Behandlung dieser Krebserkrankung erfolgreich abgeschlossen?	Ja ☐1 Nein ☐2 (PAK) Weiß nicht ☐3
14	Leiden Sie an Herz-Kreislauf-Erkrankungen? Wenn ja, an welchen? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
15	Leiden Sie an Bluthochdruck?	Ja ☐1 Nein ☐2 Weiß nicht ☐3
15a	Wenn ja, ist dieser medikamentös stabil eingestellt?	Ja ☐1 Nein ☐2(PAK)
16	Leiden Sie an Lungenerkrankungen? Wenn ja, an welchen? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
17	Leiden Sie an Diabetes?	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
18	Leiden Sie an chronischen Infektionskrankheiten? Wenn ja, an welchen?	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3

	_____ _____	
19	Hatten Sie jemals einen Schlaganfall? Wenn ja, wann? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
20	Leiden Sie an einer Schilddrüsenerkrankung? Wenn ja, an welcher? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
21	Leiden Sie an neurologischen oder mentalen Erkrankungen? Wenn ja, an welchen? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
22	Leiden Sie an rheumatologischen Erkrankungen?	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
23	Leiden Sie an anderen chronischen Erkrankungen? Wenn ja, an welchen? _____ _____	Ja ☐1 (PAK) Nein ☐2 Weiß nicht ☐3
24	Ist in den nächsten 6 Monaten eine Operation geplant?	Ja ☐1 (SAK) Nein ☐2
25	Haben Sie Heuschnupfen?	Ja ☐1 (SAK) Nein ☐2 Weiß nicht ☐3
26	Nehmen Sie derzeit Medikamente ein? a) bei Bedarf? b) regelmäßig?	Ja ☐1 (SAK) Nein ☐2 Ja ☐1 (SAK) Nein ☐2 Ja ☐1 (SAK) Nein ☐2
26a	Medikamenteneinnahme bei Bedarf? Wenn ja, welche? _____ _____	Ja ☐1 (SAK) Nein ☐2
26b	Dauermedikation? Wenn ja, welche? _____ _____	Ja ☐1 (SAK) Nein ☐2
27	Nehmen Sie Nahrungsergänzungsmittel/ Vitaminpräparate? Wenn ja, welche? Wie häufig? _____ _____ _____ _____ _____ _____ _____ _____ _____ _____	Ja ☐1 (SAK) Nein ☐2
28	Betreiben Sie Sport? Wenn ja, welchen? Wie häufig? _____ _____ _____ _____ _____ _____ _____ _____ _____ _____	Ja ☐1 (SAK) Nein ☐2
Kontraindikationen Lungenfunktionstest		
29	Haben Sie ein Aortenaneurysma? (Aufdehnung der Hauptschlagader)	Ja ☐1 (SAK) Nein ☐2
30	Hatten Sie einen Herzinfarkt in den letzten 3 Monaten?	Ja ☐1 (SAK) Nein ☐2
31	Hatten Sie eine Operation im Brust- oder Bauch-bereich in den letzten 3 Monaten?	Ja ☐1 (SAK) Nein ☐2 Weiß nicht ☐3
32	Hatten Sie eine Augenoperation im letzten Monat?	Ja ☐1 (SAK) Nein ☐2 Weiß nicht ☐3

33	Waren Sie in den letzten 3 Monaten schwanger?	Ja ☐1 (SAK) Nein ☐2 Weiß nicht ☐3
34	Nehmen Sie Tuberkulosemedikamente ein?	Ja ☐1 (SAK) Nein ☐2
Kontraindikationen Bronchospasmolyse		
35	Stillen Sie momentan?	Ja ☐1 (SAK) Nein ☐2
Kontraindikationen Blutabnahme		
36	Leiden Sie an Hämophilie?	Ja ☐1 (SAK) Nein ☐2 Weiß nicht ☐3
36a	Nehmen Sie Medikamente ein, die die Blutgerinnung hemmen? Wenn ja, welche? _____	Ja ☐1 (SAK) Nein ☐2
Kontraindikationen Nasallavage		
37	Leiden Sie an Verletzungen oder offenen Wunden im Nasenraum?	Ja ☐1 (SAK) Nein ☐2

Appendix 4: General questionnaire

S1	Datum (TT.MM.JJJJ)		|__|__| . |__|__| . |__|__|__|__| T T M M J J J J	
S2	Uhrzeit (Std. Min.)		|__|__| . |__|__| Std. Min.	
S3	Probanden-ID		|__|__|__|	
S4	Untersucher-ID		|__|__|__|	

Soziodemografische Faktoren und Sozioökonomischer Status

SF1	Geschlecht	☐1 ☐2	Männlich Weiblich	
SF2	Wann sind Sie geboren? (TT.MM.JJJJ)		|__|__| . |__|__| . |__|__|__|__| T T M M J J J J	
Ethnizität				
SFE1	Wo sind Sie geboren? Bitte verwenden Sie die heutige Staatsbezeichnung!	☐1 ☐2	Deutschland (in den heutigen Grenzen) In einem anderen Land und zwar: Geburtsland	
Familienstand				
SFF1	Welchen Familienstand haben Sie? Mit „Verheiratet“ meinen wir auch eingetragene Lebenspartnerschaft	☐1 ☐2 ☐3 ☐4 ☐5	Verheiratet mit Ehepartner/in zusammen lebend Verheiratet mit Ehepartner/in getrennt lebend Ledig Geschieden Verwitwet	
SFF2	Haben Sie einen festen Partner/ Partnerin? **INT**: Nicht fragen, wenn SFF1=1	☐1 ☐2	Ja Nein	

Ausbildung

SFA1	Welchen höchsten allgemeinen Schulabschluss haben Sie? Sagen Sie es mir bitte anhand dieser Liste.	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8	Schulabgang ohne Abschluss Hauptschulabschluss / Volksschulabschluss Realschulabschluss / Mittlere Reife/ Fachschulreife Polytechnische Oberschule 10. Klasse (vor 1965: 8. Klasse) Fachhochschulreife / fachgebundene Hochschulreife / Fachoberschule allgemeine Hochschulreife (Abitur, EOS, Berufsausbildung mit Abitur) anderer Schulabschluss: _____ noch in Schulausbildung, Art der Schule: _____	

Erwerbsstatus

SFEs1	Üben Sie derzeit eine berufliche Tätigkeit aus?	☐ 1 ☐ 2	Ja Wenn ja, welche?_____ Nein	
SFEs2	Waren Sie jemals arbeitslos?	☐ 1 ☐ 2	Ja Nein	
SFEs3	Wie lange sind Sie insgesamt arbeitslos gewesen?		Vor 1990: |__|__| Jahre **oder** |__|__| Monate arbeitslos Seit 1990: |__|__| Jahre **oder** |__|__| Monate arbeitslos	

Medizinische Anamnese

Stoffwechselerkrankungen

		1) Wurde bei Ihnen jemals von einem Arzt [Erkrankung] diagnostiziert? 1: Ja 2: nein 3: Weiß nicht	2) In welchem Jahr oder in welchem Alter wurde zum ersten Mal [Erkrankung] diagnostiziert? Jahr |__|__|__|__| oder Alter |__|__|	3) Hatten Sie in den letzten 12 Monaten [Erkrankung]? 1: Ja 2: nein 3: Weiß nicht	4) Wurden Sie in den letzten 12 Monaten wegen [Erkrankung] behandelt? 1: Ja 2: nein 3: Weiß nicht
MASt1	Stoffwechselerkrankungen (z.B. Diabetes, erhöhte Blutfette, Gicht/ Harnsäureerkrankung)	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3

