Cardiovascular and pulmonary health effects of air pollution

Long-term effects in Sweden and effects of wood smoke

Leo Stockfelt

Department of Occupational and Environmental Medicine
Institute of Medicine,
Sahlgrenska Academy at the University of Gothenburg,

UNIVERSITY OF GOTHENBURG
Gothenburg 2016

Cover illustration: Wood smoke from a Swedish summer house. Photo credits: Anita Ahlerup.

Cardiovascular and pulmonary health effects of air pollution
© Leo Stockfelt 2016
Leo.stockfelt@amm.gu.se

ISBN 978-91-628-9726-0 (printed)
ISBN 978-91-628-9797-7 (e-pub)

Printed in Gothenburg, Sweden 2016
Printed by Ineko AB

Prevention is better than cure

Cardiovascular and pulmonary health effects of air pollution

Long-term effects in Sweden and effects of wood smoke

Leo Stockfelt

Department of Occupational and Environmental Medicine, Institute of Medicine, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

ABSTRACT

Exposure to air pollution is associated with increased morbidity and mortality in cardiovascular and pulmonary diseases. Main suggested mechanisms are airway and systemic inflammation, affecting hemostasis in the short term and atherosclerosis in the long term. Few studies have investigated the effects over decades, or which time-windows of exposure are the most relevant. In Sweden and many other countries wood burning is one of the largest sources of air pollution. The main aims of this thesis are to increase the knowledge of the mechanisms through which wood smoke causes respiratory and cardiovascular diseases, and the effects of long-term exposure to air pollution in a Swedish cohort.

In an experimental chamber study in healthy adults, short-term exposure to two types of wood smoke was associated with symptoms and biomarkers of airway effects, but not with biomarkers of systemic inflammation or coagulation. This indicated that relatively low doses of wood smoke induce effects on airway epithelial permeability and possibly airway inflammation. In a long-term cohort study of residential exposure to nitric oxides (NO_x) in Gothenburg, we observed a time trend of decreasing exposure. Back extrapolation of exposure was fairly correct for 5-7 years but not for longer time spans, showing that historical dispersion models and residential history are important for accurate long-term exposure estimations. Total non-accidental mortality was associated with residential NO_x exposure. The effect estimates were similar for NO_x exposure the last year, the mean NO_x exposure the last 5 years, and the mean NO_x exposure since enrolment. The effect estimates for cause-specific cardiovascular mortality were similar to those for total mortality. The effect was near linear with no evidence of any threshold, and only marginally affected by confounders and effect modifiers.

Keywords: Air pollution, wood smoke, human exposure studies, dispersion modelling, cohort studies, cardiovascular disease

ISBN: 978-91-628-9726-0 (printed) / 978-91-628-9797-7 (e-pub)

SAMMANFATTNING PÅ SVENSKA

När man utsätts för luftföroreningar ökar risken att insjukna och dö i hjärtkärl- och lungsjukdomar. Sannolikt beror detta framförallt på att små partiklar som stannar kvar i lungorna orsakar luftvägsinflammation som i sin tur leder till systemisk inflammation. Detta kan på kort sikt öka risken för att drabbas av blodproppar, och på lång sikt påskynda utvecklingen av åderförkalkning. Vedeldning är en av de största källorna till luftföroreningar i Sverige och världen, men det är osäkert om vedrök är mer eller mindre farligt än andra luftföroreningar. I denna avhandling har jag studerat effekter av kort tids exponering för vedrök på biologiska markörer hos friska försökspersoner, och effekterna av lång tids exponering för luftföroreningar på sjuklighet och död i en grupp äldre män i Göteborg.

När friska frivilliga försökspersoner i tre timmar exponerades för vedrök i en experimentell kammare fann vi effekter på biomarkörer som tydde på lättare inflammation och barriärskada i luftvägar. Vi fann dock inga tecken på systemisk inflammation eller ökad risk för blodproppar.

Halterna av luftföroreningar (kväveoxider) i Göteborg har under de senaste 35 åren nästan halverats. Att extrapolera exponering längre än 5-7 år gav felaktiga värden, vilket visar hur viktigt det är att kontinuerligt följa studiedeltagarna och ha historiska exponeringsmodelleringar för att kunna göra en tillförlitlig exponeringsbedömning. Högre luftföroreningsnivå vid bostaden var kopplat till ökad risk att dö i förtid. Riskökningen var lika tydligt kopplad till sista årets exponering som till längre perioders exponering. Sambandet mellan luftföroreningar och dödlighet förändrades i liten grad av att vi tog hänsyn till rökning, socioekonomi eller andra riskfaktorer. Vi fann inga tecken på att det finns en säker nivå, utan allt tyder på att minskade luftföroreningshalter kan minska risken för förtida död även när nivåerna redan är låga.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Stockfelt L, Sallsten G, Olin A-C, Almerud P, Samuelsson L, Johannesson S, Molnár P, Strandberg B, Almstrand A-C, Bergemalm-Rynell K, Barregard L. Effects on airways of short-term exposure to two kinds of wood smoke in a chamber study of healthy humans. Inhalation Toxicology 2012; 24, 47-59.

II. Stockfelt L, Sallsten G, Almerud P, Basu S, Barregard L. Short-term chamber exposure to low doses of two kinds of wood smoke does not induce systemic inflammation, coagulation or oxidative stress in healthy humans. Inhalation Toxicology 2013; 25, 417-425.

III. Molnár P, Stockfelt L, Barregard L, Sallsten G. Residential NO_x exposure in a 35-year cohort study. Changes of exposure, and comparison with back extrapolation for historical exposure assessment. Atmospheric Environment 2015; 115, 62-69.

IV. Stockfelt L, Andersson EM, Molnár P, Rosengren A, Wilhelmsen L, Sallsten G, Barregard L. Long term effects of residential NO_x exposure on total and cause-specific mortality and incidence of myocardial infarction in a Swedish cohort. Environmental Research 2015; 142, 197-206.

CONTENT

SELECTED ABBREVIATIONS

ACS	American Cancer Society
ANS	Autonomic Nervous System
ATS	American Thoracic Society
BMI	Body Mass Index
BP, SBP, DBP	Blood Pressure, Systolic & Diastolic Blood Pressure
CC16	Club Cell (formerly Clara cell) secretory protein 16
CRP	C-Reactive Protein
EBC	Exhaled Breath Condensate
FVII	Coagulation Factor VII
FVIII	Coagulation Factor VIIIc
FENO	Fraction of Exhaled Nitric Oxide
IARC	International Agency for Research on Cancer
IHD	Ischemic Heart Disease
IL	Interleukin
MI	Myocardial Infarction
NO_x	Nitric Oxides. Mainly NO_2 and NO.
PAH	Polycyclic Aromatic Hydrocarbons
PM	Particulate Matter, often of a defined size category.
SAA	Serum Amyloid A
sICAM-1	Soluble Intercellular Adhesion Molecule-1
sVCAM-1	Soluble Vascular Cell Adhesion Molecule-1
TEOM	Tapered Element Oscillating Microbalance
VOC	Volatile Organic Compounds
WBC	White Blood Cell
WHO	World Health Organization
vWf	Von Willebrand Factor

1 INTRODUCTION

1.1 Air pollution and health

Air pollution is a major risk factor for mortality and morbidity, estimated to cause around 5.5 million premature deaths annually in the world (1). The estimated numbers of annual excess deaths are around half a million in Europe (2, 3) and several thousand in Sweden (4), making air pollution the single largest environmental health risk.

1.1.1 The history of air pollution research

Making and controlling fire from biomass burning was a crucial step in the development of mankind. Where there's fire, however, there's smoke. Bad air quality has been considered a cause for ill health at least since antiquity, and is mentioned in for example the Hippocratic Corpus and early roman medical advice (5). The growth of coal burning and eventually industrialization led to very high levels of air pollution in urban areas. During the 20th century a number of air pollution episodes occurred such as the Meuse valley fog in 1930 (6, 7), the Donora smog in 1948 (8, 9) and the London smog episodes of 1948 and 1952 (10-12). The London smog of 1952, initially estimated to have caused about 3000-4000 and later as many as 12000 excess deaths (13), is often considered the event that led to the birth of modern air pollution research.

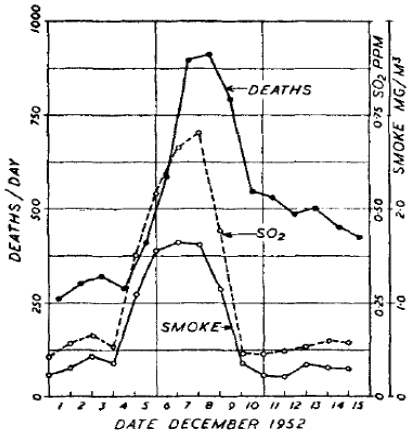

Figure 1. Air pollution and mortality during the London smog 1952. Reproduced with permission, from Wilkins 1954 (12).

Figure 2. Air pollution in London in the 1950s.

1.1.2 Air pollution epidemiology

The short-term effects of high levels of air pollution on mortality, mainly in cardiovascular and respiratory diseases, have been verified by consistent findings in hundreds of time series studies (14-16).

Long-term exposure to air pollution has also been shown to be harmful. In the 1990s two landmark cohort studies in the US, the (fig 3) (17) and the American Cancer Society (ACS) studies (18), showed an association between fine particulate matter (PM) air pollution and total and cardiopulmonary mortality. Similar results were found in European cohort studies (19-24) as well as in reanalyses (25) and extended analyses (26-31) of the Six Cities and ACS cohorts. A recent European multicenter study found a relatively strong effect of exposure to fine particles on all-cause mortality but no significant effect on cardiovascular mortality (32, 33).

In addition to mortality, exposure to air pollution has also been associated with incidence of cardiovascular and respiratory diseases, as well as with lung cancer, diabetes, cognitive impairment, low birth weight and preterm birth (34).

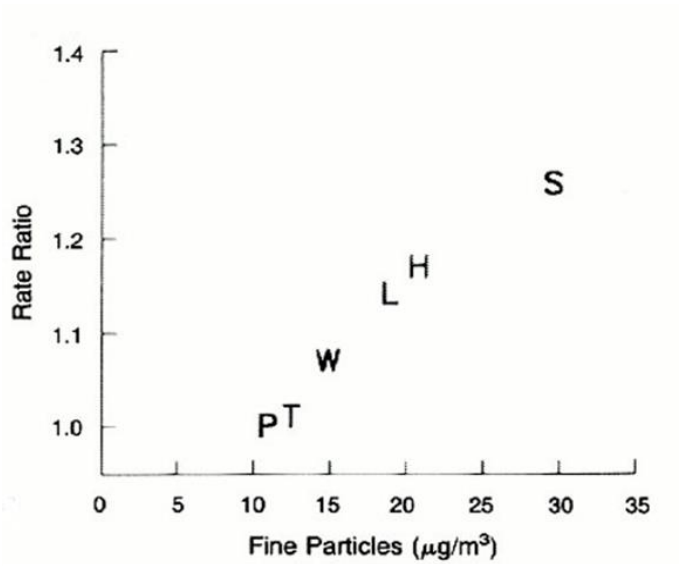

Figure 3. Estimated adjusted mortality-rate ratios for and pollution levels in six US cities, each letter denoting one city. Reproduced with permission, from Dockery 1993 (17). Copyright Massachusetts Medical Society.

Effect estimates are generally larger in long-term studies conducted over years than in short-term studies of days-weeks (15). For comparison, for each

increment of 10 µg/m^3 of $PM_{2.5}$ a recent review of time series studies found an increase of 1.04% in all-cause mortality (14), while a recent review of long-term effects reported a pooled effect of 6.2% (35).

The associations found between air pollution and health effects have usually been near linear, and observed also at low exposure levels with no evidence of a lower threshold where exposure is "safe". Health impact assessments have thus usually assumed a linear effect and compared the actual exposure levels with theoretical "lowest possible exposure" scenarios, as if there were no anthropogenic emissions. Commonly the 6% increase in all-cause mortality per 10 µg/m^3 of annual average $PM_{2.5}$ from the extended analysis of the ACS study (27) have been used. The recent WHO HRAPIE project (36) recommended using the results of Hoek et al 2013 for health risk assessments (35), but the difference is slight (6% in the ACS study vs 6.2% in Hoek et al, per 10 µg/m^3 of $PM_{2.5}$). Short-term associations with mortality are assumed to be included in the long-term effects when the excess mortality is estimated.

Using these effect estimates the relative risk increases due to air pollution exposure are for each individual slight at most common exposure levels, compared with traditional risk factors such as smoking. However, the impact on the population is large since only some are active smokers while the entire population is exposed to air pollution constantly. In addition, people's ability to protect themselves from harmful exposures should be considered. Smoking is voluntary – breathing is not.

1.1.3 The present situation

Air quality has improved in Sweden and much of the Western world in the last few decades, but globally the development has not been as positive. The

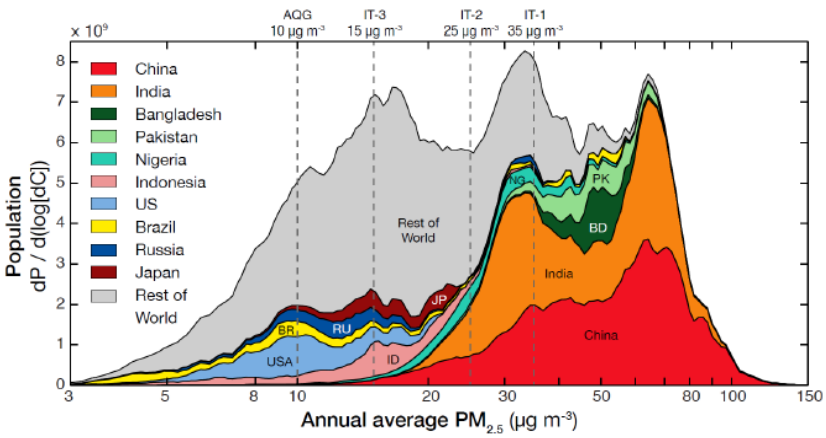

Figure 4. Global and regional distributions of population as a function of annual (2013) average ambient $PM_{2.5}$ concentration for the world's 10 most populous countries. Dashed vertical lines indicate WHO Interim Targets (IT) and the WHO Air Quality Guideline (AQG) of 10 µg/m^3 of $PM_{2.5}$. Reproduced with permission, from Brauer et al 2015 (37).

majority of highly exposed people now live in low- or middle-incomes countries such as China, India and Southeast Asia (Fig 4) (37). Annual average exposure levels in the developing world are often many times higher than air quality guidelines, both for rural people using solid fuels for heating and cooking and for residents of polluted megacities.

Short-term exposures can be extremely high. At the time I am writing this paragraph (December 1st 2015) the $PM_{2.5}$ levels in Beijing are an astounding 524 $\mu g/m^3$ (Fig 5) (38), and a large part of Southeast Asia has been covered in smoke from Indonesian forest fires for several months. In the Western world as well there are still alarms of high air pollution levels, such as the Paris smog episodes of 2015. In this context it is also worth noting that even the lower levels in the developed world are not safe; recent studies such as the ESCAPE study reporting effects on mortality also at very low exposure levels (mean annual $PM_{2.5}$<15$\mu g/m^3$) (32).

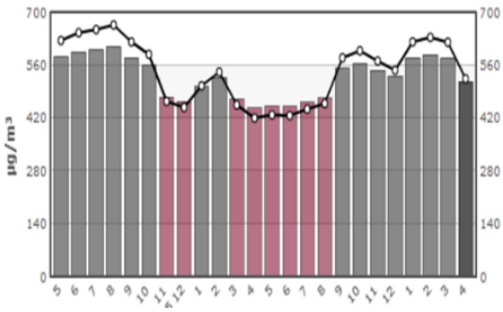

Figure 5. Levels of $PM_{2.5}$ and Air Quality Index (AQI) at the US Embassy in Beijing December 1, 2015 (38). The bars represent the AQI and the connected white rings $PM_{2.5}$ concentrations. From the US Department of State Air Quality Monitoring Program.