Block Diabetes Ende

MASt2	Erhöhte Blutfette (Cholesterin, Triglyceride)	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MASt3	Gicht/ Harnsäureerkrankungen	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
Rheumatologische und muskuloskelettale Erkrankungen					
MAR1	Arthrose: Hüft-/ Knie-/ Fingergelenke	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAR2	Entzündliche rheumatische Erkrankungen (Rheumatoide Arthritis, entzündliche Wirbelsäulenerkrankungen)	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAR2a	Rheumatoide Arthritis/ chronische Polyarthritis	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAR2b	Entzündliche Wirbelsäulenerkrankungen / ankylosierende Spondylitis, Morbus Bechterew	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAR3	Autoimmunerkrankungen (z.B. Lupus erythematodes, Sjörgen-Syndrom)	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
		1) Wurde bei Ihnen jemals von einem Arzt [Erkrankung] diagnostiziert? 1: Ja 2: nein 3: Weiß nicht	2) In welchem Jahr oder in welchem Alter wurde zum ersten Mal [Erkrankung] diagnostiziert? Jahr |__|__|__|__| oder Alter |__|__|	3) Hatten Sie in den letzten 12 Monaten [Erkrankung]? 1: Ja 2: nein 3: Weiß nicht	4) Wurden Sie in den letzten 12 Monaten wegen [Erkrankung] behandelt? 1: Ja 2: nein 3: Weiß nicht
Allergien					
MAA1	Allergien (z.B. Heuschnupfen, Nahrungsmittelallergien, Tierhaarallergien, Hausstauballergie)	☐1 ☐2 ☐3			
MAA2	Heuschnupfen	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAA3	Nahrungsmittelallergie	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAA4	Hausstauballergie	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAA5	Tierhaarallergie	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
Magen-Darm-Erkrankungen					
MAMD1	Sodbrennen/ Reflux	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3

MAM D2	Colitis ulcerosa, Morbus Crohn	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
		1) Wurde bei Ihnen jemals von einem Arzt **[Erkrankung]** diagnostiziert? 1: Ja 2: nein 3: Weiß nicht	2) In welchem Jahr oder in welchem Alter wurde zum ersten Mal [Erkrankung] diagnostiziert? Jahr |__|__|__|__| oder Alter |__|__|	3) Hatten Sie in den letzten 12 Monaten **[Erkrankung]?** 1: Ja 2: nein 3: Weiß nicht	4) Wurden Sie in den letzten 12 Monaten wegen **[Erkrankung]** behandelt? 1: Ja 2: nein 3: Weiß nicht
Hauterkrankungen					
MAH1	Hauterkrankungen (z.B. Neurodermitis, Schuppenflechte)	☐1 ☐2 ☐3			
Neurologische und mentale Erkrankungen					
MAN M1	Neurologische und psychische Erkrankungen (z.B. Krampfanfall, Parkinson-Syndrom, Depression, Multiple Sklerose, Migräne)	☐1 ☐2 ☐3			
MAN M2	Depression	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAN M3	Migräne	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
Krebserkrankung					
MAK1	Krebserkrankung	☐1 ☐2 ☐3	Jahr |__|__|__|__| oder Alter |__|__|	☐1 ☐2 ☐3	☐1 ☐2 ☐3
MAK1a	Um welche Krebserkrankung handelt es sich?		_____ **Krebserkrankung**		

Alkohol

A1	Haben Sie jemals Alkohol getrunken?	☐ 1 ☐ 2	Ja Nein	Block -ende
A2	Wie oft haben Sie in den letzten 12 Monaten im Durchschnitt alkoholische Getränke, also z.B. ein Glas Wein, Bier, Mixgetränk, Schnaps oder Likör getrunken?	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	Nie Nur zu besonderen Anlässen 1 mal pro Monat oder seltener 2-4 Mal pro Monat 2-3 Mal pro Woche 4 Mal oder mehrmals pro Woche aber nicht täglich Täglich	A7
A3	Wie viel Bier, Wein, Sekt, Apfelwein oder Alkopops trinken Sie gewöhnlich an Werktagen (von Montag bis Donnerstag)?		Insgesamt über alle Werktage |__|__|__|,|__|__|__| Liter	
A4	Wie viel Bier, Wein, Sekt,		Insgesamt am Wochenende	

	Apfelwein oder Alkopops trinken Sie gewöhnlich an Wochenenden (Freitag, Samstag, Sonntag)?		|__|__|__|,|__|__|__| Liter	
A5	Wie viele Zentiliter Spirituosen, Likör, Cocktails trinken Sie gewöhnlich an Werktagen (von Montag bis Donnerstag)? *Ein Schnaps: 2cl* *Ein Cocktail: 4cl, Likör: 4cl*		Insgesamt über alle Werktage |__|__|__| cl	
A6	Wie viele Zentiliter Spirituosen, Likör, Cocktails trinken Sie gewöhnlich an Wochenenden (Freitag, Samstag, Sonntag)? *Ein Schnaps: 2cl* *Ein Cocktail: 4cl, Likör:4cl*		Insgesamt am Wochenende |__|__|__| cl	Block -ende
A7	Warum haben Sie in den letzten 12 Monaten keinen Alkohol getrunken? *Mehrfachantworten möglich*	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8	Vorliegen einer Erkrankung Andere gesundheitliche Gründe/ zum Schutz meiner Gesundheit Empfehlung eines Arztes Finanzielle Gründe Religiöse Gründe Ich bin trockene/r Alkoholiker/in Andere Gründe Weiß nicht	

Sportliche Aktivität

Denken Sie an all Ihre **anstrengenden** Aktivitäten in den **vergangenen 7 Tagen. Anstrengende** Aktivitäten bezeichnen Aktivitäten die starke körperliche Anstrengungen erfordern und bei denen Sie deutlich stärker atmen als normal. Denken Sie dabei nur an körperliche Aktivitäten, die Sie für mindestens 10 Minuten ohne Unterbrechung verrichtet haben.

SA1	An wie vielen **der vergangenen 7 Tagen** haben Sie anstrengende körperliche Aktivitäten wie schweres Heben, Aerobic oder schnelles Fahrradfahren betrieben? *Denken Sie dabei nur an körperliche Aktivitäten, die Sie für mind. 10 Minuten ohne Unterbrechung verrichtet haben.*	☐ 1 ☐ 2	|__| Tage pro Woche Keine anstrengenden körperlichen Aktivitäten	UI1
SA1a	Wie viel Zeit haben Sie für gewöhnlich an einem dieser Tage mit anstrengender körperlicher Aktivität verbracht?	☐ 1 ☐ 2 ☐ 3	|__|__| Stunden pro Tag |__|__|__| Minuten pro Tag keine anstrengenden körperl. Aktivitäten	

Umweltbelastung Innenraum

UI1	Wie würden Sie den Verkehr in der Straße, in der Sie wohnen beschreiben?	☐1 ☐2 ☐3	Anwohnerverkehr (in Wohngebieten) Durchgangsverkehr Berufsverkehr	
UI2	Kommt es in den Hauptverkehrszeiten regelmäßig zu Staubildung in der Straße, in der Sie wohnen?	☐ 1 ☐ 2	Ja Nein	
UI3	Befindet sich das Haus, in dem Sie wohnen, in einer Straßenschlucht von mindestens 100 Metern Länge? *Straßenschlucht: überwiegend geschlossene Häuserzeilen auf beiden Seiten der Straße; Querstraßen und einzelne Hauseingänge und*	☐ 1 ☐ 2	Ja Nein	

	Hofeinfahrten sind erlaubt			
UI4	Würden Sie Ihre Wohnung als feucht bezeichnen?	☐1 ☐2	Ja Nein	
UI5	Gab oder gibt es Schimmel- oder Stockflecken in Ihrer Wohnung (außer auf Nahrungsmitteln)?	☐1 ☐2	Ja Nein	
UI6	Benutzen Sie einen Gasherd zum Kochen?	☐1 ☐2	Ja Nein	

Abschluss des Interviews/ Interviewende

Sie haben jetzt alle Fragen des Interviews beantwortet. Vielen Dank für Ihre Mitarbeit.

AI	Uhrzeit Interview Ende		__|__| . |__|__| Std. Min.	

Kommentar von Probanden oder Interviewer/in zum Interview:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Appendix 5: Memory aid documents

Studieninformation

Studie zu Feinstaubquellen in Innenräumen und deren gesundheitlicher Wirkung

Liebe Probandin, lieber Proband,
wir möchten uns für Ihr Interesse und Ihre Bereitschaft, an diesem Forschungsprojekt mitzuwirken, ganz herzlich bedanken.
Im Folgenden werden wir Ihnen das Forschungsprojekt sowie die durchzuführenden Untersuchungen genauer beschreiben. Die Untersuchungen werden in Duisburg stattfinden.

1 Warum führen wir diese Studie durch?

Seit einigen Jahren gibt es intensive öffentliche Diskussionen um die gesundheitsschädigende Wirkung von Feinstaub in der Außenluft. In zahlreichen Studien konnte gezeigt werden, dass sowohl kurzfristige als auch langjährige Feinstaubbelastungen, wie sie z.B. durch erhöhtes Verkehrsaufkommen oder die Industrie entstehen, zu erheblichen Beeinträchtigungen der Gesundheit führen können. Negative Auswirkungen auf das Herz-Kreislauf-, Atemwegs- und das zentrale Nervensystem sowie erhöhte Sterberaten wurden beobachtet.
Obwohl in Innenräumen mitunter sehr hohe Feinstaubkonzentrationen vorkommen, wurden diese, bis auf das Zigarettenrauchen (z.B. Rauchverbot in Gaststätten, öffentlichen Gebäuden etc.) bislang kaum beachtet. Der Feinstaub in Innenräumen setzt sich zusammen aus Partikeln, die aus der Außenluft in den Innenraum gelangen,

und aus Partikeln, die bei verschiedenen Aktivitäten wie Kochen, Backen, Kerzen abbrennen oder durch Kaminfeuer erzeugt werden. Da wir uns die meiste Zeit des Tages und der Nacht in Räumen aufhalten, sind wir dieser Art von Feinstaub besonders lange und zeitweilig sehr intensiv ausgesetzt. Es ist daher immens wichtig, mögliche Auswirkungen auf die Gesundheit genau zu untersuchen.
Das Ziel dieses Forschungsvorhabens ist es zu untersuchen, inwieweit im Alltag regelmäßig vorkommende Innenraumbelastungen gesundheitliche Auswirkungen haben. In Abhängigkeit von bestimmten Aktivitäten im Innenraum wird untersucht, ob es zur Veränderung biologischer Faktoren wie z.B. Entzündungsmarkern im Blut oder der Lungenfunktion kommt. Wir können damit erkennen, welche Belastungen längerfristig gesehen gesundheitsschädigend sein könnten. Somit kann ein jeder durch Veränderung von Alltagsaktivitäten die Risiken für seine Gesundheit und die seiner Mitbewohner minimieren.

2 Von wem wird die Studie durchgeführt?

Die *Studie zu Feinstaubquellen in Innenräumen und deren gesundheitlicher Wirkung* wird vom Umweltbundesamt gefördert. Die durchführenden Institutionen sind:

Institut für Energie- und Umwelttechnik e.V. (IUTA)
Bliersheimer Str. 60
47229 Duisburg

IUF - Leibniz Institut für Umweltmedizinische Forschung
An der Heinrich Heine Universität gGmbH
Auf m Hennekamp 50
40225 Düsseldorf

Die Studienkoordination wird von Frau Dr. Vanessa Kinner (wissenschaftliche Mitarbeiterin des IUF) übernommen. In diesem Rahmen kümmert Sie sich um den reibungsloser Ablauf der Studie, die Betreuung der Probanden sowie die wissenschaftliche Auswertung der Daten.
Die Untersuchungen werden von speziell geschultem Personal durchgeführt und der gesamte Ablauf unterliegt der Aufsicht einer Studienärztin/eines Studienarztes.

3 Was wird genau untersucht?

Wie erfolgen die Untersuchungen zu den Innenraumbelastungen?
Bei den Innenraumbelastungen (Expositionen) werden unterschiedliche Szenarien aus dem Alltag nachgestellt wie u.a. Kochen oder Backen.
Die Expositionen finden in einem Prüfraum am Institut für Energie- und Umwelttechnik (IUTA) in Duisburg statt. Nach einer ca. 30-minütigen Akklimatisierungsphase (Gewöhnungsphase) mit Umgebungsluft werden Sie jeweils einer Feinstaubquelle für maximal vier Stunden im Prüfraum ausgesetzt. Während dieser Zeit können Sie sich frei im Prüfraum bewegen und z.B. Lesen, am Laptop arbeiten, Musik hören etc. Danach werden während weiterer 6 Stunden in einem Raum mit Umgebungsluft verschiedene kurz dauernde Gesundheitsuntersuchungen durchgeführt (genauer Zeitplan siehe Tabelle 1). Zwischen den einzelnen Untersuchungen können Sie sich wiederum frei im Raum bewegen und ihren persönlichen Beschäftigungen nachgehen. Nach insgesamt maximal 10 Stunden sind die Untersuchungen abgeschlossen. Am nächsten Morgen bitten wir Sie noch einmal für einen kurzen Zeitraum ins Studienzentrum zu kommen. Dann möchten wir gerne einige Messungen durchführen, bei denen wir mit einer leicht verzögerten Reaktion rechnen. Diese Untersuchung dauert ca. 2h.
Da verschiedene Feinstaubquellen getestet werden, wird dieser Messablauf zunächst 5 x durchgeführt. Im Detail heißt das, dass zunächst 4 Szenarien mit Feinstaubquellen aus dem Alltag nachgestellt werden und einmal eine Untersuchung mit gefilterter Luft stattfindet. Treten nach den Expositionen keine physiologischen Veränderungen auf, ist die Testserie hiermit beendet. Kommt es allerdings nach einer oder mehrerer Expositionen zu einer Veränderung bestimmter biologischer Marker, so werden in einem zweiten Untersuchungsabschnitt diese Expositionssituationen mit niedrigerer Dosierung wiederholt. Durch dieses zweistufige Design können die gesundheitlich wirkenden Quellen weiter untersucht und mögliche Schwellenwerte bestimmt werden.
Um die Rahmenbedingungen möglichst konstant zu halten, werden die Untersuchungen einmal pro Woche jeweils am gleichen Wochentag stattfinden. Es ist geplant, dass jeweils 3-4 Probanden gleichzeitig getestet werden.

Welche Gesundheitsuntersuchungen werden gemacht?

Fragebogenerhebung
Sie werden einmalig gebeten, einen Fragebogen zu Ihrem Alter, Geschlecht, Größe, Gewicht, Lebensstil, Erkrankungen, Medikation sowie beruflichen und privaten Belastungen gegenüber Feinstaub auszufüllen.

Zudem werden wir Sie bitten, jeweils direkt am Morgen vor den Expositionen einen Fragebogen zu Ihrem aktuellen Gesundheitszustand auszufüllen (siehe Tabelle 1). Im Falle z.B. akuter Infektionskrankheiten muss der Testtermin verschoben werden, da z.B. eine Erkältung sowohl die Reaktion auf die Feinstäube verändern kann, als auch die Messungen beeinträchtigt.