1.2 The properties of air pollution

Air pollution is a complex heterogeneous mixture of solid and liquid particles and gases, several of which may have negative health effect individually or synergistically. Negative health effects have been most consistently associated with particles, but also with gaseous pollutants including nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and ozone (O_3). Different air pollutants are often highly correlated, making it difficult to discern in epidemiological studies which pollutant (or combination of pollutants) that are causally associated with the negative health effects.

Air pollution comes from many emission sources, both anthropogenic and natural, such as combustion and road wear from road traffic, industrial processes, volcanoes, windblown sand, salt and soil, pollen and combustion of biomass. Air pollutants can be classed as primary – directly emitted from a process, or secondary – formed by chemical reactions between the primary pollutants in the air.

1.2.1 Particulate matter air pollution and deposition

Particles in the air are generally measured in mass (µg/m^3) and/or number (/m^3) and divided into size fractions depending on aerodynamic diameter. Particles with an aerodynamic diameter of <10 µm are called *thoracic particles* (PM_{10}) and particles <2.5 µm *fine particles* ($PM_{2.5}$). Particles between 10 and 2.5 µm are often called *coarse particles*, and particles <0.1 µm *ultrafine particles* (UFP, or $PM_{0.1}$) (15). The larger particles naturally account for a greater part of the weight measurements and the smaller particles for a greater part of the particle numbers. In the urban environment coarse particles generally comes from wear of roads and tyres while a higher fraction of fine and ultrafine particles come from combustion (traffic, heating and cooking or industry and power generation). Particle sizes and emission sources are described in figure 6, from (39).

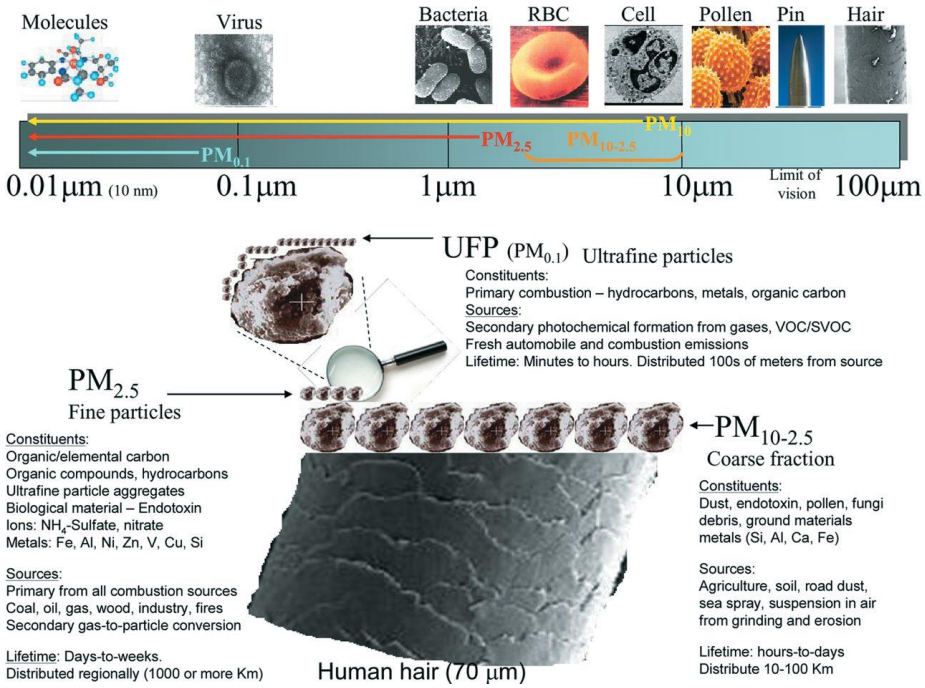

Figure 6. Size, sources and composition of PM air pollution. Reproduced with permission, from Brook 2008 (39).

The health effects of particles depend on to what extent and where they deposit in the respiratory system. In general, particles above 4 µm and below 0.01 µm deposit primarily in the upper respiratory tract, while particles 0.01-0.2 µm and particles 1-4 µm can penetrate deeper into the lungs and deposit

in the bronchial and alveolar regions. For particles 0.2-0.7 µm the fraction that is deposited is low, below 20% (Fig 7) (40). The upper airways are better able to eliminate foreign particles through mucociliary clearance.

The size of the particles also affects how they can disperse in the atmosphere. Coarse and ultrafine particles remain within a smaller geographical area since smallest particles rapidly merge and combine into larger particles in the atmosphere, and the largest particles (PM_{10}) are removed by sedimentation. Particles of around 2.5 µm, however, can remain suspended in the air for a long time and be transported by winds for hundreds of kilometers. In Gothenburg and Sweden most $PM_{2.5}$ is long-distance transported (4).

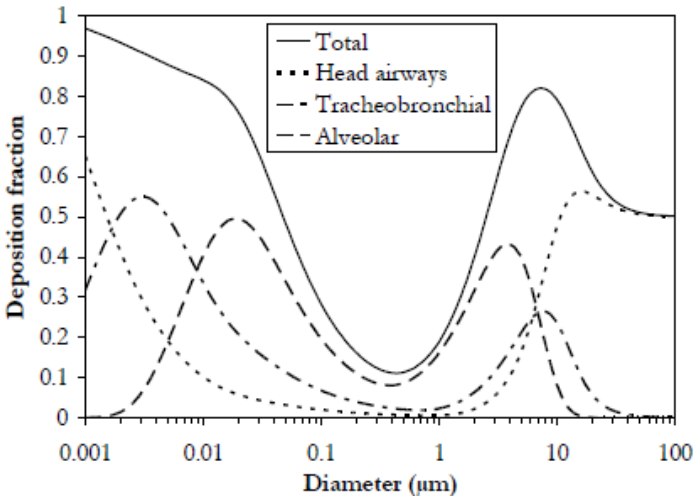

Figure 7. Total and regional respiratory tract deposition of unit density spheres for a sitting male adult according to the ICRP model. Mouth breathing, tidal volume 0.75 L/min and a frequency of 12 breaths/min. Reproduced with permission, from ICRP 1994 (40).

Most combustion-derived particles are small, in a size-range where the fraction being deposited in the airways decreases with increasing size. Since the relative humidity in the lungs is close to 100%, inhaled particles can grow by absorbing water and the hygroscopicity of particles affects the probability of deposition (41). The variation between individuals is large, indicating one possible reason for differences in individual sensitivity to air pollution.

Epidemiological studies of the health effects of air pollution generally use the mass measurements of the particles that are regulated and measured as exposure (such as $\mu g/m^3$ of $PM_{2.5}$, PM_{10}, or gases like NO_2). However, particles of an aerodynamic diameter of less than 10 or 2.5 µm are not uniform (Fig 8). The chemical composition of a given particle may be sea

salt, soot, dust and contain varying levels of endotoxins, transitional metals with oxidative potential or carcinogenic polycyclic aromatic hydrocarbons (PAHs). Other aspects than mass or number measurements are thus important for toxicity, such as total particulate surface area on which reactions can occur (relatively larger for smaller particles), oxidative stress potential, solubility, charge and stability (in atmosphere and tissue), as well as the deposition patterns discussed above (15). Some of the more common constituents of airborne PM are carbon (elemental and organic), nitrates, sulfates, PAHs, metals and biological compounds (42).

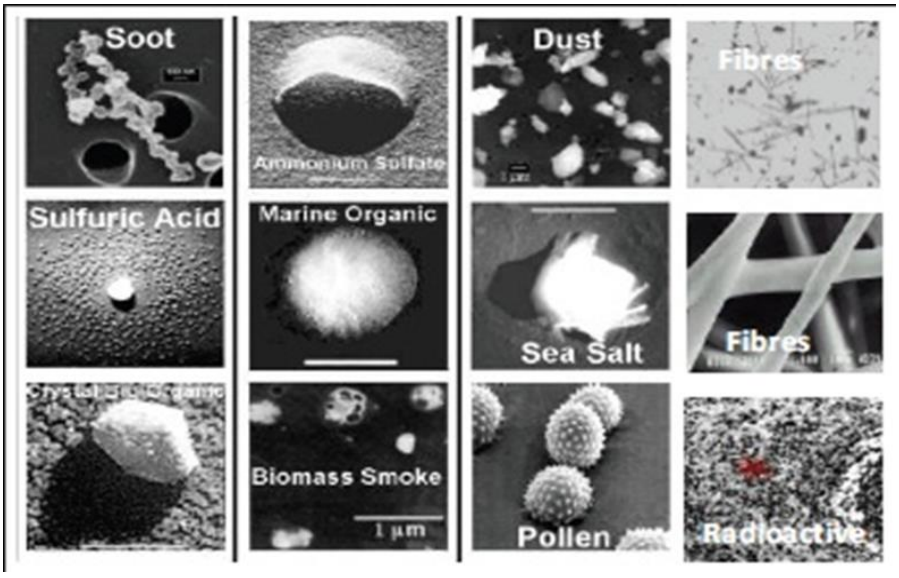

Figure 8. An electron microscopy picture of different types of particles.

1.2.2 Nitrogen Oxides

Nitrogen oxides (NO_x) consist of NO and NO_2 as well as larger molecules (NO_3, N_2O_4, N_2O_5). The main source of NO_x in ambient air is combustion of fossil fuels in road traffic or industrial processes. NO_2 has been studied the most since it is commonly regulated and measured, and since NO from combustion to a large extent is converted to NO_2 (42). NO_2 or NO_x are often used as proxies for combustion-derived particles, but might also have an independent effect on mortality (43). NO_2 is a free radical and can thus cause inflammation and injury to the airways, and has been implicated in number of airway disorders (44).

1.2.3 Other components of air pollution

Carbon monoxide (CO) is created during combustion with insufficient oxygen to produce carbon dioxide (CO_2). CO is very toxic, sometimes fatal in enclosed spaces, since inhaled CO enters the blood stream and binds to hemoglobin, reducing the capacity of erythrocytes to transport oxygen (44). SO_2 is a noxious irritating gas emitted during industrial processes and combustion of fuels containing sulfur. SO_2-emissions have decreased in the western world in the last few decades. Exposure to SO_2 leads to mainly respiratory health effects (45). Ground level O_3 is a secondary pollutant created in urban areas by atmospheric reactions between NO_x, O_2, hydrocarbons and ultraviolet light. O_3 levels are therefore high in the summertime in sunny areas. O_3 is a powerful oxidant that can induce inflammation in the respiratory tract and impair pulmonary function. O_3 exposure has also been associated with increased risks of cardiovascular events (45). PAHs are a class of compounds formed during incomplete combustion of carbonaceous materials. PAHs in air can be gaseous or bound to particles. Many PAHs are carcinogenic and mutagenic, and have been connected with other adverse health outcomes. Benzo(a)pyrene is most studied and often used as an indicator compound for PAH exposure (44).

1.3 Wood smoke

1.3.1 Residential combustion of solid fuels

Household combustion of solid fuels is one of the ten largest causes of loss of life in the world. In the latest global burden of disease estimate (1) approximately half of the excess mortality due to air pollution is due to ambient PM and the other half due to household use of solid fuels, representing 2.9 million deaths each, globally. Most of the health effects from combustion of solid fuel are among poor people in developing countries.

Around half the people in the world still use solid fuels (such as wood, coal, dung, agricultural waste) for indoor cooking and heating (46).

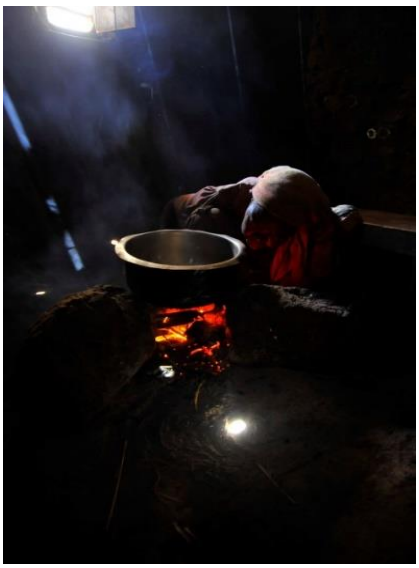

Figure 9. Kenyan woman cooking in dark and smoky room. Photograph Alex Kamweru for Global Alliance for Clean Cookstoves. Reproduced with permission.

Exposure levels can be very high, several hundreds of µg/m^3 of PM for the users, as well as locally/regionally in communities (47).

Wood burning for residential heating or recreation is also a large source of PM air pollution in parts of the developed world, including the Nordic countries. In the wintertime wood burning can contribute as much as 70% of $PM_{2.5}$ in northern Sweden (48), up to 90% in Seattle (49), and more than 90% in Christchurch, New Zealand (50). The contribution of wood burning to PM emissions is likely to increase in the future as emissions from road traffic decrease and there is a shift towards renewable energy sources. One assessment of annual $PM_{2.5}$ emissions estimated that for 15 EU countries domestic wood stoves contributed 25% in the year 2000, and expected an increase to 38% in 2020 (51).

1.3.2 Landscape fires

Landscape fires (wild and prescribed forest fires, tropical deforestation fires, peat fires, agricultural burning and grass fires) are a large source of air pollution globally. Wildfire smoke exposure has been associated with cardiovascular and especially respiratory symptoms and morbidity (52). There are few studies demonstrating an association with mortality, but one recent estimation was that wildfires cause 339.000 annual excess deaths (53). During fire events regional air pollution levels can be very high, reaching hundreds of µg/m^3 of $PM_{2.5}$, and affect air quality at great distances. The incidence of large uncontrolled wildfires has increased in recent years (54).

In Sweden, wildfires are seldom considered as a source of air pollution since they have been relatively rare and well controlled, with a few exceptions such as the fire in Västmanland in 2014. However, the expected increase in summer temperatures due to global warming in the coming decades is likely to increase the risk of major forest fires in Sweden in the future.

1.3.3 The composition of wood smoke

Wood smoke contains both smaller particles from relatively complete combustion consisting of mainly alkali salts, and larger particles from more incomplete combustion containing more carbonaceous material (55). The physiochemical properties of wood smoke vary with combustion conditions but can be classified into three fine particle classes: spherical organic carbon particles (50-600 nm) generated under low-temperature and incomplete combustion conditions, inorganic ash (15-125 nm) generated under high-temperature and complete combustion conditions, and aggregate soot particles (20-50 nm) generated under high-temperature and incomplete

combustion conditions. (55). Normally, combustion conditions vary during the combustion cycle and the smoke consists of a mixture of particle types.

One recent study of biomass smoke particles from pellet combustion showed that, taking hygroscopicity into account, the fraction deposited in human airways was low (0.21-0.23 of particle numbers) since the majority of particles were in a size-range with a low probability of deposition (0.2-0.7 µg) (56). However, a follow-up study burning wood in a common wood stove (more comparable to paper I and II in this thesis) showed a higher deposition fraction (around 0.34) (57). This is still a somewhat lower deposition fraction than for diesel exhaust particles, which are generally more hydrophobic and thus increase their size less in the humid airways (58).

The relative toxicity of PM from combustion of wood compared to other PM is unclear. The evidence for associations with mortality is not as strong as for ambient air pollution or diesel exhaust particles, but two reviews did not find any evidence for less respiratory health effects by wood smoke-derived PM compared to other PM (47, 59), and a recent position paper stated that there is not enough evidence to conclude that residential biomass emissions are less harmful than particles from fossil fuel combustion, calling for comparative studies (60). In vitro studies have indicated that particles emitted from incomplete combustion could be more toxic (55, 61)

1.4 Biological mechanisms

It is intuitively easier to expect respiratory health effects of inhaling air pollution than cardiovascular health effects. However, both studies of short-term exposures such as the London smog of 1952 (and subsequent time series studies) and the long-term cohort studies such as the Six Cities study and the ACS study clearly showed an increase in cardiovascular mortality as well. Indeed, since cardiovascular disease is a much more common cause of death than respiratory disease in the Western world, the large majority of deaths due to air pollution are cardiovascular. However, the causality of the observed associations between air pollution and mortality have been questioned, especially the biological plausibility of how low concentrations of inhaled PM could cause death in cardiovascular disease (62). Partially in response to this, a large and growing number of experimental and epidemiological studies have explored potential pathophysiological mechanisms and provided supporting evidence in the last few decades.

1.4.1 Cardiovascular health effects

The proposed biological mechanisms of how exposure to PM air pollution increases the risk of cardiovascular events have been examined and found support in a number of animal, in vitro, human chamber and epidemiological studies and are described in several recent reviews and position papers (15, 39, 42, 63, 64). The main pathways are summarized in Fig 10. Briefly, PM deposited in the airways may induce local pulmonary inflammation/oxidative stress, leading to the release of systemic inflammatory/pro-oxidative mediators. Inflammatory cytokines are also involved in the activation of the coagulation cascade, and thus in the short term connected to increased risks of thrombosis. In the long term inflammation is associated with the progression of atherosclerosis and cardiovascular disease. Particles or particle constituent may also translocate into the systemic circulation and directly affect vascular function and blood constituents. A third suggested mechanism is that particles may interact with irritant receptors in the lung affecting the autonomic nervous system (eg reducing activity in the parasympathetic nervous system and/or increasing activity in the sympathetic) thus increasing the risk of arrhythmias. These three pathways are not mutually exclusive.

1.4.2 Respiratory health effects

Most of us have all experienced respiratory symptoms in relation to air pollution exposure, perhaps in a large city, a night-club filled with cigarette smoke, next to a campfire or when we let the food burn on the stove because we are mentally formulating the defense of our dissertation. It is therefore not surprising that there is strong epidemiological evidence for an association between air pollution exposure and respiratory symptoms, and exacerbations of airway diseases such as asthma and chronic obstructive pulmonary disease. There is also evidence that air pollution increases the risk of developing these diseases as well as allergy and respiratory infections (65, 66).

Inhaled particles trigger inflammation in the smaller airways, probably through generation of reactive oxygen species and oxidative stress. PM can also interfere with clearance and inactivation of bacteria in the lungs (67). Inflammatory mediators are generated and inflammatory cells recruited and activated. Exposure to air pollution can also weaken pulmonary defense mechanisms by increasing epithelial permeability, decreasing mucociliary clearance and depressing the function of macrophages. There is substantial variations in individual susceptibility to health effects of air pollution (65). Epigenetic effects of air pollution have also been suggested (15).

1.4.3 Cancer

In addition to cardiopulmonary health effects, air pollution increases the risk of lung cancer. Outdoor air pollution, diesel engine exhaust and household combustion of coal have all by IARC been classed as carcinogenic to humans (group 1) and indoor emissions from biomass combustion and cooking in developing countries has been classed as probably carcinogenic (group 2a). A number of individual constituents in air pollution such as benzene have also been classed as carcinogenic with varying degrees of certainty (68-70).

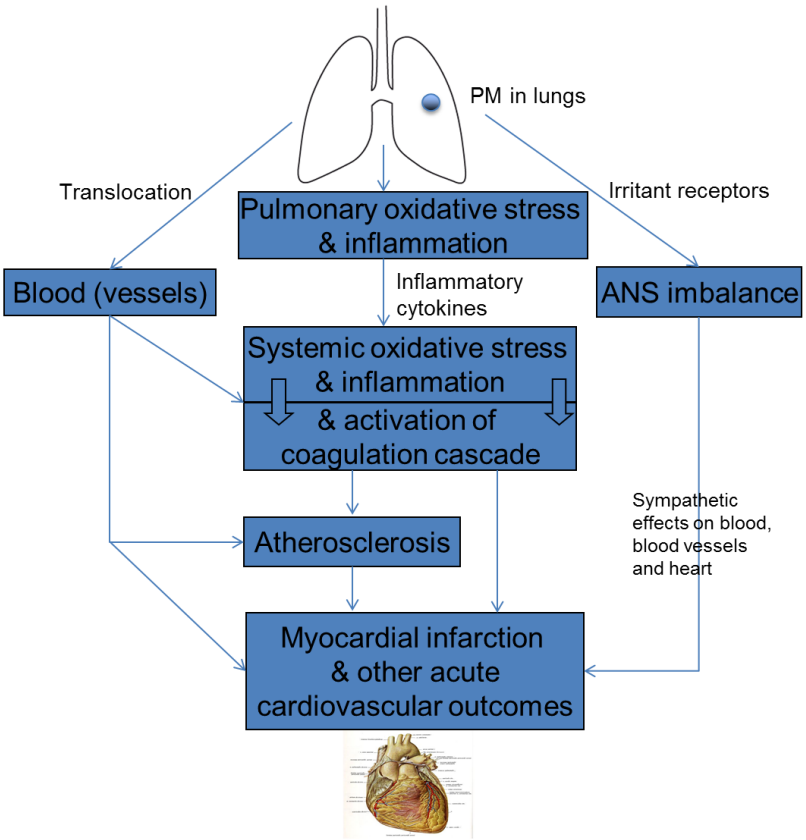

Figure 10. The main mechanisms through which PM air pollution causes cardiovascular disease, adapted from Brook 2010(15).

2 AIMS

The overall aims of this thesis were to investigate the mechanisms through which wood smoke causes respiratory and cardiovascular diseases, and the effects of long-term exposure to air pollution in a Swedish cohort.

Specific aims were to:

- Determine if short-term exposure to wood smoke in healthy adults affects biomarkers of airway inflammation, oxidative stress and endothelial permeability, and symptoms (Paper I), or biomarkers of systemic inflammation, coagulation and oxidative stress (Paper II).
- Compare the relative toxicity of two types of wood smoke (Paper I and II).
- Describe the long-term time trends and spatial contrast of the population's residential exposure to NO_x in Gothenburg (Paper III).
- Assess how the exposure assessment was affected by extrapolation and by relocations (Paper III).
- Determine if long-term residential NO_x exposure is a risk factor for overall and cause-specific mortality, or incident myocardial infarction, in a population-based cohort of men in Gothenburg (Paper IV).
- Evaluate if shorter or longer time-windows of exposure are more important for the effect of long-term NO_x exposure on mortality (Paper IV).

3 MATERIALS AND METHODS

3.1 Overview

The following section contains a summary of the methods used in all studies, for further details see papers I-IV. Papers I and II are based on an experimental chamber study of effects on biomarkers (mainly) in healthy adults by wood smoke exposure. Paper III is an exposure modelling study based (mainly) on a large cohort of men in Gothenburg and paper IV is an epidemiological study of total and specific cardiopulmonary mortality and incident myocardial infarction (MI) in that cohort. All studies were reviewed and approved by the Regional Ethical Review Board in Gothenburg.

Table 1. Study design, population, methods and outcomes in each paper

Paper	I	II	III	IV
Design	Experimental chamber study		Exposure modeling study	Epidemiological cohort study
Time frame	Short (hours-days)		Long (years-decades)	
Study population	13 healthy adults		7494 men in Gothenburg	
Exposure	Wood smoke (two types)		NO_x	
Exposure levels	Mean PM mass 146/295 vs <15 µg/m^3		Median annual NO_x 17-44 µg/m^3	
Outcomes	Biomarkers of airway effects, and symptoms	Biomarkers of systemic inflammation, coagulation and oxidative stress	Yearly residential exposure, temporal and spatial contrast, extrapolation and relocation analysis	Total & cause-specific cardiopulmonary mortality, incident MI
Statistical methods	Wilcoxon signed rank test, Spearman rank correlations	Wilcoxon signed rank test, T-tests, Spearman rank correlations	Descriptive statistics, Pearson correlations, T-tests	Cox proportional hazards regression models, generalized additive models

3.2 Study population and data collection

3.2.1 Paper I and II

The study group of paper I and II initially consisted of 16 healthy never-smoking adults (8 men and 8 women) aged 20-57 years of age (mean age 31) recruited from the staff of our department and students. We excluded three participants before data analysis because upper airway infections emerged

just before or between the sessions, and the results are thus based on 13 persons. All had normal spirometry values and none had symptomatic allergy, and none took any medication for at least two days before each session.

3.2.2 Paper III and IV

The study population of paper III and IV was the Primary Prevention Study cohort (71), followed during the study period of January 1st 1973 to December 31st 2007. A random third (n=10,004) of all men in the city of Gothenburg born between 1915 and 1925, except for 1923, were enrolled in 1970–1973 (n=7,494, participation rate 75%), and screened again in 1974–1977 (n=7,121). On both occasions participants filled out questionnaires on background data and potential cardiovascular risk factors (occupation, smoking habits, occupational and leisure time physical activity, a diagnosis of diabetes mellitus, hypertensive medication, psychological stress, and family history of coronary events) and were examined by health care professionals (height, weight, systolic and diastolic blood pressure and cholesterol levels).

Questionnaire information on tobacco smoking habits from both examinations was used with participants categorized into five groups: "never-smokers", "ex-smokers, screening 1", "smokers quitting between screening 1 and screening 2", "light smokers" (a consumption of cigars, pipe tobacco, and cigarettes equivalent to 1–15 cigarettes/day), and "intermediate and heavy smokers" (the equivalent of >15 cigarettes/day). Occupation was classified into classes according to a socioeconomic classification system of Statistics Sweden, as described in (72). The classes were: (1) higher civil servants, executives and self-employed professionals, (2) intermediate non-manual employees, (3) foremen in industrial production and assistant, non-manual employees, (4) skilled workers, (5) unskilled and semi-skilled workers, and (6) others (mainly men with disability pensions, and farmers).

Physical activity during leisure time was categorized into (1) mainly sedentary activity (2) moderate physical activity (3) regular strenuous physical activity or athletic training or competitive sports several times per week. A diagnosis of diabetes mellitus at baseline was used as a dichotomous variable, as well as a family history of coronary events, and psychological stress ("persistent stress", yes/no).

BMI was calculated from height and weight, and the mean BMI across the two measurements categorized into quintiles. Systolic and diastolic blood

pressure was measured after 5 minutes of rest to the closest 2 mmHg, and values from screening 1 included in the model as a continuous variable. Participants with a systolic blood pressure =>140 mmHg, a diastolic blood pressure= >90 mmHg, or taking antihypertensive medication were defined as hypertensive (yes/no). Fasting serum cholesterol concentrations at screening 1 were used as a continuous variable.

Although the Primary Prevention Study was originally an intervention trial against smoking, hypercholesterolemia, and hypertension, no significant differences in risk factors or outcomes were found between the intervention group and a control group when a subsample of 20% were re-examined ten years after the first examination (73). Consequently, any changes brought about by the intervention were taking place in the general population as well, and the cohort was therefore considered to be representative of the city population.

Selected background characteristics of the population are shown in Table 2. At baseline in 1973, participants were middle aged, with ages evenly distributed between 48 and 58 years. Over half of the participants were initially smokers, but a relatively large fraction (9% of the cohort) quit between the first and the second screening. Around half were employed in white collar jobs, and half in blue collar jobs. Most reported a moderate amount of physical activity in their leisure time. A quarter reported a family history of coronary events, and 16% said that they were feeling constantly stressed. Only 2% had a diagnosis of diabetes mellitus. The median BMI was 25 kg/m^2, with very few underweight or obese participants. Systolic and diastolic blood pressures were relatively high on average. Only a minority regularly took hypertensive medication. The distribution of background covariates was similar in the whole population and in those with missing NO_x exposure data (Table 2).

A small number of participants (n=46) died before the start of the study period and were excluded from the analysis, as were the few individuals (n=4) for whom no address information was found.

Table 2. Selected characteristics for all participants and those with baseline NO_x exposure data (adapted from paper IV).

		All	With baseline NO_x
Mean NO_x at baseline (range)	µg/m³		42 (5–186)
Number		7,494	6,557
Age at baseline, mean (range)	years	53 (48–58)	53 (48–58)
Smoking categories, % (n)	Never-smokers	25 (1,851)	25 (1,648)
	Ex-smokers at scr 1	22 (1,671)	22 (1,434)
	Quit before scr 2	9 (703)	10 (627)
	Light smokers	28 (2,061)	28 (1,811)
	Heavy smokers	16 (1,201)	16 (1,032)
Occupational class % (n)	High rank white collar	11 (812)	11 (716)
	Mid rank white collar	17 (1,266)	17 (1,117)
	Low rank white collar	19 (1,400)	19 (1,233)
	Skilled manual labor	26 (1,914)	26 (1,681)
	Unskilled man. labor	23 (1,691)	23 (1,475)
	Other	5 (405)	5 (330)
Leisure time physical	Sedentary	26 (1,920)	26 (1,682)
activity, % (n)	Moderate	58 (4,308)	59 (3,809)
	Intermediate/vigorous	16 (1,166)	15 (981)
Psychological stress, % (n)	Persistent stress	16 (1,100)	15 (950)
	No persistent stress	84 (5,950)	85 (5,220)
Diabetes mellitus, % (n)	Yes	2 (149)	2 (126)
	No	98 (7,301)	98 (6,395)
Family history of acute MI, % (n)	Yes	24 (1,799)	24 (1,565)
	No	76 (5,695)	76 (4,992)
BMI, median (5^{th}–95^{th} %iles)		25 (21–31)	25 (21–31)
S-cholesterol, mean (5^{th}–95^{th} %iles)	mmol/L	6.5 (4.8–8.5)	6.5 (4.8–8.4)
SBP, mean (5^{th}–95^{th} %iles)	mmHg	149 (118–190)	149 (118–190)
DBP, mean (5^{th}–95^{th} %iles)	mmHg	95 (76–118)	95 (76–118)
Antihypertensive meds., % (n)	Yes	16 (1,221)	17 (1,081)
	No	84 (6,273)	84 (5,476)

3.3 Study design and exposure assessment

3.3.1 Paper I and II

Study design

Participants were in a chamber exposed to first filtered indoor air, one week later to wood smoke from the start-up phase of the wood-burning cycle, and another week later to wood smoke from the burn-out phase of the wood-burning cycle. Each session lasted for three hours and were identical, apart from the exposure. Blood, urine and breath condensate samples were taken before the participants entered the chamber, and at several time points after exposure. To make sampling practically possible a new participant started the schedule every 10 minutes, so that one whole session lasted 5 hours 40 minutes. In the chamber the participants read or chatted, at rest. In the middle of the exposure they had a small snack (sandwich), and they had free intake of soft drinks and water. The participants were not allowed to eat closer than one hour before the first blood samples. Because a nitrate-rich meal can increase the fraction of exhaled nitric oxide (FENO) levels (74) participants were instructed not to eat green salad, spinach, sausage, ham or >4 potatoes before NO-measurements.

The exposure chamber & generation of wood smoke

The exposure chamber at the Swedish National Testing and Research Institute (SP) measured 29 m^3 (7.4 x 6 x 2.9 m). The walls, floor and ceiling were covered with Teflon-impregnated glass fiber fabric and were cleaned between exposure sessions. Wood smoke was generated in a small cast iron wood stove, chosen to represent a typical stove in Scandinavia, just outside the chamber. A partial flow of the generated wood smoke was mixed with HEPA-filtered indoor air and led into the chamber. The $PM_{2.5}$ mass concentration was controlled online to maintain a target concentration of about 200 $\mu g/m^3$. A mixture of hardwood and softwood was used (50% birch, 50% spruce, moisture content 14-16% and 17-19% respectively). A 2.5-3 kg batch combining small and large logs was ignited, and approximately every 40 minutes another three logs of 1.5 kg were added until the session was over.