Gesundheitsuntersuchungen
Folgende Gesundheitsuntersuchungen werden zu unterschiedlichen Zeitpunkten durchgeführt (Zeitplan siehe Tabelle 1).
FeNO-Test
Bei dem FeNO-Test handelt es sich um einen Test, bei dem die Konzentration von Stickstoffmonoxid (NO) in der Ausatemluft gemessen wird. Die Konzentration von NO spiegelt das Entzündungsausmaß in den Atemwegen wieder. Die Messung ist schmerzfrei und schnell. Sie werden 10 Sekunden lang in ein kleines Röhrchen ausatmen. Das Testergebnis liegt in kürzester Zeit vor.
Lungenfunktionstest/Bronchospasmolysetest
Bei diesem Test atmen Sie über ein Mundstück in ein Messgerät (Spirometer). Dadurch können wir Ihre Lungenfunktion messen, das heißt, wie viel Luft Sie maximal ausatmen können und ob Ihre Atemwege verengt sind. Es handelt sich hierbei um einen einfachen und völlig schmerzfreien Test. Um festzustellen, ob eine rückgängig zu machende Einschränkung Ihrer Lungenfunktion vorliegt, wird der Test nochmals nach der Inhalation eines Mittels, das die Bronchien erweitert (Bronchodilatator), durchgeführt. Dieser Test ist sehr wichtig, um verschiedene Formen von Lungenfunktionseinschränkungen voneinander unterscheiden zu können. Die einmalige Gabe des Bronchodilatators hat bei Gesunden keine Nebenwirkungen und wird von oder in Gegenwart eines(r) Arztes/Ärztin durchgeführt.
Blutdruckmessung
Die Blutdruckmessung erfolgt indirekt über eine aufblasbare Manschette am Arm. Die Manschette wird stark aufgepumpt und während des Ablassens der Luft werden über die Strömungsgeräusche des Blutes die Blutdruckwerte bestimmt.
Messung der Gefäßsteifigkeit
Dabei handelt es sich um eine Untersuchung, mit der wir die kardio-vaskulären Funktionen, wie z.B. die arterielle Steifigkeit und den zentralen Blutdruck, beurteilen können. Die Messung erfolgt in sitzender Position durch das Auflegen eines Druckmessers am Handgelenk und ist für Sie komplett schmerzfrei.
Messung der Funktion der Blutgefäße
Diese Untersuchung gibt darüber Auskunft, wie gut und schnell sich die Arterien weiten und zusammenziehen können. Diese sogenannte endotheliale Funktion lässt Rückschlüsse auf mögliche Einschränkungen Ihres Gefäßsystems zu. Bei der Untersuchung werden in sitzender Position 2 Kappen auf die Zeigefinger gesetzt sowie an einem Arm eine Blutdruckmanschette für 5 Minuten aufgepumpt. Nach Lösen der Blutdruckmanschette kommt es zu einer Weitstellung der Gefäße, die an der Fingerkuppe gemessen wird. Die Dauer der Untersuchung beträgt ca. 10 Minuten und kann zu einem unangenehmen Gefühl auf der Seite der Blutdruckmanschette führen. Es ist wichtig, dass Sie während der Untersuchung nicht aufstehen und sich nicht stark bewegen.
Neurokognition (PEG-Board Test)
Der PEG-Board Test ist ein Testverfahren zur Messung der Konzentrationsfähigkeit, der Geschicklichkeit und der Hand- und Fingerfunktionen.

Laboruntersuchungen
Für die Laboruntersuchungen werden folgende Untersuchungen durchgeführt:
Nasallavage
Bei der Nasallavage (Nasenspülung) wird Ihnen eine isotonische Kochsalzlösung in den Nasenraum eingeträufelt und anschließend in einem Röhrchen durch Neigung des Kopfes nach vorne wieder aufgefangen. Wir können daraufhin bestimmen, ob und in welcher Konzentration sich Entzündungszellen und andere Entzündungsmarker in der ausgeschwemmten Flüssigkeit befinden.
Blutabnahme
Die Blutabnahme erfolgt zur Bestimmung von Entzündungsmarkern, Markern der Blutgerinnung, des oxidativen Stresses und der Funktion der inneren Blutgefäßwand. Diese lassen Rückschlüsse auf die Wirkmechanismen der Feinstäube zu. Wir behalten uns vor, Blutproben einzufrieren, um später bei Bedarf weitere Untersuchungen machen zu können. Es werden zusätzliche Blutproben für das Umweltbundesamt abgenommen, welche aber ausschließlich in anonymisierter Form weitergegeben werden. Ein Rückschluss auf Ihre Person ist nicht möglich. Die Blutabnahme wird von oder in Gegenwart eines(r) Arztes/ Ärztin durchgeführt.

Urin (Rückstellproben)
Wir bitten Sie, uns Urinproben zur Verfügung zu stellen. Diese werden wir aufheben um gegebenenfalls später noch weitere Untersuchungen von Entzündungswerten durchführen zu können.

Auf der nächsten Seite sehen Sie eine Tabelle, die Ihnen den Überblick über die unterschiedlichen Untersuchungen und die jeweiligen Zeitpunkte der Untersuchung gibt. Zwischen den Untersuchungen haben Sie Zeit zum Lesen, Arbeiten am Laptop, Musik hören, etc.

Tabelle 1: Zeitplan der Fragebogenerhebung, Gesundheits- und Laboruntersuchungen

	vor Exposition	während Exposition	nach Exposition	+2h	+4h	+24h
Allgemeiner Fragebogen	x					
Fragebogen zu akuten gesundheitlichen Störungen	x					
Fragebogen zu akuter Befindlichkeit	x	x	x	x	x	x
FeNO-Test	x			x		x
Nasallavage	x			x		
Lungenfunktionstest	x				x	x
Gefäßsteifigkeit	x		x	x	x	x
Blutabnahme	x			x		x
Blutdruck	x	x	x	x	x	x
Neurokognition	x				x	x

4 Können irgendwelche gesundheitlichen Risiken durch die Feinstaubexpositionen oder die Untersuchungen auftreten?

Durch die geplante kontrollierte Expositionsstudie treten **keine gesundheitlichen Risiken** für Sie auf. Es geht vielmehr darum, erste Reaktionen des Körpers wie z.B. eine Veränderung von bestimmten Entzündungsmarkern im Blut bzw. bei der Nasallavage festzustellen, die durch den Feinstaub möglicherweise verursacht wurden. Kommt es tatsächlich zu einer Veränderung dieser Marker, so lassen diese ersten Reaktionen weiterführend darauf schließen, ob bei längerer und höherer Exposition gesundheitliche Einschränkungen zu erwarten wären. Die Expositionen werden so gewählt, wie sie in Höhe und Dauer auch im Alltag im privaten Bereich regelmäßig vorkommen können. Während der Expositionen werden Sie in dem Prüfraum visuell überwacht, so dass bei ersten Anzeichen von Unwohlsein der Versuch abgebrochen werden kann.
Auch die Gesundheits- und Laboruntersuchungen sind, wie unter Punkt 3B beschrieben, für Sie weitgehend komplikations- und risikofrei. Durch das Inhalieren der Kochsalzlösung bei der ***Nasallavage*** (Nasenspülung) kann es zu einem kurzfristigen unangenehmen Gefühl in der Nase und den Nasennebenhöhlen kommen. Die ***Lungenfunktionstests*** umfassen das Ausatmen in einen Apparat, was anstrengend sein kann, aber nicht unangenehm. Durch das tiefe Ein- und Ausatmen kann es in seltenen Fällen zu einem leichten und vorübergehenden Schwindelgefühl kommen. Bei der *Blutentnahme* bestehen nur die Risiken, die mit einer normalen Blutentnahme verbunden sind. Die Entnahme einer Blutprobe ist in der Regel nur mit einem sehr geringen Risiko verbunden. An der Einstichstelle kann es zu leichten Schmerzen kommen oder es kann ein Bluterguss (blauer Fleck) entstehen, der evtl. einige Tage sichtbar ist. In äußerst seltenen Fällen kann auch die Bildung eines Blutgerinnsels (Thrombose), eine örtlich begrenzte Entzündung oder eine Infektion an der Einstichstelle auftreten oder es kann zu dauerhaften Schädigungen von Blutgefäßen oder Nerven kommen.

5 Wer kann teilnehmen?

In Frage kommen generell alle gesunden Personen, Männer und Frauen im Alter zwischen 18 und 75 Jahren. Die Studienteilnehmer sollen keine chronischen Erkrankungen, keine Dauermedikation und keine beruflich bedingte hohe Partikelbelastung haben.

6 Die Teilnahme ist freiwillig

Die Teilnahme ist freiwillig und Sie haben zu jedem Zeitpunkt der Studie die Möglichkeit, Ihr Einverständnis ohne Angabe von Gründen zurückzuziehen. Für die Teilnahme an dieser Studie erhalten Sie eine Aufwandsentschädigung von 50,00 € pro Expositionstag. Wenn Sie sich dafür entschließen, an der Studie teilzunehmen, bitten wir Sie, die beigefügte Einverständniserklärung zu unterschreiben.

Wenn Sie noch Fragen haben, können Sie sich gerne an folgende Ansprechpartnerin wenden.

Ansprechpartnerin für Ihre Fragen:

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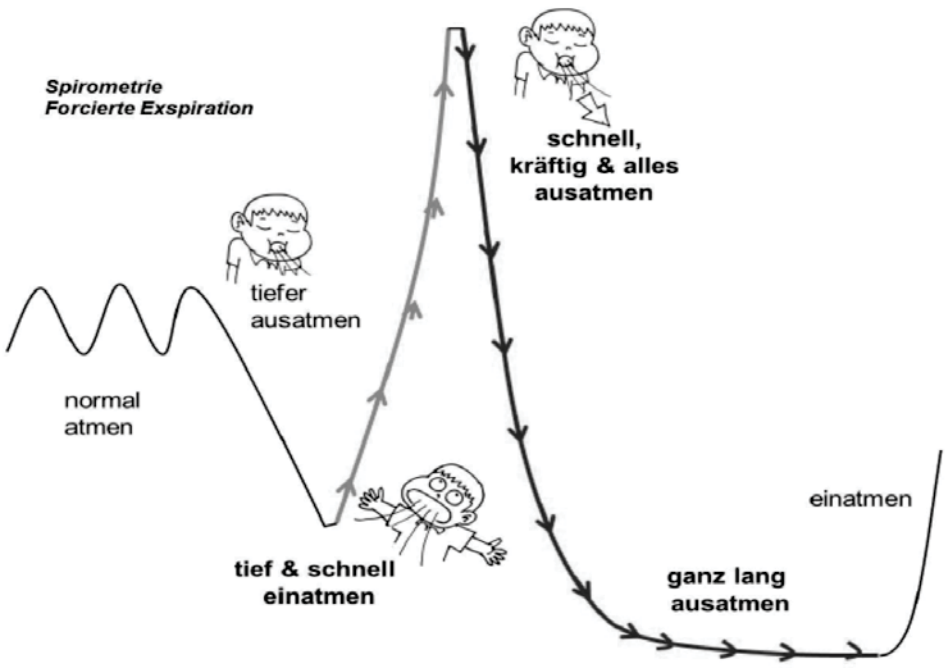
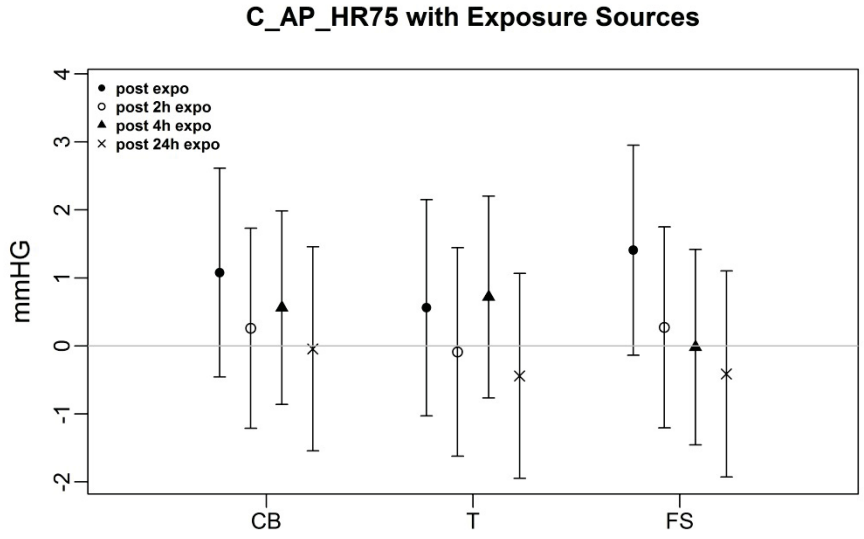
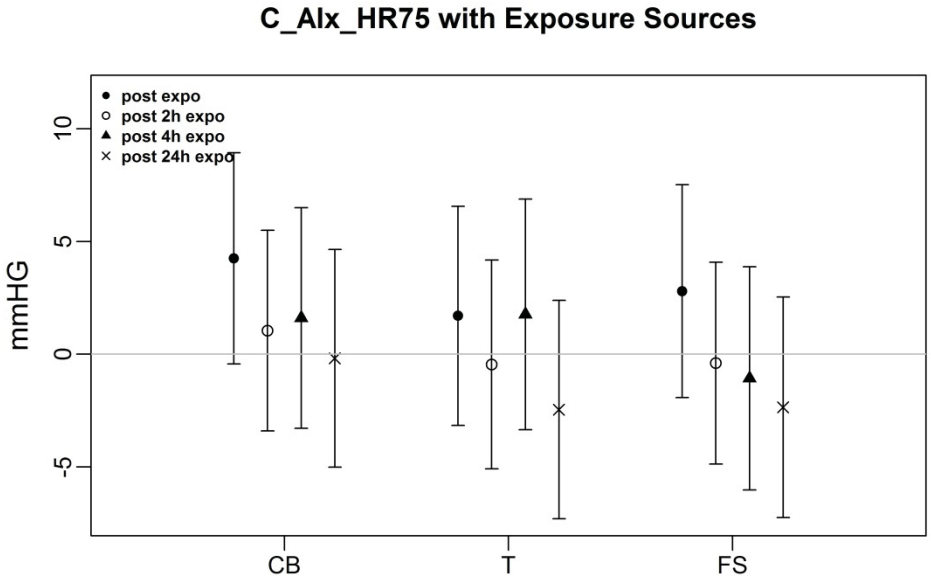
KLEINE GEDÄCHTNISSTÜTZE - ABLAUF DER LUNGENFUNKTIONSMESSUNG



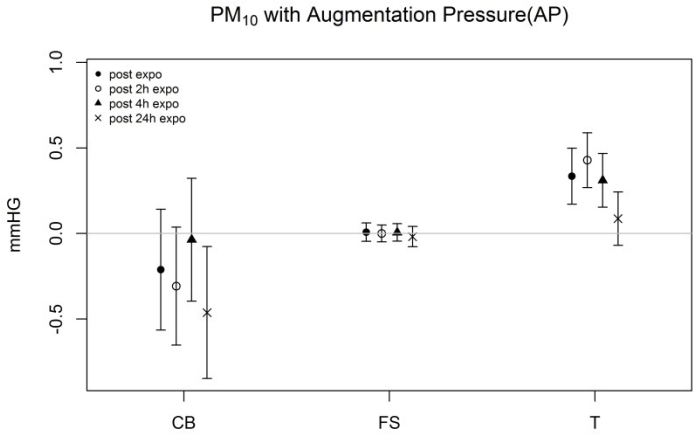
Appendix 6: Model I – Figure (Crude – Model)