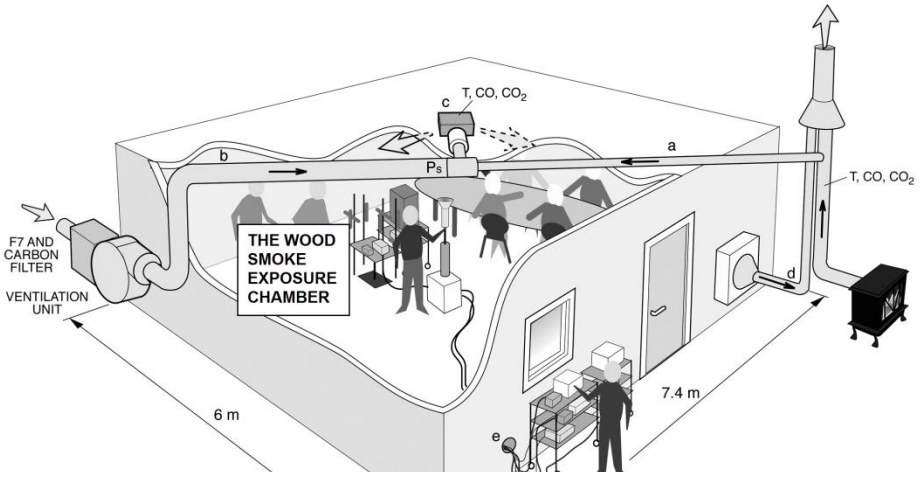

Figure 11. The wood smoke exposure chamber used in the study. Partial flow of flue gas (a), flow of filtered indoor air (b), supply of air terminal device (c), ventilation outlet (d), and connection hole for instruments (e). Adapted from Sallsten et al 2006.

In the session when wood smoke from the start-up phase was generated, smoke was supplied to the chamber for 12-14 minutes starting immediately after new wood logs were added. In the session using smoke from the burn-out phase, smoke was supplied for 15 minutes starting 25 minutes after wood was added. In both sessions the fire was started one hour before the sessions to warm the wood stove.

Sampling and characterization of wood smoke

We measured the $PM_{2.5}$ mass concentration online using a tapered element oscillating microbalance (TEOM), and $PM_{2.5}$ and PM_1 mass concentrations using cyclones and sampling pumps. Number concentrations and size distributions of particles (0.007–6.7 µm) were measured by an electric low pressure impactor (ELPI). Some filters were analyzed for trace elements using an energy dispersive X-ray fluorescence (EDXRF) spectrometer (75), and for black carbon (BC) content by an optical method. Other filters were analyzed for particulate PAHs using high-resolution gas chromatography and low resolution mass spectrometry (HRGC/LRMS). We measured NO + NO_2 online using a chemiluminescence technique and CO_2 + CO using infrared technique.

Stationary measurements of benzene and 1,3-butadiene were performed using SKC-Ultra diffusive samplers filled with Carbopack X and the samples analyzed with an automatic thermal desorber (ATD) and gas chromatograph

flame ionization detection (76). Active sampling of formaldehyde and acetaldehyde was performed using pumps and Sep-Pak DNPH-silica cartridges, and the samples analyzed using high performance liquid chromatography (HPLC) (77). Measurements of naphthalene, toluene, ethylbenzene and xylenes were made using with Perkin Elmer ATD tubes filled with Tenax TA. The samples were analyzed using ATD and gas chromatograph flame ionization detection (76, 78). Finally, we registered the temperature and relative humidity in the chamber. All measurements were taken in the center of the chamber throughout the whole session.

3.3.2 Paper III and IV

For all participants' individual yearly addresses for the entire study period were retrieved and assigned geographical coordinates. We manually checked all addresses and corrected inconsistencies, such as spelling mistakes, and assigned some of the older addresses using maps in the city archives. Each year, some participants (5–10%) had insufficient address information for assigning precise coordinates, or the assigned coordinates were outside our modeled area and could therefore not be assigned a NO_x value. Some of the addresses just outside the border of the modeled area (within about 200 m), however, could reliably be assigned using a somewhat larger model calculation for the year 1990 (as described in paper III).

Yearly mean levels of NO_x for the Gothenburg area were modeled in 75,000 (250*300) 50-meter squares for the years 1975, 1983, 1991, 1997, 2004, and 2009 by the Gothenburg Environment Department in a Gaussian dispersion model using the EnviMan AQPlanner and historical and current emission databases (EDBs). The EDBs contain information on emissions from road traffic, shipping, industries, larger energy and heat producers, small-scale heating and construction machinery. For 1975 and 1983 emissions from industries and shipping were estimated based on later emission data and adjusted by reported production data. Since emissions from industries and shipping were less than half of the traffic emissions and for most participants not close to their homes, the impact of this uncertainty was minor.

We linearly interpolated NO_x levels for the years between the modeled years so each individual had an exposure value at the residential address for each year. When address information for the participant's last year was missing (due to death or emigration before addresses were collected for that year), we used the NO_x exposure of that year at the preceding year's address. For the years 1973 and 1974, the NO_x values for 1975 were used.

At baseline 6946 participants were alive and residing in Gothenburg, for which we could model exposure for 6563 (95%). We assigned a total of 160,568 addresses during the study period, 146,675 of which could be given a NO_x value (91%).

By using the dispersion model for the year 2009 and the time trend at the central monitoring station, we compared the back-extrapolated NO_x levels at the participants' homes with the "true" levels for the previous modeled years, 2004, 1997, and 1975, for the participants' correct addresses. Subsequently, we investigated the effects of extrapolating forwards and backwards with and without taking relocations into account.

We compared exposure levels in the cohort with exposure levels in the entire modelled area, with the exposure in the whole population for the years 1975, 1990, and 2004, and with rooftop measurements at a central location.

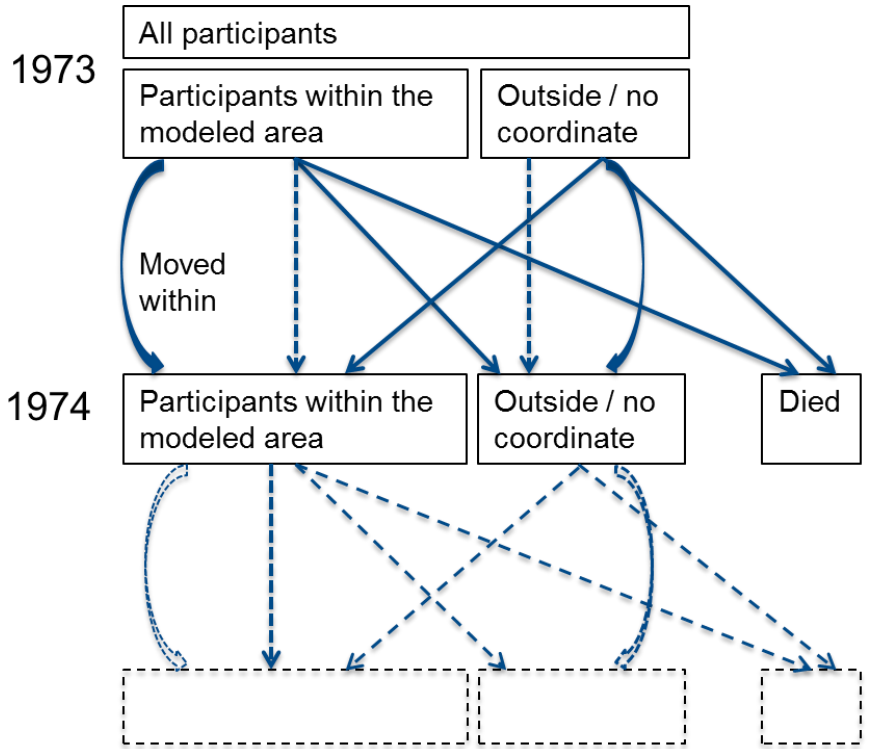

Figure 12. A flowchart of inclusion and exclusion from the cohort based on participants' residence and survival. Adapted from paper III.

3.4 Outcomes

3.4.1 Paper I and II

Symtoms

Subjective symptoms were measured and scored (0-10) according to the Borg scale (79), using a self-administered questionnaire in the last 15 minutes of each session. The symptoms included in the questionnaire were headache, dizziness, nausea, tiredness, chest pressure, cough, shortness of breath, irritation of the eyes, irritation of the nose, unpleasant odor, irritation of the throat and bad taste in the mouth.

Biomarkers

We collected venous blood for serum, plasma and blood cell counts. Blood counts by flow cytometry were performed on the same day, while the serum and plasma aliquots were stored frozen in polyethylene cryotubes until analysis. We collected timed urine samples in polypropylene bottles, males discarding the first 100 mL to eliminate post-renal excretion of Club cell protein 16 (CC16) from the prostate (80), registered the volumes and froze aliquots until analysis.

Exhaled breath condensate (EBC) was collected using an RTube, and the exhaled volume measured with an ECoVent. Participants wore a nose clip, rinsed their mouth with distilled water for 30 seconds before sampling, and breathed tidally through the RTube until 80 litres had been exhaled. Samples were frozen at -20ºC and analyzed using HPLC as described in (81). We measured FENO with a chemiluminescence analyser, in duplicates and at several exhalation flow rates according to the 2005 ATS/ERS recommendation. A plus or minus 10% deviation of the instant flow and a plus or minus 5% of the mean flow during the plateau phase was accepted.

CC16 in serum and urine (S/U-CC16), surfactant protein D (SP-D), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), serum amyloid A (SAA), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble P-selectin (sP-selectin) were analysed using commercial enzyme-linked immunosorbent assay (ELISA) kits. D-dimer and von Willebrand factor (vWf) antigen were determined using sandwich ELISA. Surfactant protein A (SP-A) was determined using a home-made ELISA using two different antibodies against human SP-A, one polyclonal and one monoclonal (82). High-sensitivity serum C-reactive protein (CRP) was measured by immunoturbidometry. Fibrinogen in plasma was determined based on the coagulation time at high

thrombin concentration, and factor VII (FVII) using a one-step thromboplastin method. Functional factor VIIIc (FVIII) was determined using a thrombolyzer. 8-Iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) was analyzed in urine using a radioimmunoassay as developed by Basu (83). U-CC16 and urinary 8-iso-$PGF_{2\alpha}$ values are presented after adjustment for creatinine levels in urine.

3.4.2 Paper IV

During the study period 1973-2007, all participants were followed based on their unique Swedish personal identification number. We obtained data on cause-specific mortality according to the International Classification of Diseases (ICD)-8, -9, and -10 from the Swedish national register on cause of death. We examined total non-accidental mortality, cardiovascular mortality, death from ischemic heart disease (IHD), death from acute MI, death from cerebrovascular diseases, and death from respiratory diseases excluding lung cancer. For incident MI, we combined data from the hospital discharge register, the Gothenburg Registry of Myocardial Infarctions, and the national register on cause of death.

During the study period, 5669 deaths (76% of participants) occurred in the cohort. Almost all were non-accidental, and almost half were caused by cardiovascular diseases (Table 3). A total of 1722 incident MI occurred.

Table 3. ICD codes and numbers of deaths from selected causes and incident MI in the cohort during the study period. Adapted from paper IV.

Cause of death	Number	% of deaths
Total mortality	5669	100%
Non-accidental (ICD8 and ICD9 001-779 and ICD10 A00-R99)	5457	96%
Cardiovascular (ICD8 and ICD9 400-440 and ICD10 I10.0-I70)	2465	43%
Ischemic heart disease (ICD8 and ICD9 410-414, ICD10 I20-I25)	1584	28%
Myocardial infarction (ICD8 and ICD9 410, ICD10 I21, I22)	953	17%
Cerebrovascular disease (ICD8 and ICD9 430-438, ICD10 I60-69)	442	8%
Respiratory disease, except cancer (ICD8+9 460-519, ICD10 J00-J99)	373	7%
Incident myocardial infarction (ICD8 and ICD9 410, ICD10 I21)	1722	n.a.

n.a. = not applicable.

3.5 Statistical methods

Data analyses were, except where otherwise stated, performed using the SAS software package, for papers I and II with version 9.2 and for paper IV

version 9.3. In paper IV R version 3.0.2 was used in some analyses. In paper III we used Sigma-plot 11.0 and Microsoft Excel for descriptive statistics and statistical analysis. All p-values presented are two-sided, and an α-level of 0.05 was used.

3.5.1 Paper I and II

For all biomarkers we calculated intra-individual differences at each time point by subtracting the change after exposure to filtered air from the change after exposure to each of the two wood smoke sessions, making each individual his or her own control. Statistical significance was tested with t-tests for normally distributed biomarkers (FVII, FVIII, vWf, f8/vWf, leukocytes, sP-selectin, platelets) and Wilcoxon's signed rank test for biomarkers not normally distributed (CRP, fibrinogen, SAA, 8-iso-$PGF_{2\alpha}$, sICAM-1, sVCAM-1, D-dimer, IL-6, TNF-α, S-CC16, U-CC16, SP-A, SP-D, MDA). Point estimates (in %) of all significant differences are also presented, expressed as the median of the intra-individual differences divided by each individual's baseline value before wood smoke exposure. Associations between biomarkers were assessed using Spearman's rank correlation coefficient (r_S), separately for each sample time, and in all unexposed morning samples combined. Biomarker samples below the detection limit (L) or missing were assigned the value $L/\sqrt{2}$ as described in (84).

3.5.2 Paper III

We imported the geocoded data (addresses and modeled NO_x levels) into QGIS version 2.4.0-Chugiak (85) and used overlay analyses with the function *join attributes by location*. Descriptive NO_x statistics and exposure contrasts during the study period were presented. We assessed linear associations between continuous variables, using the Pearson correlation coefficient (for source emissions and participants´ exposure) and R^2 values (for dispersion models vs back extrapolation).

To investigate whether relocation patterns affected the exposure trends, we analyzed the differences between NO_x exposure the first year at the new address and NO_x the same year at the old address, as if they still had resided at the old address. We used t-tests to test the mean difference for the whole study period, as well as for three time periods and three age groups.

3.5.3 Paper IV

We estimated the associations between residential NO_x exposure and different mortality outcomes using Cox proportional hazards models with age as the time scale. We used three different exposure time windows: (1) NO_x

exposure in the last year, (2) the 5 year mean NO_x exposure, and (3) the mean NO_x exposure since enrolment. For all three exposure windows, the NO_x exposure was included as an annual, time-dependent variable. For exposure windows 2 and 3, we excluded estimates that were based on <80% of the intended data. Participants were censored at the end of the study period, at the time of death due to cause of death other than the relevant outcome, or when exposure information was missing. For incident MIs the procedure was the same, but we also censored participants after the first MI.

Analyses for each outcome were performed for all three exposure windows using three models. Model 1 was a crude model only including NO_x, age as time scale, and calendar year as a third degree polynomial. Model 2 also included smoking status and occupational class since these covariates were associated with both NO_x exposure levels and mortality outcomes. Model 3 included all available potential explanatory variables, except those excluded because of collinearity. Model 2, including the "true" confounders, was considered our main model and used in all subsequent analyses (see Discussion section 4.2.14 and Fig 25 for further discussion).

Potential effect modification was tested by including the interaction term NO_x * covariate in Model 2 for each covariate (as binary variables); thereafter, stratified results were examined. We performed sensitivity analysis by including the covariates previously excluded due to collinearity, and by changing the time scale to "time in study". Analyses were also performed with/without calendar year in the model, and with calendar year as linear variable instead of a third degree polynomial.

In the proportional hazards model, NO_x was included as a linear effect. We also allowed the effect to be non-linear, using generalized additive models (GAMs) and the mgcv package in R, version 3.0.2 (86). A Poisson regression model (for Model 2) was fitted where the effect of both NO_x exposure last year and calendar year was included as smooth functions. The smooth terms were represented using penalized regression splines.

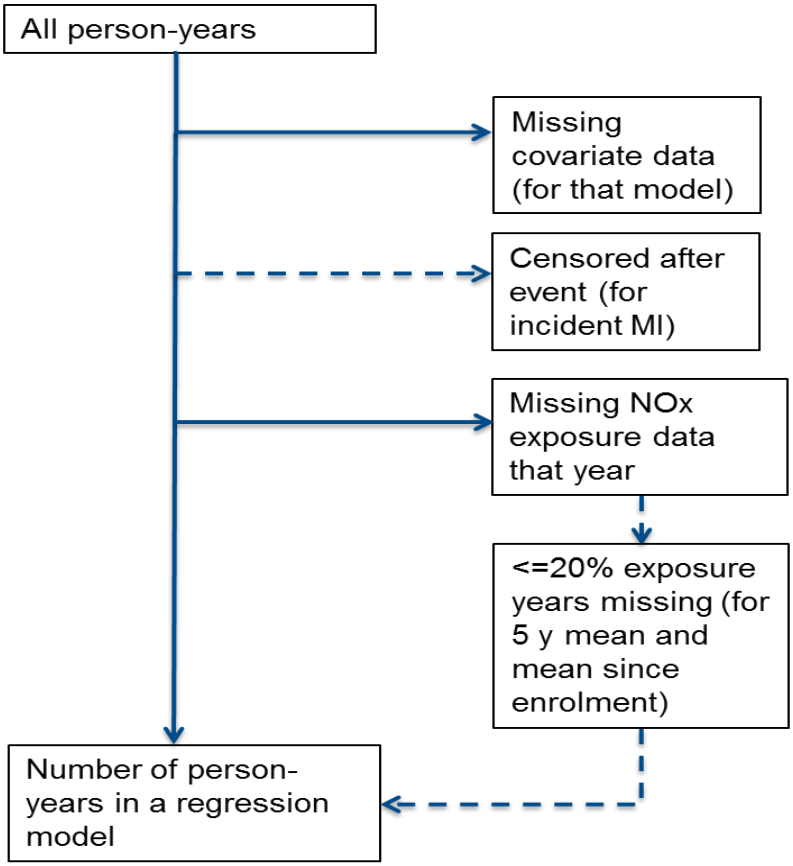

Figure 13. Flowchart describing the exclusion, and inclusions, in the cox regression models.

4 RESULTS & DISCUSSION

In this section the main results of the four papers in the thesis are summarized and discussed, including some additional analyses. Further details can be found in paper I-IV.

4.1 Paper I and II

4.1.1 Exposure

Exposure levels in the control session and the two wood smoke exposure sessions are presented in Table 4. In general, all exposures were very low in the control session with filtered air. Most exposures were higher in the start-up wood smoke session compared to the burn-out session.

PM mass concentrations were considerably higher in the start-up session compared to the burn-out session: Measured using cyclones and pumps $PM_{2.5}$ mass was almost twice as high in the start-up session compared to the burn-out session (295 vs 146 $\mu g/m^3$). Measured with TEOM $PM_{2.5}$ mass concentration was also higher in the start-up session compared to the wood smoke session (221 vs 148 $\mu g/m^3$) despite an under-estimation in the start-up session where the highest exposure peak was missing due to instrument limitations (Fig 14a). The temporal variation in exposure was considerable, especially in the start-up session. Black carbon levels were slightly higher in the burn-out session compared to the start-up session.

The particles were on average smaller in the start-up session (geometric mean diameter 38 nm vs 83 nm), and the fraction of particles being ultrafine correspondingly higher (68% vs 40% of the total number). A large part of the particles can thus be considered “nano-particles”. Average PM number concentrations (PNC) were only somewhat higher in the start-up session. The concentrations of trace elements (K, Zn, Rb, Pb) were relatively similar in the start-up session and the burn-out session, except for the levels of Pb that were more than twice as high in the burn-out session. All particle measurements and trace elements were very low in the filtered air session, often below the limit of detection.

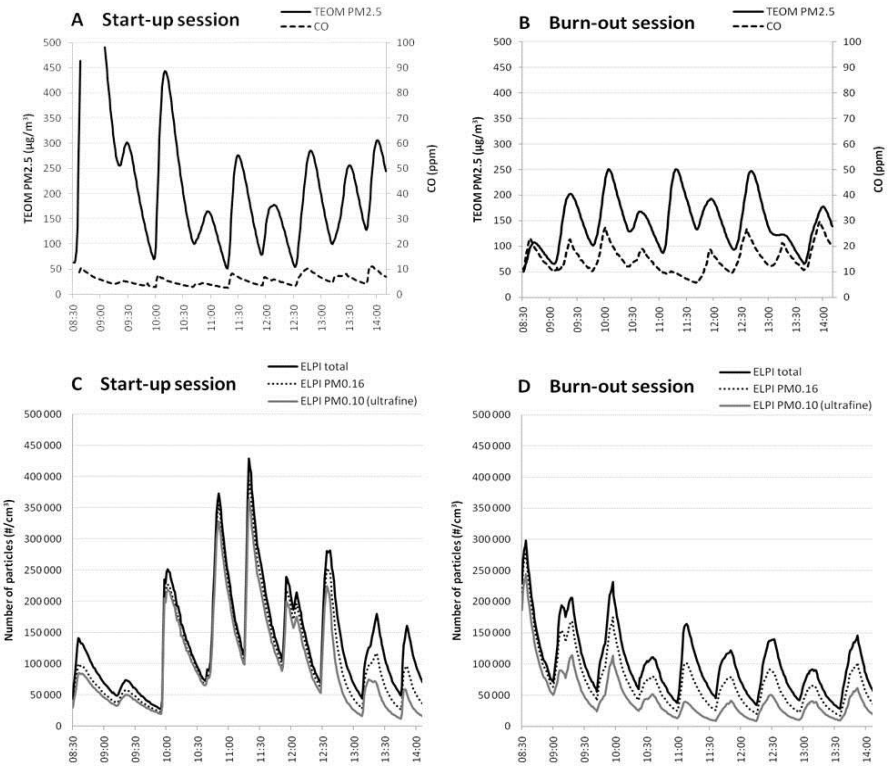

Figure 14. $PM_{2.5}$ and CO concentration during 5.8 h in the exposure chamber for both wood smoke sessions (A and B), measured online using a TEOM. Particle number concentrations (C and D) during the same period, in total and in the smallest size intervals ($PM_{0.10}$, or $PM_{0.16}$), as measured using an ELPI. From paper I.

The concentrations of particulate PAHs were higher in the start-up wood smoke session than the burn-out session, and very low in the filtered air session. For example, the average benzo(a)pyrene concentration was 3600 times higher in the start-up session and 480 times higher in the burn-out session compared to filtered air. In the start-up session we found strong correlations between almost all PAHs and between the PAHs and PM (r_s = 0.83-1.0). In the burn-out session there were fewer significant correlations. The concentrations of most Volatile Organic Compounds (VOCs) were also higher in the start-up session compared to the burn-out session, several (1,3-butadiene, naphthalene and acetaldehyde) about twice as high. All VOCs except xylenes and toluenes were much lower in the filtered air session.

The levels of NO, NO_2 and CO were highest in the burn-out session; mean NO_2 levels 0.05 ppm (=101 µg/m^3), vs 0.03 ppm (=61 µg/m^3) in the start-up

session and 0.01 ppm (=20µg/m^3) in the filtered air session. The highest peak concentrations also occurred in the burn-out session (NO_2=0.1 ppm = 203 µg/m^3, and CO=30 ppm). The concentrations of CO_2 were similar in all sessions, about 1300 ppm, roughly 3-4 times normal outdoor levels. The mean temperature in the exposure chamber was similar in all sessions, and the relative humidity lowest in the burn-out session.

Table 4. Time weighted averages of the online measurements (PM mass, particle number concentration, NO, NO_2, CO), means of replicate filter samples (PM mass, BC, elements and PAHs) and VOCs. From paper I.

	Filtered air session			Start-up session			Burn-out session		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
$PM_{2.5}$ (µg/m^3) (TEOM)	C	8.4	2.0	C	221*	121	C	148	48
PM mass (µg/m^3)	7	<15		16**	295	43	1	146	15
PNC (1000#/cm^3)	C	2.9	0.77	C	140	83	C	100	51
Ultra fine particles (%)	C	55	5.8	C	68	21	C	40	15
BC (µg/m^3)	-	-		6	90	17	6	115	10
Trace elements (ng/m^3)									
K	6	<1500		6	9700	4500	6	8800	1200
Zn	6	70	14	6	2400	720	6	3100	340
Rb	6	<30		6	73	30	6	105	18
Pb	6	47	43	6	170	24	6	400	55
Particulate PAHs (ng/m^3)									
Benzo(b)fluoranthene	3	0.03	0.05	6	20	6	6	4.9	1.3
Benzo(k)fluoranthene	3	<0.01		6	23	8.6	6	4.5	1.1
Benzo(a)pyrene	3	0.01	0.006	6	36	15	6	4.8	1.6
Perylene	3	<0.01		6	5.5	2.1	6	1.1	0.26
Indeno(1,2,3-cd)pyrene	3	0.02	0.02	6	49	14	6	14	2.3
Dibenzo(a,h)anthracene	3	<0.01		6	4.3	1.9	6	3.0	0.50
Benzo(g,h,i)perylene	3	<0.01		6	41	9.6	6	11	1.4
VOCs (µg/m^3)									
Benzene	3	2.0	0.20	3	33	0.78	3	21	1.1
1,3-Butadiene	3	0.16	0.02	3	8.5	0.17	3	4.2	0.11
Toluene	2	15	3.0	3	28	0.95	3	17	0.49
Ethylbenzene	2	4.8	0.56	3	4.0	0.25	3	4.7	0.28
Xylenes	2	19	0.58	3	13	1.3	3	20	1.4
Naphthalene	2	1.6	0.08	3	10	0.79	3	4.1	0.60
Formaldehyde	2	11	0	3	94	4.7	3	81	9.5
Acetaldehyde	2	13	0	3	71	4.2	3	37	4.4
Gaseous (ppm)									
NO	C	0.08	0.02	C	0.14	0.10	C	0.30	0.07
NO_2	C	0.01	0.004	C	0.03	0.01	C	0.05	0.02
CO	C	0.73	0.04	C	5.6	2.0	C	15	5.1

C = continuous measurements

* = registration missing for half an hour, ** = one sample omitted due to leakage

4.1.2 Biomarkers of airway effects

Airway biomarkers before and after exposure to start-up/burn-out wood smoke and filtered air are presented in Table 5. Several biomarkers of airway effects increased after exposure to wood smoke compared to the control session, indicating effects on airway epithelial permeability and airway inflammation.

FENO – Fraction of Exhaled Nitric Oxide

FENO is a marker of airway inflammation, associated with asthma and other airway diseases (87). FENO is increasingly used and now recommended by the ATS in the management of asthma (88). Exhaled nitric oxide at 50 ml/s exhalation flow rate ($FENO_{50}$) mainly represents the conducting airways, and exhaled nitric oxide at 270 ml/s exhalation flow rate ($FENO_{270}$) mainly represents the alveolar compartment.

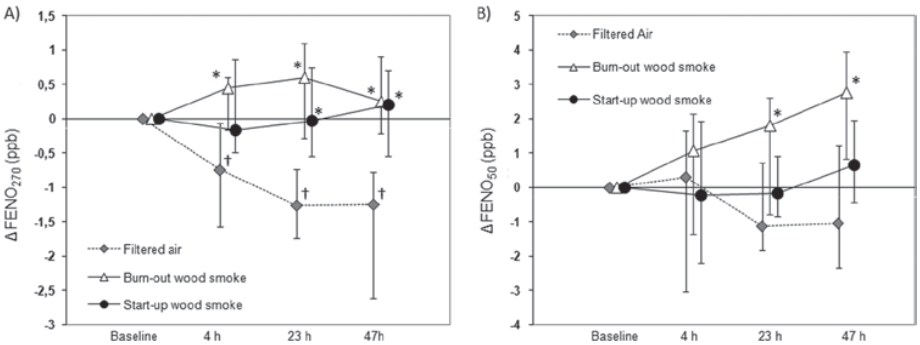

*Figure 15. Median changes (Δ) from baseline and 90% confidence intervals for (A) $FENO_{270}$ and (B) $FENO_{50}$ at all sample times in the filtered air session and both wood smoke sessions. *Significant net increase after wood smoke exposure. †Significant decrease from baseline. From paper I.*

$FENO_{270}$ increased after both wood smoke exposures compared to filtered air at almost all sample times (point estimates 18-32%, Fig 15A). Although highly significant results, a cautious interpretation is appropriate since this relative increase was to a large extent due to an unexplained relatively high $FENO_{270}$ baseline the morning of the filtered-air session, and consequently a decrease after exposure to filtered air (Fig 15A). Without adjustment for changes in the control session only a non-significant tendency to increase after exposure to burn-out wood smoke remained (point estimates 5-14%).

$FENO_{50}$ increased after exposure to wood smoke from the burn-out phase of the wood-burning cycle. Adjusted for filtered air, the changes were significantly higher after wood smoke exposure both the following mornings

(point estimates 12% and 19%, Fig 15B). After exposure to wood smoke from the start-up session, $FENO_{50}$ increased only marginally compared to after filtered air.

While we believe the best interpretation of the findings on $FENO_{50}$ and $FENO_{270}$ is as signs of distal and proximal airway inflammation by exposure to wood smoke, some precautions should be kept in mind. The increase in both $FENO_{50}$ and $FENO_{270}$ after wood smoke exposure was stronger in the burn-out session, where most exposures were lower than the start-up session. It seems unlikely that the higher levels of gaseous pollutants in the burn-out session could have caused the effect on FENO. Furthermore, in our previous wood smoke exposure chamber study using higher exposure an increase in $FENO_{270}$ but not $FENO_{50}$ was observed (89). Null effects on FENO have been reported in some other chamber studies of wood smoke exposure (90-92) as well as in other experimental studies of air pollution (93-95). However, several studies of outdoor air pollution have reported an association with FENO (96-101).

Pneumoproteins

Club cell protein 16, believed to protect the respiratory tract against inflammation and oxidative stress, is secreted by club cells into the epithelial lining fluid (ELF) of the lung. A small fraction normally passes through the lung epithelial barrier into serum where it is rapidly eliminated through renal clearance, leading to increased urinary levels. CC16 can thus be measured both in ELF, blood and urine. Increased levels of CC16 in serum may come from increased production/secretion into the respiratory tract, increased leakage through the lung-blood barrier or decreased renal clearance (102). Increased leakage is believed to be more important than increased synthesis in acute exposure situations (103).

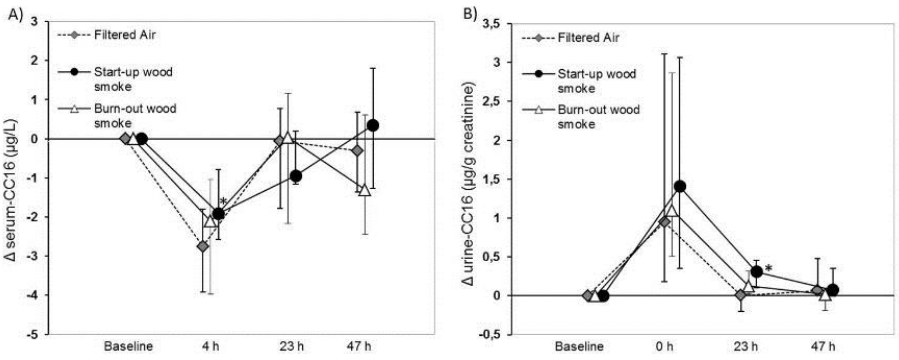

*Figure 16. Median changes (Δ) from baseline and 90% confidence intervals for (A) S-CC16 and (B) U-CC16 at all sample times in the filtered air session and the two wood smoke sessions. *significant net increase after wood smoke exposure. From Paper I.*

The surfactant protein A (SP-A) and surfactant protein D (SP-D), produced by alveolar type II cells, are important for surfactant homeostasis and pulmonary immunity (104). While larger than CC16 they can still penetrate the lung-blood barrier, and increased serum levels have been observed in pulmonary diseases (105).

In the start-up wood smoke session S-CC16 was significantly increased compared to after filtered air four hours after exposure (point estimate 19%, Fig 16A), and U-CC16 was increased in the next morning (point estimate 59% Fig 16B). No significant changes in CC16 were found in the burn-out session. The surfactant proteins SP-A and SP-D showed no significant changes in the start-up session, while there was a significant small net decrease of SP-D four hours after exposure in the burn-out session (4%).

We interpret the increase in S-CC16 four hours after exposure start as increased leakage through the lung-blood barrier due to minor epithelial damage. Rapidly increased synthesis/secretion of CC16 cannot be excluded as an alternative explanation though, since we did not take BAL samples of CC16. However, another recent chamber study of wood smoke exposure in healthy humans did analyze CC16 in BAL and did not see any increase or decrease after wood smoke exposure compared to clean air (92).