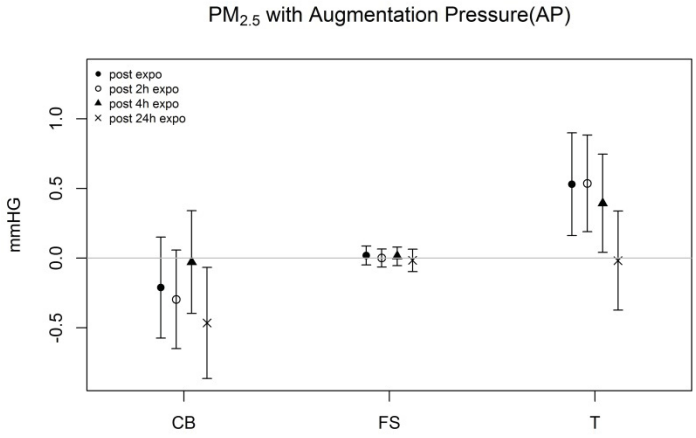


Appendix 6 – Figure 1: Mean effect estimates and 95% Confidence Intervals (CI) for changes in augmentation pressure (AP) and augmentation index (AIx) depending on exposure scenario (candle burning CB, toasting bread T and frying sausages FS) in the crude model

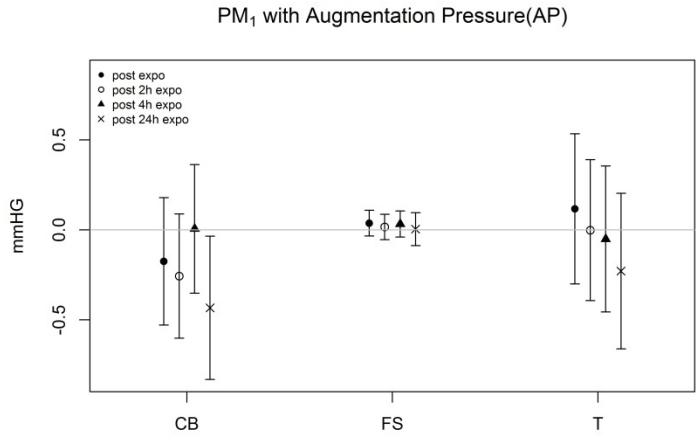
Appendix 7: Model II – Metric Figures for Augmentation Pressure(AP)



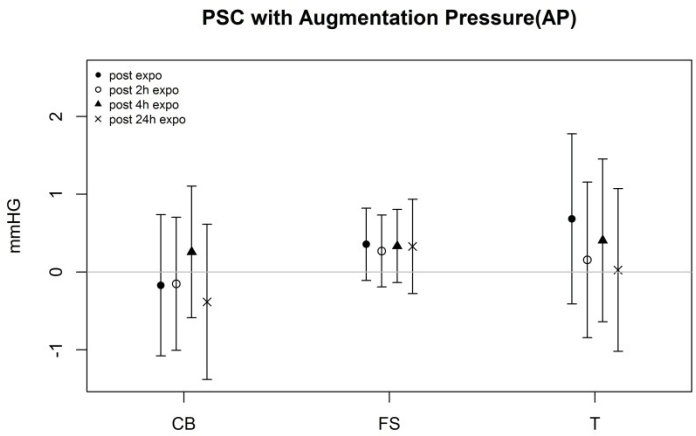
Appendix 7 - Figure 1**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP associated with an increase of 10 µg/m^3 PM_{10} during candle burning (CB), frying sausages (FS) and toasting bread (T) in the fully adjusted model**



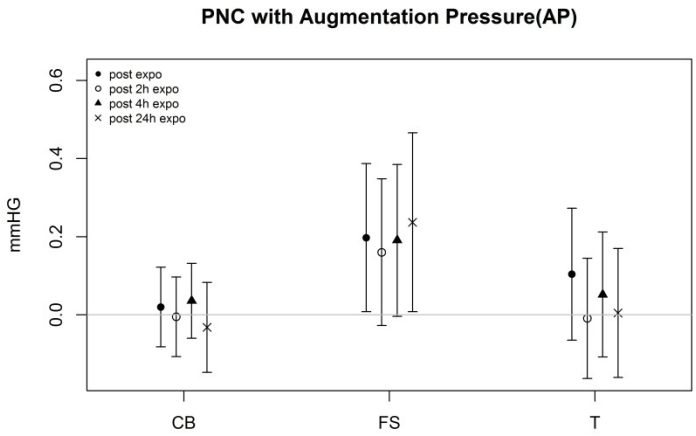
Appendix 7 - Figure 2**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP associated with an increase of 10 µg/m^3 $PM_{2.5}$ during candle burning (CB), frying sausages (FS) and toasting bread (T) in the fully adjusted model**



Appendix 5 - Figure 3**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP associated with an increase of 10 µg/m^3 PM_{10} during candle burning (CB), frying sausages (FS) and toasting bread (T) in the fully adjusted model**

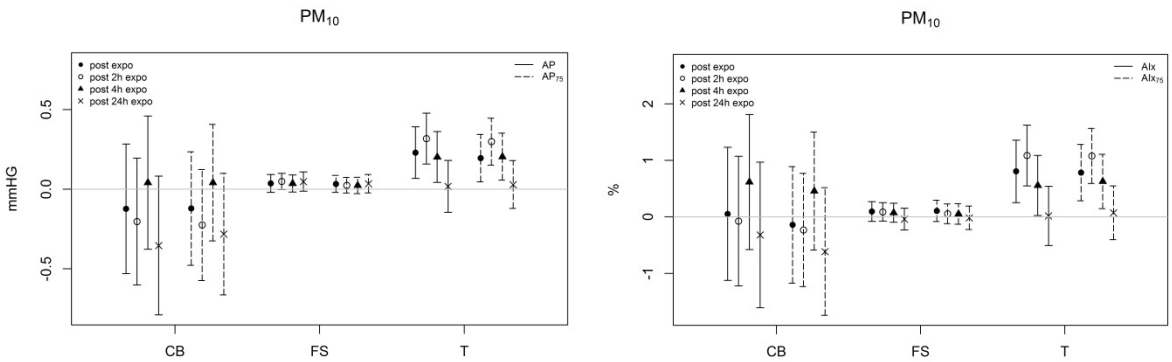


Appendix 7 - Figure 4**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP associated with an increase of 1,000 µm^2/cm^3 PSC during candle burning (CB), frying sausages (FS) and toasting bread (T) in the fully adjusted model**