S-CC16 was normalized already in the sample the following morning, indicating that the effect was minor and rapidly repaired. Instead, levels in urine were increased the morning after exposure, which is biologically consistent since CC16 is cleared through urinary secretion. Further support for the validity of these findings are that similar effects were found in our previous wood smoke exposure study (89). However, it should be noted that two other chamber studies did not find any changes in S-CC16 after wood smoke exposure compared to clean air (92, 106). Increased S-CC16 has also been found in other studies after short-term exposure to other airway irritants (107-110). Studies of smokers (82, 111-113), and residential wood burners (114) indicate that longer-term exposures instead decrease levels of CC16, probably due to loss of club cells. CC16 is a small protein with a high concentration gradient, and subtle defects in the lung epithelial barrier may be enough to cause a detectable increase in S-CC16 (102). The larger size of SP-A and SP-D is one possible reason for why these biomarkers were not affected as CC16. Limited power to detect small variations due to larger inter-assay variability is also a possible explanation, especially for SP-A.

Interestingly, *decreases* in SP-D and CC16 were recently observed after acute airway exposure in ski waxers (115). A major function of collectins such as

SP-A and SP-D is to bind targets to facilitate phagocytosis (e.g. microorganisms and allergens, but they have also been shown to bind carbon nanotubes (116)). A possible explanation for the decrease of serum-SpD after wood smoke exposure might be that the surfactant is consumed by binding small carbonaceous particles. However, chance is an alternative explanation.

Table 5. Median levels (ranges) of biomarkers of airway effects before and after exposure. Adapted from paper I.

Parameter	Before	**After filtered air**			Before	**After start-up smoke**			Before	**After burn-out smoke**		
Sample	1	2	3	4	1	2	3	4	1	2	3	4
S-CC16 (serum, µg/L)	8.6 (5.3-18)	6.7 (2.5-12)	8.1 (4.7-13)	7.2 (4.3-19)	8.3 (3.9-16)	6.7* (3.1-14)	8.3 (4.9-14)	8.3 (5.8-17)	8.1 (5.7-13)	6.4 (0.01-13)	8.7 (5.2-11)	7.8 (3.7-13)
U-CC16 (urine, µg/g krea)	0.07 (0.02-3.2)	1.7 (0.06-19)	0.4 (0.03-3)	0.5 (0.03-3.7)	0.12 (0.02-5.5)	1.4 (0.18-17)	0.5* (0.04-5.9)	0.3 (0.03-3.7)	0.2 (0.01-3.7)	1.3 (0.03-14)	0.4 (0.03-4)	0.12 (0.02-3)
$FENO_{270}$ (exhaled air, ppb)	6.3 (3.4-13)	5.1 (1.3-13)	5.1 (2.1-11)	5.0 (1-9.5)	4.7 (2.6-10)	5.2 (2.9-11)	5.0* (2.6-10)	4.9* (3-13)	5.1 (2.7-9.9)	7.1* (3.4-10)	6.2* (2.2-10)	5.0* (3.5-12)
$FENO_{50}$ (exhaled air, ppb)	17 (7.8-52)	16 (6.9-44)	19 (7.6-44)	17 (8.8-46)	14 (6.8-43)	17 (8.4-47)	17 (5.9-47)	17 (8.3-53)	15 (6.9-45)	19 (7.9-43)	18* (8.8-44)	19* (7.7-50)
SP-A (serum, ng/ml)	6.0 (0.2-240)	6.7 (0.3-230)	6.1 (0.3-250)	6.5 (0.2-250)	6.1 (0.2-270)	6.0 (0.2-250)	6.1 (0.3-240)	5.9 (0.2-240)	5.9 (0.2-230)	5.5 (0.2-220)	5.6 (0.2-250)	5.8 (0.2-260)
SP-D (serum, mg/ml)	61 (31-180)	63 (28-150)	70 (30-160)	73 (35-180)	65 (29-170)	56 (30-150)	61 (28-190)	57 (31-190)	59 (28-180)	54† (29-150)	63 (34-170)	70 (30-150)
MDA (breath, µmol/ml)	20 (3-80)	20 (3-60)	10 (3-70)	30 (3-100)	10 (3-90)	40 (3-530)	20 (3-70)	10 (3-70)	20 (3-70)	10 (3-70)	20 (3-80)	10 (3-70)

* Significant increase (two-sided p-value <0.05) compared to filtered air
† Significant decrease (two-sided p-value <0.05) compared to filtered air

4.1.3 Biomarkers of systemic effects

Biomarkers of systemic inflammation, coagulation and oxidative stress before and after exposure to start-up/burn-out wood smoke and filtered air are presented in Table 6. In general, biomarkers did not increase after exposure to wood smoke, compared with filtered air. Figure 17 gives a simplified picture of the relationship between biomarkers used as outcomes in the thesis, and the biological functions they are associated with.

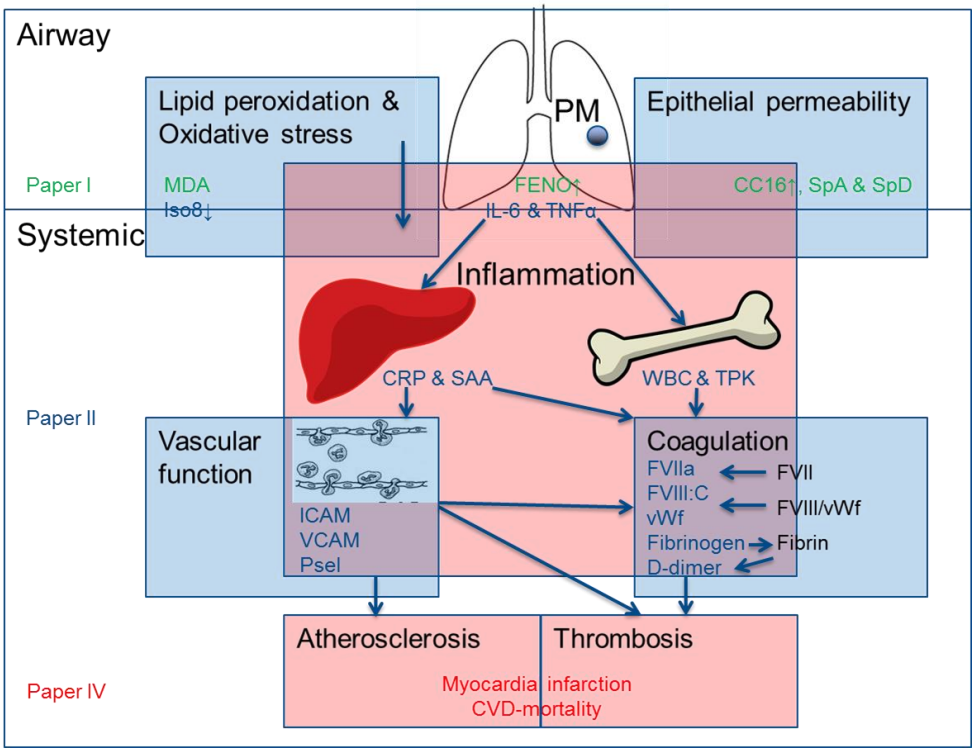

Figure 17. The outcomes used in paper 1, II and IV, and a schematic depiction of their relationships. Overlapping indicate that the biological processes are associated, for example coagulation and vascular function are affected by inflammation. Biomarkers on the border between airway and systemic effects can originate both in the airways and elsewhere.

Systemic inflammation

IL-6 and TNF-α are inflammatory cytokines that induce the production of acute phase reactants in the liver such as CRP and SAA. During inflammation these biomarkers increase in serum. So does white blood cell (WBC) count, and the soluble forms of the Cellular Adhesion Molecules (CAMs) that facilitate recruitment of WBC to the inflamed tissue. There is a

well-established connection between inflammation and cardiovascular disease, and increased serum levels of inflammatory biomarkers have been associated with cardiovascular disease and mortality (117-120).

The main biomarkers of systemic inflammation did not increase after exposure to wood smoke compared with exposure to filtered air, nor did the levels of soluble CAMs. The levels of IL-6, TNF-α, SAA, WBC, and sP-selectin did not change significantly after exposure at any sampling time. CRP was slightly lower four hours after exposure in the start-up session compared to filtered air (point estimate -5%). Soluble ICAM-1 increased only 47 hours after exposure in the burn-out session (point estimate +7%), which is not biologically plausible and was mainly due to simultaneous decrease after filtered air. Soluble VCAM-1 was significantly but marginally decreased four hours after exposure in both wood smoke sessions compared to exposure to filtered air (point estimates -2% and -5%). Decreases in sVCAM-1 or CRP would not indicate an increased risk of cardiovascular disease, but a protective effect by wood smoke exposure also seems improbable. While these changes might be true effects of wood smoke exposure, they are small and erratic and chance is also a possible explanation. Our interpretation of these results is that we did not in healthy adults find evidence of systemic inflammation after wood smoke exposure at these doses and types of wood smoke, that would have indicated an increased risk of cardiovascular diseases. The results are of course not evidence against a link between wood smoke exposure and cardiovascular disease.

Decreases in biomarkers of systemic inflammation after wood smoke exposure have been found in other studies as well for IL-6 (106, 121, 122), and CRP (122), while others have found no changes in IL-6 or other biomarkers of systemic inflammation (92). One study with relatively high exposure doses (see Table 7 below) did however see an increase in neutrophils in both blood and BAL and in IL-1β, indicating an inflammatory response (123). On the other hand, a study of people living in a reconstructed Viking house with very high exposure to wood smoke for five days did not see any increase in biomarkers of systemic inflammation, a decrease in sICAM-1 and a tendency towards a decrease in sVCAM-1 (124).

Coagulation

Inflammation is closely linked to coagulation, with many of the biomarkers increased in inflammation increasing the risk of thrombosis. Fibrinogen is an acute phase reactant that is increased in inflammatory conditions and is an important determinant of blood viscosity and platelet aggregation. Plasma levels have been associated with cardiovascular diseases (125-128). Platelets

are important in both inflammation and thrombosis and are linked to the progression of atherosclerosis (129). Circulating levels of D-dimer reflects the degradation of cross-linked fibrin, and higher levels have been associated with coronary heart disease (130). The coagulation factors VII and VIII and the von Willebrand factor are part of the coagulation cascade. Factor VIII and vWf are acute phase reactants and have been associated with inflammation and cardiovascular disease (131-133).

The effects of wood smoke on biomarkers of hemostasis were ambiguous. Fibrinogen decreased slightly but significantly in the morning after exposure in both wood smoke sessions, compared to filtered air (point estimates-4% and -5%). In the burn-out session fibrinogen was decreased also four hours after exposure (point estimate -8%, Fig 18). Platelet counts were also slightly lower four hours after exposure in the burn-out session (point estimate -6%) and in the morning after exposure in both wood smoke sessions (point estimate -6% in both). Furthermore, D-dimer showed a significant net decrease two mornings after exposure in the start-up session (point estimate -20%), but not at previous sampling times. However, in the burn-out session, FVII showed increased significantly compared to filtered air four hours after exposure, as well as 47 hours after exposure (point estimates +6% and +12%, Fig 18). In the same session, FVIII showed a non-significant tendency to increase four hours after wood smoke exposure (point estimate +14%, p=0.09, Fig 18), and was significantly increased the next morning (point estimate +37%). At the same sample time the FVIII/vWf ratio was increased (point estimate +47%). In the start-up session, FVIII also showed a tendency to increase the morning after exposure (point estimate +13%, p=0.08), but was significantly *decreased* four hours after exposure (point estimate -29%, Fig 18).

There were thus both statistically significant increases and decreases in biomarkers of coagulation after wood smoke exposure but no clear pattern. The increases in FVII and FVIII could be interpreted as a true effect indicating an increased risk of coagulation, and consequently cardiovascular disease, by wood smoke exposure. An increase in FVIII (but not FVII) was found in a previous wood smoke exposure study (121). However, the number of biomarkers of coagulation decreasing after wood smoke exposure was greater than the number increasing, making this explanation less plausible (assuming that they react in the same way to wood smoke exposure). Also, the few biomarkers of coagulation that have been measured in other chamber studies have been unaffected by wood smoke exposure (106, 123).

Another possible interpretation of the results is that the decrease in fibrinogen and platelets represents a true effect of wood smoke exposure. Though the effects were too slight to be clinically relevant, they were consistent for the same three sampling times for both biomarkers, and could possibly represent an early consumption coagulopathy as discussed in (134). However, if

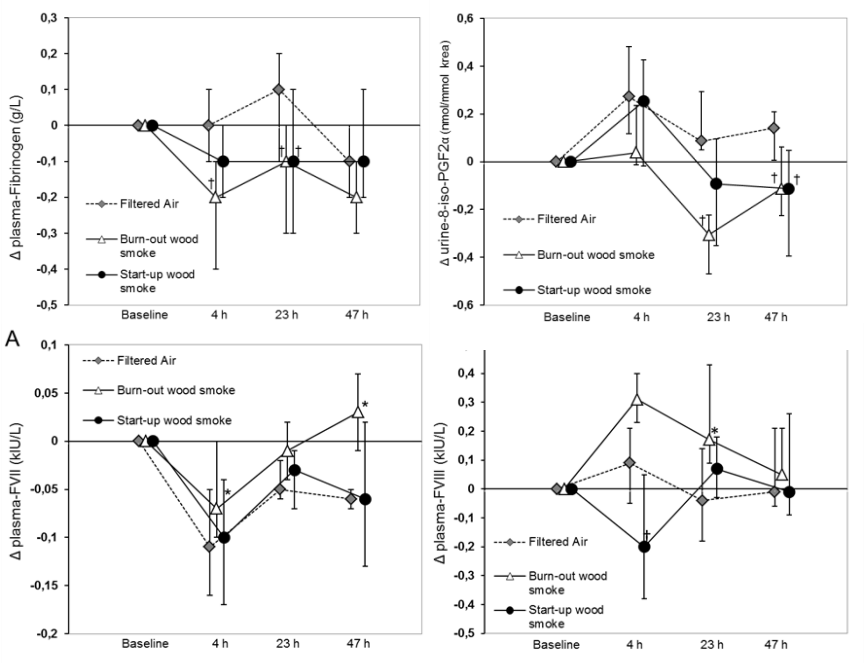

*Figure 18. Median changes (Δ) from baseline and 90% confidence intervals for biomarkers of coagulation and lipid peroxidation. *Significant net increase after wood smoke exposure; †Significant net decrease after wood smoke exposure compared to after filtered air.*

fibrinogen was consumed, an increase in D-dimer would be expected rather than the observed decrease. Furthermore, the short half-life of FVII (about 4-7 hours) argues against a true sustained increase 47 hours after exposure. Taking the changes in all biomarkers of coagulation into account, and the limited findings of other wood smoke exposure studies (106, 121, 123), our interpretations of the results in paper II is that we did not find evidence of an increased risk of thrombosis in healthy adults by these doses of short-term wood smoke exposure.

Lipid peroxidation

Malondialdehyde (MDA) is an indicator of lipid peroxidation that can be measured in EBC, and that was previously found to increase after wood

smoke exposure (89). So was 8-Iso-$PGF_{2\alpha}$, a major F_2-isoprostane in urine, considered to be a reliable marker of lipid peroxidation and oxidative stress *in vivo*. Elevated levels of F_2-isoprostanes have also been associated with cigarette smoking and cardiovascular diseases (135), and increases in markers of oxidative stress have been reported in many animal and in vitro studies of air pollution (136).

MDA showed a tendency (p=0.1) to increase four hours after exposure in the start-up session but did not change significantly at any times. However, the analysis of MDA in EBC was limited by a large fraction of the samples (26%) being below the detection limit. A main reason for this was probably the method of collecting breath condensate, the RTube, chosen for being transportable and practical in this study with a high number of participants being tested in a short time. However, the RTube collects less volume and leads to more rapid loss of heat labile substances compared with another method, ECoScreen, that can maintain lower temperatures (137). The use of the latter method was not possible in this study for practical reasons.

Urinary isoprostane 8-iso-$PGF_{2\alpha}$, adjusted for creatinine, was surprisingly decreased after exposure to wood smoke; In the burn-out wood smoke session on the first and second mornings after exposure (point estimates -82% and -71%) and in the start-up session the second morning after exposure (point estimate -53%, Fig 18). The changes were large and highly significant. This might in part be explained by an increase after exposure in the filtered air session, but not completely since there was a decrease after wood smoke even without adjustment for filtered air (significantly so in the morning after exposure in the burn-out session). Our hypothesis was an increase in 8-Iso-$PGF_{2\alpha}$, and we cannot find a plausible biological explanation for a decrease. No other wood smoke exposure study has to our knowledge measured MDA in EBC or 8-Iso-$PGF_{2\alpha}$ in urine, and the study that measured 8-Iso-$PGF_{2\alpha}$ in EBC did not see any effect of wood smoke exposure (91).

4.1.4 Circadian rhythm and correlations

In all sessions CC16 had a clear circadian rhythm with lower S-CC16 and higher U-CC16 in the afternoon than in the mornings, concurrent with previous findings (80, 138). SP-D had lower afternoon than morning levels, and was correlated with increasing age. The WBC and 8-iso-$PGF_{2\alpha}$ showed a clear circadian rhythm with higher levels in afternoons than in the mornings. At most sample times, fibrinogen was highly correlated with increasing age, sP-selectin and leukocyte counts, CRP with SAA, FVIII with vWf and D-

dimer, and FVII with platelet counts. $FENO_{50}$ and $FENO_{270}$ were highly correlated with each other (R_S=0.82 for unexposed morning samples).

Table 6. Biomarkers of systemic inflammation, coagulation and lipid peroxidation. Medians in serum, plasma and urine in the filtered air session and the two sessions of wood smoke exposure at all sample times. Adapted from paper II.

Parameter	Before	**After filtered air**			Before	**After start-up smoke**			Before	**After burnout smoke**		
Sample #	1	2	3	4	1	2	3	4	1	2	3	4
(plasma, pg/ml)	0.77	0.75	0.61	0.73	0.67	0.77	0.66	0.74	0.66	0.83	0.66	0.58
TNF-α (plasma, pg/ml)	0.99	0.75	0.97	0.90	1.01	0.94	0.96	0.99	1.00	0.88	0.95	1.04
SAA (serum, mg/L)	1.6	1.8	2.0	1.7	1.8	1.5	1.8	1.9	2.5	1.7	2.2	2.4
Leukocytes (blood, ×10^9/L)	5.6	7.1	5.6	6.2	5.6	6.6	5.6	5.6	5.9	6.6	5.6	5.7
CRP (serum, mg/L)	0.87	0.76	0.62	0.62	0.64	0.60†	0.52	0.63	0.84	0.74	0.72	0.69
sP-selectin (serum, ng/mL)	71	70	69	77	81	68	73	74	77	60	64	72
sICAM-1 (serum, ng/mL)	113	106	108	110	115	103	106	105	113	103	110	110*
sVCAM-1 (serum, ng/mL)	604	601	566	578	620	618†	600	619	651	637†	611	634
FVII (plasma, kIU/L)	1.02	0.89	0.97	0.96	1.04	0.94	0.97	0.97	0.88	0.85*	0.87	0.95*
FVIII (plasma, kIU/L)	1.1	1.2	1	1.1	1.1	1†	1.2	1.2	0.9	1.4	1.2*	1.1
vWf (plasma, kIU/L)	1.2	1.2	1	1.1	1	0.9	0.9	0.9	1.1	1.2	1	1
Fibrinogen (plasma, g/L)	2.6	2.5	2.7	2.5	2.6	2.4	2.5†	2.5	2.6	2.5†	2.7†	2.7
Platelets (blood, x10^9/L)	251	246	239	234	259	269	250†	255	282	264†	256†	267
D-dimer (plasma, mg/L)	0.15	0.15	0.15	0.16	0.20	0.17	0.18	0.15†	0.16	0.16	0.17	0.16
8-iso-$PGF_{2α}$/creatinine (urine, nmol/mmol krea)	0.47	0.82	0.57	0.59	0.67	0.82	0.46	0.50†	0.50	0.59	0.17†	0.41†

* Significant increase (two-sided p-value <0.05) compared to filtered air exposure

† Significant decrease (two-sided p-value <0.05) compared to filtered air exposure

4.1.5 Symtoms

Symptom-scores were as expected higher during exposure to wood smoke than filtered air and slightly higher in the start-up session than in the burn-out session. Irritation of the eyes increased significantly in both wood smoke sessions compared to filtered air, and irritation of the nose in the burn-out session. The most commonly reported symptoms in all sessions were (in this order) tiredness, irritation of the eyes, irritation of the nose and throat, and headache. However, subjective symptoms were weak, most participants scoring 0-2 on most symptoms and a few scoring 0 on all symptoms in all sessions. Similar findings have been reported in other experimental wood smoke exposure studies (90, 139, 140). However, surprisingly the wood smoke exposure chamber study with the highest exposure doses in terms of PM mass (see Table 7) reported no increase in symptoms after wood smoke exposure, despite increases in biomarkers (123). The type of wood and the combustion conditions may be more important for symptoms than PM mass.

Though the smell of wood smoke may be pleasurable, it thus seems like exposure levels so low that they do not cause any alarming symptoms do cause measurable effects in biomarkers in healthy adults. However, it also seems like our senses are very sensitive at detecting wood smoke air pollution, at least as sensitive as the biomarkers that have been used in chamber studies. In most cases, "If you can't smell the wood smoke, there's no *short-term* risk" therefore seems a reasonable guideline for everyday behavior and risk communication, even if it cannot on this basis be stated for certain that short-term exposure to wood smoke at lower exposure levels than we can sense (and be irritated by) do not cause relevant health effects. This is the opposite to the message concerning health effects of *long-term* exposure to ambient air pollution – where epidemiological studies have indicated that there is no safe exposure level, even at exposure so low we cannot perceive them with our senses (see discussion on paper IV).

4.1.6 Comparing the two wood smoke sessions

Since the composition of wood smoke varies with combustion conditions, health effects are also likely to vary. Therefore, one aim was to compare the effects on airways by two kinds of wood smoke. Keeping the setup, combustion equipment and participants the same to isolate the effects of just combustion conditions we used wood smoke from the start-up phase of the wood-burning cycle and the burn-out phase of the wood burning cycle. Our *a priori* theory was that the combustion would be less complete and the wood smoke would contain more toxic carbonaceous material in the session using wood smoke from the start-up phase of the wood burning cycle. We expected

the combustion in the burn-out phase to be more complete, producing smoke with more particles consisting of mainly alkali salts, and possibly less toxic.

The levels of particulate PAHs and of most VOCs were as expected higher in the start-up session than in the burn-out session, and levels of trace elements more similar, indicating that this was true. However, PM mass and numbers and the levels of UFP were substantially higher in the start-up session, limiting our possibility to make conclusion of differences in wood smoke toxicity by comparing effects on biomarkers in our subjects in the two wood smoke exposure sessions.

Of the biomarkers where we observed effects of wood smoke exposure, CC16 in serum and urine increased after wood smoke in the start-up session but not the burn-out session, while FENO increased more in the burn-out session. The differences are not distinct enough to draw certain conclusions of differences in biological effects. Symptoms were also somewhat more pronounced in the start-up session. An *in vitro* study using wood smoke particles from the same combustion equipment as the present study did not find any difference in cytotoxicity between particles from the different phases of the combustion cycle (61), indicating that the observed differences are due to particle dose rather than toxicity. The effects of wood smoke exposure on biomarkers of systemic effects were (as described in section 4.1.3 above) dubious, but if anything more pronounced in the burn-out session.

4.1.7 Comparisons with other chamber studies of wood smoke

Tables 7 and 8 summarizes the setup, the exposure, the estimated inhaled dose, and the effects on biomarkers in the chamber studies of wood smoke exposure in humans published to date. It is apparent that the studies so far are heterogeneous, and it is difficult to draw conclusions by comparing the studies since they differ in both exposure and outcomes measured. The majority of the findings are non-positive, or weak, and some negative, and the overall picture is thus less clear for wood smoke than for diesel exhaust particles. All studies were short-term exposures performed on relatively young and healthy subjects. A strength of paper I and II is that the setup and outcomes were similar to those in Barregard 2006 & 2008, allowing for comparisons.

Paper I and II in this dissertation indicate an effect of wood smoke on epithelial permeability and/or airway inflammation (S/U-CC16, FENO), but a decrease in a marker of lipid peroxidation. Our previous wood smoke

exposure studies (89, 121, 139, 141) mainly support these findings and also found indications of airway oxidative stress, inflammation/coagulation and lipid peroxidation (the *opposite* effect on 8-iso-$PGF_{2\alpha}$ compared to paper II). However, there are also non-positive studies for most biomarkers, and studies reporting effects opposite to the hypothesis (Table 7 and 8).

While one study (123) found an increase in neutrophils in both blood and BAL indicating an inflammatory response, another (92) instead reports a *decrease* in cell counts of neutrophils and other cell counts in BAL and BW. The latter did at the same time observe an increase in some leukocytes in endobronchial tissue. They also report a tendency towards a decrease in GSH in BAL, while a previous study had found an increase, and speculated that it represented an antioxidant response to wood smoke (90).

One study found indications of cardiovascular effects of wood smoke exposure with effects on HRV and arterial stiffness (142), while two other studies (using another method) found no effects on vascular function (143, 144).

Table 7. Study design, exposure and biomarkers of systemic effects for wood smoke vs filtered air in chamber studies.

Study		Barregard 2006 Sallsten 2006 Danielsen 2008		Paper II (Stockfelt 2013)		Riddervold 2011 Forchhammer 2012 Bonlokke 2014		Unosson 2013 Muala 2015	Ghio 2011	Pope 2012
	Session	1	2	Start-up	Burn-out	Low exp	High exp			
Subjects	N	13[g]		13		20		14	10	26
	Characteristics	Healthy		Healthy		Healthy atopics		Healthy	Healthy adults	Healthy
	Mean age (range)	34 (20-56)		34 (20-57)		25 (19-55)		26 (24-27)	(18-40)	(18-25)
Setup	Burner	Cast iron wood stove		Cast iron wood stove		Cast iron wood stove		Common chimney stove	Electric heating element	Standard stove
	Wood type	Birch/spruce		Birch/spruce		Beech		Birch	Red oak	Wood
	Combustion conditions	Optimal		Optimal		Optimal (aged)		High burn rate, low O_2	Smoldering	Aged smoke
Exposure	$PM_{2.5}$ ($\mu g/m^3$)	280	241	295	146	221	354	294 (PM_1)	485	180
	PM_1 # conc ($10^3/cm^3$)	180	95	140	100	29	71	100-250	46	?
	Time (hours)	4		3		3		3	2	3
	Exercise	2*25 minutes		No		No		15/15 rest/exercise	15/15 rest/exercise	Rest
	Volume of inhaled air (l)	2580[h]		1260[h]		1260[h]		3744[j]	3015[j]	1260[h]
	Inhaled dose (μg $PM_{2.5}$)	722	622	372	184	278	446	1101	1462	227
	Inhaled dose (10^6 # PM_1)	464	233	176	126	37	89	374-936	139	NR[k]
Outcome	Symptoms	Yes (weak)		Yes (weak)		Yes (weak)		NR[k]	0	NR[k]
Blood	Factor VII	0		0	↑ (?)	-		-	-	-
Blood	Factor VIII	↑		0(↓)	↑	-		-	-	-
Blood	vWf	0		0	0	0		-	0	-
Blood	Fibrinogen	0		0(↓)	↓	-		-	-	-
Blood	Platelets	0		0(↓)	↓	-		-	-	-
Blood	D-dimer,	0		0(↓)	0	-		-	0	-
Blood	IL-1β	-		-	-	0 (?)		-	↑	-
Blood	TNF-α	0		0	0	0		0	0	-
Blood	IL-6	↓		0	0	↓		0	0	-
Blood	IL-8	-		0	0	Most below LOD		-	0	-
Blood	CRP	0		0(↓)	0	-		-	-	-
Blood	SAA	↑		0	0	-		-	-	-
Blood	sICAM-1	0[i]		0	0	-		0	-	-
Blood	sVCAM-1	0[i]		0(↓)	0(↓)	-		-	-	-
Blood	sP-selectin	↑[i]		0	0	0		-	-	-
Blood	TF, E-selectin	-		-	-	0		-	-	-
Urine	8-iso-$PGF_{2\alpha}$	↑ (?)		↓	↓	-		-	-	-
Blood	WBC	0		0	0	-		-	0	-
Blood	Neutrofiles	-		-	-	-		-	↑	-

Blood	Hb, RBC, hematocrit	0	-	-	-	-	-	-
Blood	COHb, PAI-1, plasminogen,TPA, lymphocytes, monocytes	-	-	-	-	-	0	-
Blood	LDH	-	-	-	-	-	↑	-
Blood	Cytokines[b]	-	-	-	0	-	-	-
Blood	IL-4, IL-5	-	-	-	Most below LOD	-	-	-
Blood	IL-18	-	-	-	0 (?)	-	-	-
Blood	Some WBC[c]	-	-	-	-	↑	-	-
Blood	Other WBC[d]	-	-	-	-	0	-	-
Urine	8-oxodG, 8-oxoGua	0	-	-	-	-	-	-
In PBMC[a]	Strand breaks	↓	-	-	0	-	-	-
in PBMC	hOGG1 mRNA	↑	-	-	0	-	-	-
in PBMC	DNA damage etc[e]	0	-	-		-	-	-
in PBMC	DNA damage etc[f]	-	-	-	0	-	-	-
	HRV-measures	-	-	-	0	↓	0 (↑?)	-
	Arterial Stiffness	-	-	-	-	↑	-	-
	Heart rate	-	-	-	-	↑	-	-
	Blood pressure	-	-	-	-	0	-	-
	RHI (EndoPAT)	-	-	-	0	-	-	0

a) PBMC= Peripheral Blood Mononuclear Cell.
b) IL-10, IL-12, IFNγ, GM-CSF, TGFβ1, MCP1, MIP1α & RANTES.
c) CD16+, CD56+, CD4+ HLADR+ & CD8+HLADR+ cells.
d) CD3+, CD4+, CD8+ cells.
e) hOGG1 activity, HH01 mRNA, hNUDT mRNA, FPG sites.
f) Biomarkers reported were mRNA levels of IL-6, IL-8, TNF-α, CCL2, ITGAL & HMOX1, immunofluorescence analysis of ICAM-1, ITGAL and L-selectin and the DNA damage marker Endonuclease III.
g) N=7 in round 1, 6 in round 2.
h) Estimate based on an average minute ventilation of 7 liters/minute at rest and 25 liters/minute during 70 Watt bicycle exercise.
i) Unpublished data, presented in conference abstract at ISEE 2009, Stockfelt et al.
j) Estimate based on figures in that paper, a mean body surface of 1.73 m^2 and a ventilation of 7 liters at rest.
k) NR=Not reported

Table 8. Study design, exposure and biomarkers of airway effects for wood smoke vs filtered air in chamber studies.