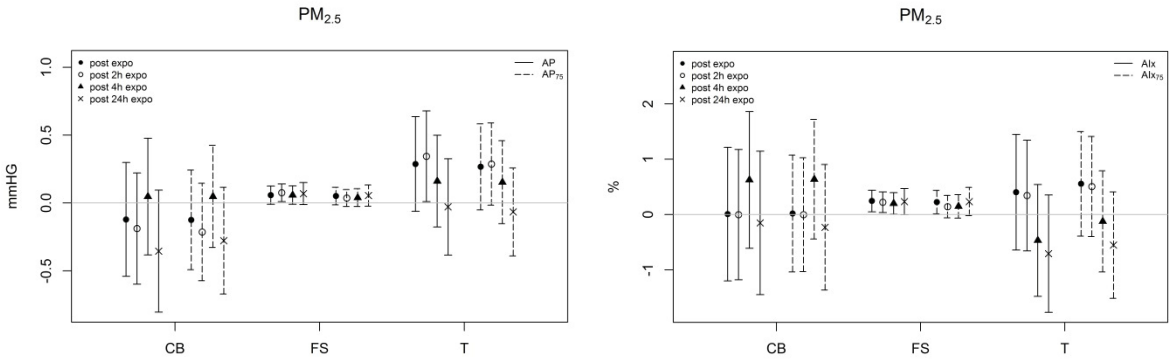


Appendix 7 - Figure 5**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP associated with an increase of 50,000 particles/cm^3 PNC during candle burning (CB), frying sausages (FS) and toasting bread (T) in the fully adjusted model**

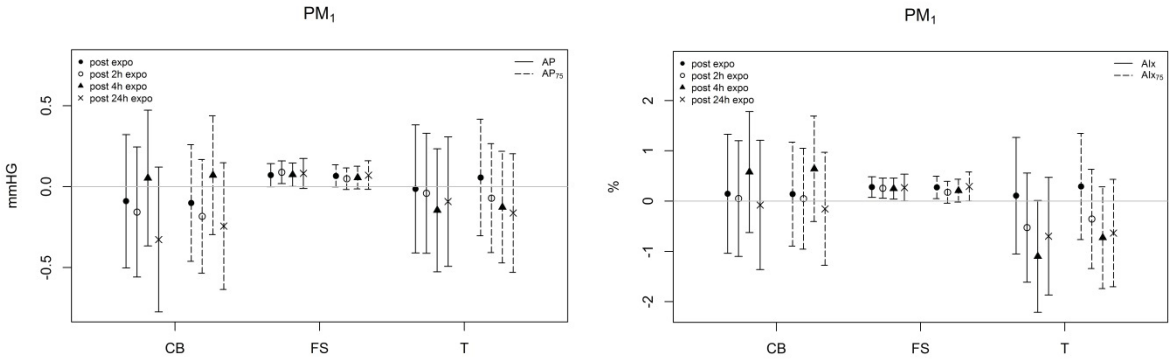
Appendix 8: Model II – Metric Figures (Crude – Model)



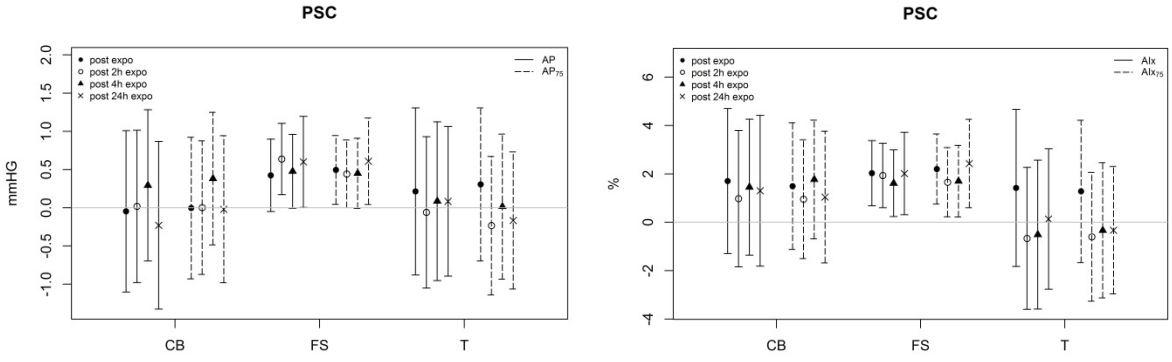
Appendix 8 - Figure 1**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP and AIx associated with an increase of 10 µg/m^3 PM_{10} during candle burning (CB), frying sausages (FS) and toasting bread (T) in the crude model**



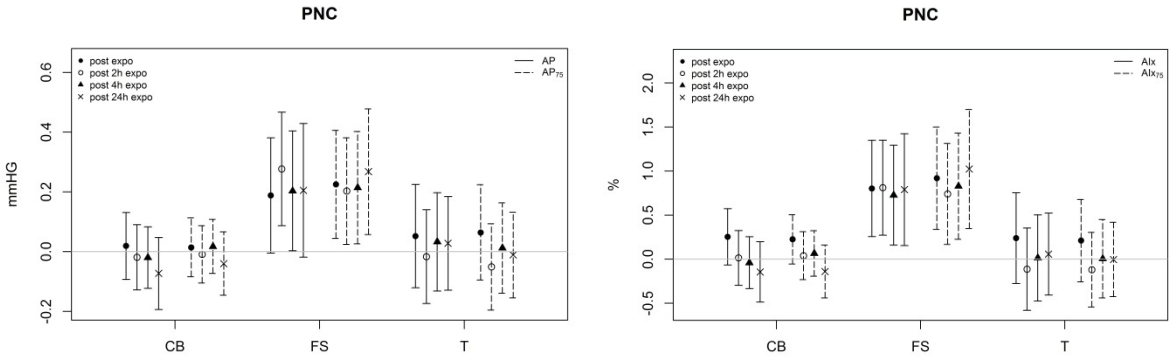
Appendix 8 – Figure 2**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP and AIx associated with an increase of 10 µg/m^3 $PM_{2.5}$ during candle burning (CB), frying sausages (FS) and toasting bread (T) in the crude model**



Appendix 8 – Figure 3**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP and AIx associated with an increase of 10 µg/m^3 PM_1 during candle burning (CB), frying sausages (FS) and toasting bread (T) in the crude model**

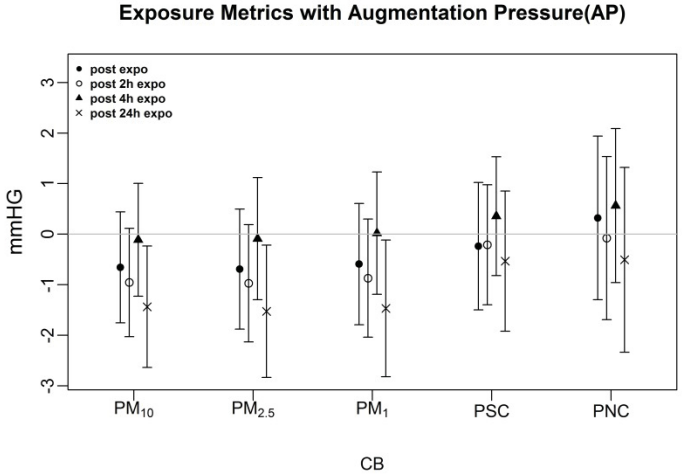


Appendix 8 - Figure 4**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP and AIx associated with an increase of 1,000 µm2/cm^3 PSC during candle burning (CB), frying sausages (FS) and toasting bread (T) in the crude model**

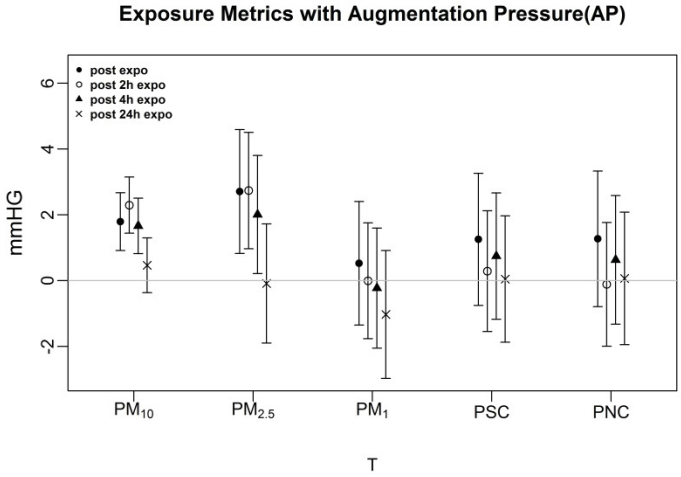


Appendix 8 – Figure 5**: Mean effect estimates and 95% confidence intervals (CI) for changes in AP and AIx associated with an increase of 50,000 particles/cm^3 PNC during candle burning (CB), frying sausages (FS) and toasting bread (T) in the crude model**

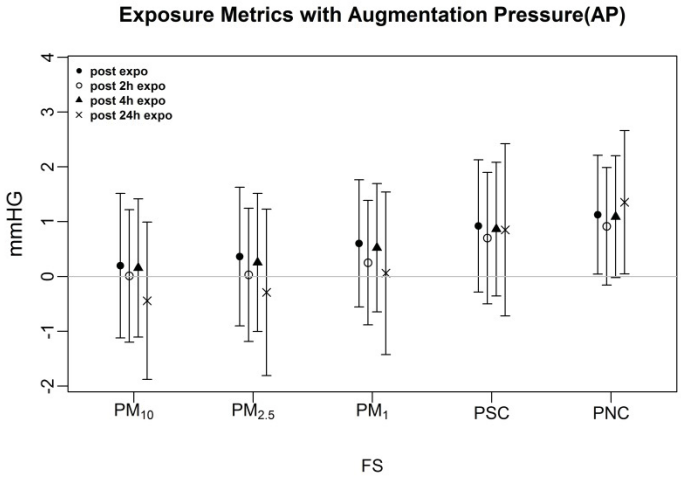
Appendix 9: Model II – IQR Figures for Augmentation Pressure (AP)



Appendix 9 - Figure 1: **Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AP during candle burning (CB) in the fully adjusted model**

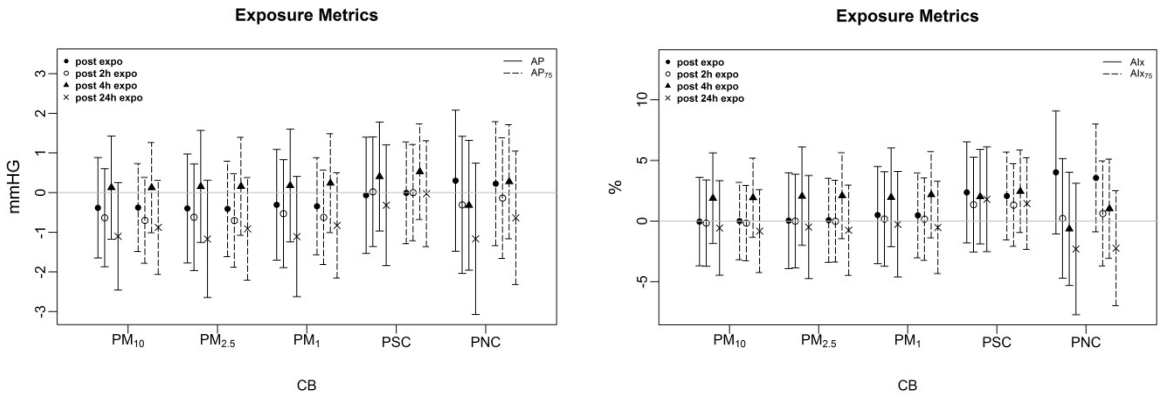


Appendix 9 - Figure 2: **Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AP during toasting bread (T) in the fully adjusted model**

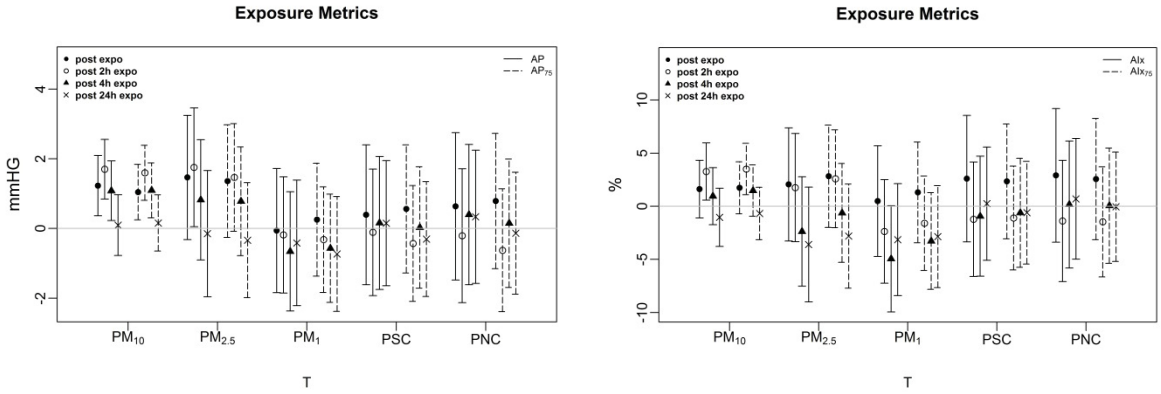


Appendix 9 - Figure 3**: Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AP during frying sausages (FS) in the fully adjusted model**

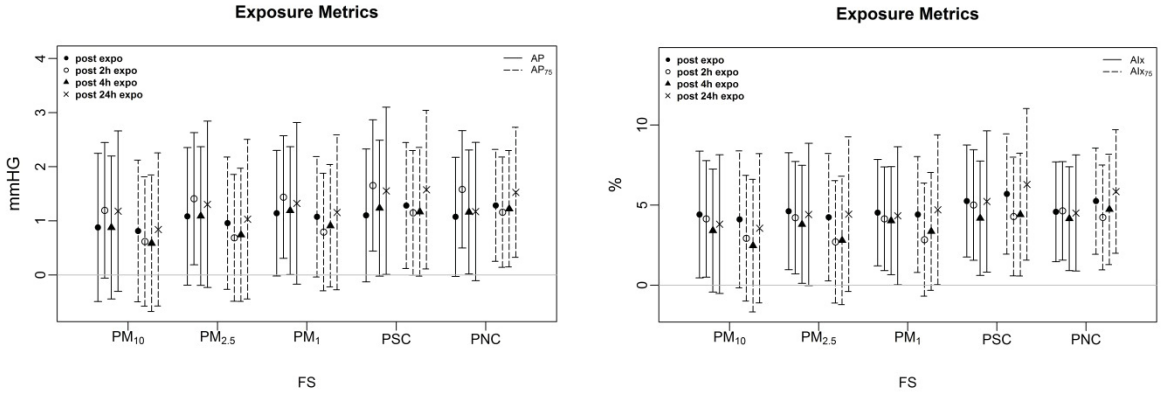
Appendix 10: Model II – IQR Figures (Crude – Model)



Appendix 10 - Figure 1**: Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AP and AIx during candle burning (CB) in the crude model**



Appendix10 – Figure 2**: Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AP and AIx during toasting bread (T) in the fully adjusted model**



Appendix 10 – Figure 3**: Mean effect estimates and 95% confidence intervals (CI) for changes per IQR (53.5 PM_{10}, 51.0 $PM_{2.5}$, 45.0 PM_1, 610,334.5 PNC and 1,835.4 PSC) in AP during frying sausages (FS) in the crude model**

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I would also like to thank all members of the work group for environmental epidemiology led by Prof. Hoffmann at the Institute of Occupational and Social Medicine – University Düsseldorf. The team of experts provided me an opportunity to join their team, encouraged me to achieve my goal and gave me access to their research facilities. Without their precious support it would not be possible to conduct this research.

Finally, I must express my very profound gratitude to my parents and to my sisters and brother for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.