Study		Barregard 2008		Paper I (Stockfelt 2012)		Sehlstedt 2010	Riddervold 2012 Bonlokke 2014		Muala 2015	Ghio 2011
	Session	1	2	Start-up	Burn-out		Low exp	High exp		
Subjects	N	13[i]		13		19	20		14	10
	Characteristics	Healthy		Healthy		Healthy	Healthy atopics		Healthy	Healthy
	Mean age (range)	34 (20-56)		34 (20-57)		24 (21-31)	25 (19-55)		26 (24-27)	(18-40)
Setup	Burner	Cast iron wood stove		Cast iron wood stove		Pellet burner	Cast iron wood stove		Common chimney	Electric heating element
	Wood type	Birch/spruce 50/50		Birch/spruce 50/50		Pine/spruce pellets/sawdust	Beech		Birch	Red oak
	Combustion conditions	Optimal		Optimal		Low O_2, high temp	Optimal (aged smoke)		High burn rate, low O_2	Smoldering
Exposure	$PM_{2.5}$ (µg/m^3)	280	241	295	146	224	221	354	294 (PM_1)	485
	PM_1 # co (10^3/cm^3)	180	95	140	100	67	29	71	100-250	46
	Time (hours)	4		3		3	3		3	2
	Exercise	2*25 minutes		No		15/15 min rest/exercise	No		15/15 rest/exercise	15/15 rest/exercise
	Volume of inhaled air (l)	2580[j]		1260[j]		3744[l]	1260[j]		3744[l]	3015[l]
	Inhaled dose (µg $PM_{2.5}$)	702	464	372	184	839	278	446	1101	1462
	Inhaled dose (10^6# PM_1)	464	245	176	126	251	37	89	374-936	139
Outcomes	Symptoms	Yes (weak)		Yes (weak)		Yes (weak)	Yes (weak)		NR[k]	0
Blood	SP-A, SP-D	-		0	0	-	0		-	-
Blood	S-CC16	↑		↑	0	-	0		0	-
Urine	U-CC16	↑		↑	0	-	0		-	-
Breath	$FENO_{270}$	↑		(↑?)	(↑?)	0	0		-	-
Breath	$FENO_{50}$	0		0	↑	0	0		0	-
Breath	$FENO_{10}$	NR[k]		NR[k]	NR[k]	-	-		0	-
EBC	8-iso-$PGF_{2\alpha}$	-		-		-	0		-	-
EBC	MDA	↑		0	0	-	-		-	-
EBC	Conductivity & pH	-		-		-	↑		-	-
	Nasal patency	-		-		-	0		-	-
NAL[a]	Inflammation[b]	-		-		-	0 (?)		-	-
	FVC, FEV1	-		-		0	0		0	0
	PEF	-		-		-	0		-	-
	DLCO	-		-		-	-		-	0

BAL[a]	GSH	-	-	↑	-	0 (↓?)	-
BW[a]	GSH	-	-	0	-	-	-
BAL[a]	GsX	-	-	-	-	↑	-
BAL[a]	GSSG	-	-	-	-	0	-
BW[a]	Neutrophils	-	-	-	-	↓	↑
BAL[a]	Neutrophils	-	-	-	-	-	↑
BW[a]	Macrophages, lymphocytes	-	-	-	-	↓	-
BAL[a]	Cytokines & proteins[c]	-	-	-	-	-	0
BAL[a]	IL-6, ICAM-1, CC16, GrzA, MPO, MMP9	-	-	-	-	0	-
BAL[a]	Airway leukocytes[d]	-	-	-	-	↓	-
BW[a]	ICAM-1, MPO, MMP-9	-	-	-	-	↓	-
BW[a]	LDH	-	-	-	-	↓	-
BW[a]	HMBGP1	-	-	-	-	0	-
BW[a]	Caspase 3	-	-	-	-	Below LOD	-
BW & BAL[a]	Cell counts, oxidative	-	-	0	-	-	-
EB[a] biopsies	Some cell counts[f]	-	-	-	-	↑	-
EB[a] biopsies	Other cell counts[g]	-	-	-	-	0	-
EB[a] biopsies	Antioxidants[h]	-	-	0	-	-	-

a) NAL=Nasal Lavage, BW=Bronchial Wash, BAL=Bronchial Lavage, EB=Endobronchial

b) Biomarkers below LOD were: IL-4, IL-5, IL-10, GM-CSF, IFN-γ, TGFβ-1, MCP-1, MIP-1α and RANTES. Some suggestions of effects but no significant changes for IL-6, IL-1β, IL-8, IL-12, IL-18.

c) IL-1β, IL-6, IL-8, TNF-α, α1-antitrypsin, protein concentration & albumin

d) Total lymphocytes, CD3+, CD4+, CD8+, CD4+ HLADR+, CD8+ CD314+, CD4+ CD25+ cells.

e) Differential cell counts (total cells, macrophages, neutrophils, lymphocytes, eosinophils, mast cells), MPO, MMP-9. Glutathione disulphide, ascorbic acid, dehydroascorbate, urate, %GSSG, total vitamin C, oxidized vitamin C, total protein, albumin.

f) Submucosal and epithelial CD3+, epithelial CD8+, submucosal mast cells

g) Submucosal and epithelial neutrophils, epithelial mast cells, submucosal and epithelial CD4+ T cells

h) Reduced GSH, % GSSG, Ascorbate, %DHA, Urate, HO-1, total protein, glutathione S-transferase

i) 7 in session 1, 6 in session 2.

j) Estimate based on an average minute ventilation of 7 liters/minute at rest and 25 liters/minute during 70 Watt bicycle exercise.

k) NR=Not reported.

4.1.8 Issues of power & multiple comparisons

It is important that negative and non-positive results are published to provide a complete picture and avoid publication bias. However, in a study with mainly non-positive results especially, like paper II, one must consider whether the study design and sample size was of sufficient size to find an effect. Using the same statistical test, the minimal differences that can be detected with a certain power depend on the variance and thus differ for each biomarker. Crude power calculations for a subset of the biomarkers of systemic effects, show that effect sizes of around 8-15% of the pre-exposure values could be detected with 80% power using the t-test. Many of the biomarkers used in this study change drastically in pathological conditions, some such as CRP or SAA by an order of magnitudes, so we consider the power sufficient to detect any relevant effects. The fact that the biomarkers of systemic effects we examined as often as not changed in the direction opposite to the hypothesis also argues against there being a true effect of this exposure. Below are some specific examples of the minimal differences in changes after wood smoke exposure compared with after exposure to filtered air, that could be detected in the current study using a t-test (calculated with PROC POWER in SAS, a two-tailed alpha-level of 0.05, n=13, a power level of 0.8, using the standard deviations of the differences in changes between the wood smoke sessions and the filtered air session):

- For the coagulation factor VII: 0,0778 kIU/L (corresponding to a difference of 8% of mean pre-exposure values)
- For platelet counts: 20,8*10^9/L (corresponding to a difference of 8% of mean pre-exposure values)
- For sP-selectin: 8,5 ng/mL (corresponding to a difference of 11% of mean pre-exposure values)
- For WBC counts: 0,88*10^9/L (corresponding to a difference of 15% of mean pre-exposure values)

When multiple comparisons are made there is a risk for false positive results. We do not consider a strict statistical correction suitable, since it would instead greatly increase the risk of false negatives. It would then be difficult to find significant effects without increasing the exposure to unethically high levels or requiring very large study populations. Limiting the number of outcomes to a very small number would also limit the potential of the study to explore possible mechanisms. It would then cost far more to perform the multiple trials necessary to investigate a comparable number of possible effects. Instead we use the standard value for statistical significance (two-sides p-values <0.05), judge the biological plausibility of statistically

significant effects, make comparisons with the findings of other similar studies, and remain cautious in interpretation until a finding has been verified in several studies. As discussed in section 4.1.7, few findings have been consistently reproduced in chamber studies of wood smoke exposure.

4.1.9 Strengths and limitations of Paper I & II

One weakness of paper I and II is that the exposure due to time limitations was not randomized, making it difficult to exclude carry-over effects between the sessions. This seems biologically unlikely however since the half-lives for most of the biomarkers used are short (hours) and the few positive effects observed were not very strong. Fibrinogen does have a half-live close to a week, but fibrinogen did not increase after exposure. Furthermore, we do not see any differences in pre-exposure morning values for the different sessions either. A formal test (one-way ANOVA with PROC GLM for normally distributed biomarkers and Kruskal-Wallis with PROC NPAR1WAY for not normally distributed biomarkers) did not show any significant differences in pre exposure values between the sessions for any of the biomarkers. The exposure was not completely blinded either. We did not inform the participants of the exposure in each session, but the smell of wood smoke is obvious and difficult to mask. Furthermore, all subjects were relatively young and healthy. The effects of wood smoke exposure might be different in those with pre-existing conditions such as elderly individuals with atherosclerosis or atopic asthmatics.

A strength is that circadian or other reasons for variations in biomarkers are controlled for by using each participant as its own control, and taking samples at the same time in each session. We were strict on excluding the participants with signs of infections emerging before the sessions.

4.2 Paper III and IV

4.2.1 Spatial exposure contrast

The spatial distribution of NO_x levels within the city of Gothenburg in the beginning of the study period (1975) is shown in Fig 19. Exposure levels were highest close to major roads and in the central city, and lower in the suburbs, especially in the southwestern part of the city. A large exposure contrast between participants is valuable in order to detect and quantify differences in health outcomes in epidemiological studies. In this cohort, the exposure contrast (the mean of the highest exposure quartile divided by the lowest) was 3-4 for most of the study period and slightly lower in end. Since

exposure levels decreased during the study period (see 4.2.2) the absolute exposure contrast was larger at the beginning of the period (50–60 µg/m^3) than at the end (20–30 µg/m^3). The 95th to 5th percentile contrast in our study was on average 4.9, and decreased somewhat in the end of the study period. This is a relatively large within-city contrast.

4.2.2 Temporal exposure contrast

The yearly mean levels of NO_x decreased during the study period. The median levels at participants' homes was 38 µg/m³ in 1975, increased to 44 µg/m^3 in 1983, and then decreased over time to 28 µg/m³ in 1993, 21 µg/m³ in 2003 and to 17µg/m^3 in 2007. The decrease is illustrated in figure 20. While NO_x levels at most participants' homes were moderate, yearly mean levels between 50 and 100 µg/m^3 were not uncommon in the 1970s and 80s. From year 2000 almost all participants had NO_x levels < 50 µg/m^3.

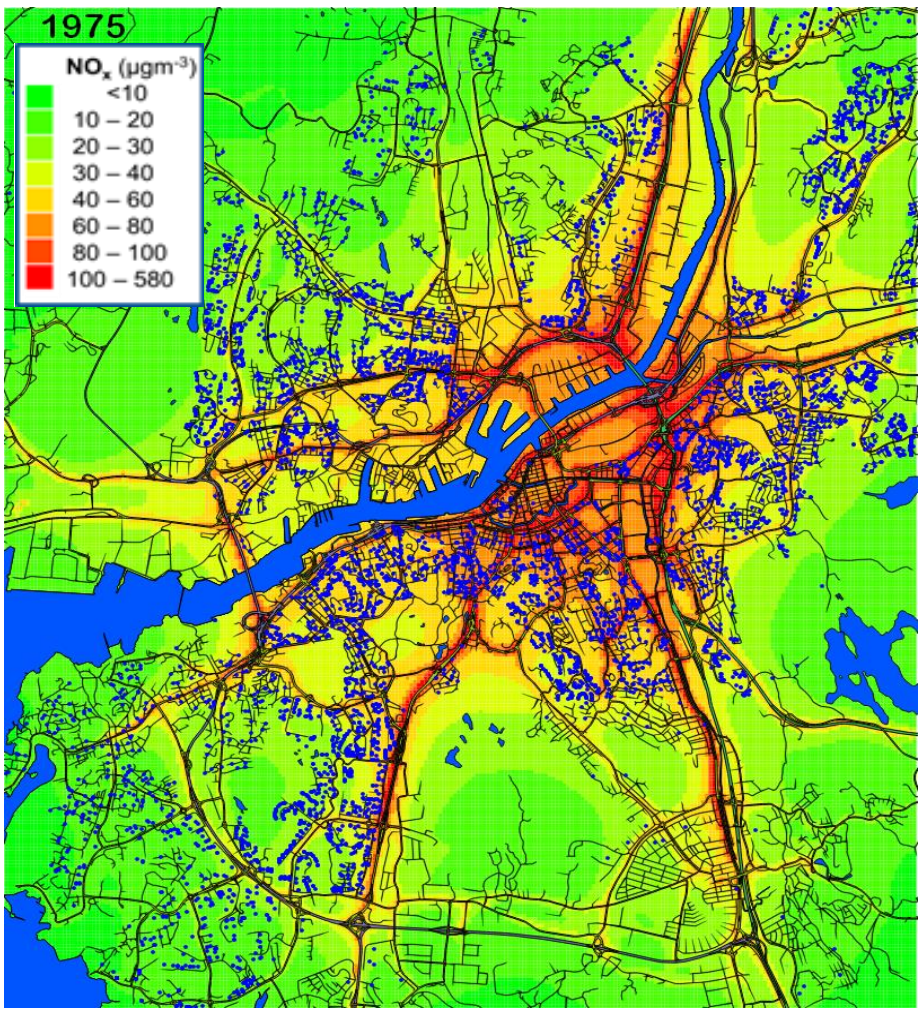

Figure 19. Modeled NOx values in Gothenburg in 1975. Each blue dot is the address of one participant. From paper III.

All major NO_x emission sources, as well as the rural background levels, decreased over time. The largest decrease by far in both absolute and relative terms was emissions from road traffic. Even though total traffic within the city increased during the study period, this was overshadowed by stronger decreases in vehicle emissions especially for passenger cars (see Table 1, paper III). In addition to technical improvements reducing emissions, such as catalysts, particulate filters, and cleaner fuels, traffic planning in Gothenburg during this time period has been aimed at redirecting traffic from the city center and housing areas to main arterial links through and around the city.

The changes in source emissions over time and median exposures among the participants were highly correlated with traffic and industry emissions, and more weakly with shipping and heating emissions. The long-range contribution of NO_2 to the participants' NO_x levels was on average about 20%, and decreased from around 10 µg/m^3 in 1982 to around 5 µg/m^3 in 2007.

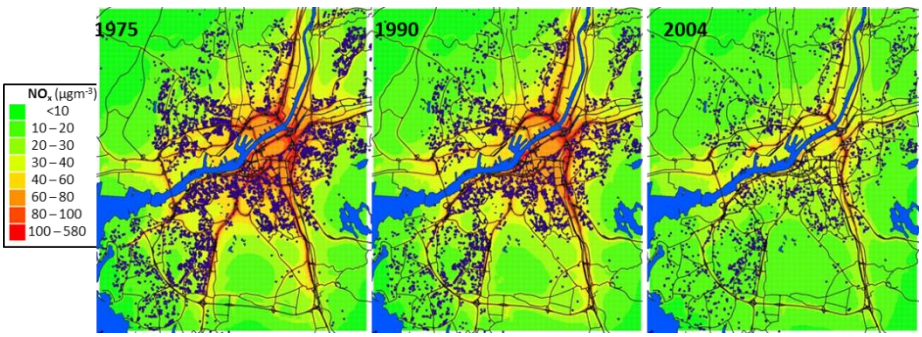

Figure 20. Modeled NO_x for three years and locations of the participants' addresses (blue dots), illustrating the declining NO_x levels and the diminishing population in the cohort (1975 N=6283, 1990 N=4133, and 2004 N=1710). From paper III.

4.2.3 Representability of the cohort's exposure

The levels at the central monitoring station were always higher than the median residential levels, while the median NO_x levels for the whole modeled area were somewhat lower than for the residential addresses. This reflects the fact that NO_x levels and population density are higher at central locations with more road traffic. For some years during the study period we had access to the residential coordinates for the whole population of Gothenburg, and could compare that exposure to the exposure in the cohort. For the years 1975, 1990 and 2004 the median levels in the whole population were 38 µg/m^3, 35 µg/m^3, and 22 µg/m^3, respectively, and compared to 38 µg/m^3, 33 µg/m^3, and 21 µg/m^3 in the cohort. The exposure in the cohort is thus considered representative for the whole population in the area. The time

trends were similar for the whole area, the cohort, and the central monitoring station. The yearly modeled concentration for the location of the monitoring station agreed well with the continuous measurements (see Paper III).

4.2.4 Time windows of NO_x exposure

Figure 21 shows the distribution of NO_x exposure in the cohort for the three different exposure time windows – the last year, the mean of the last 5 years, and the mean since enrolment. Most participants' residential NO_x exposures were between 15 and 50 µg/m³ for all three exposure windows. Exposure levels were higher for the mean exposure since enrolment compared to the 5-year mean exposure or to the last year exposure. This is because the mean since enrolment almost always included the years in the beginning of the study period when exposure levels were highest, and the mean since enrolment thus decreased slower over time than the shorter time windows.

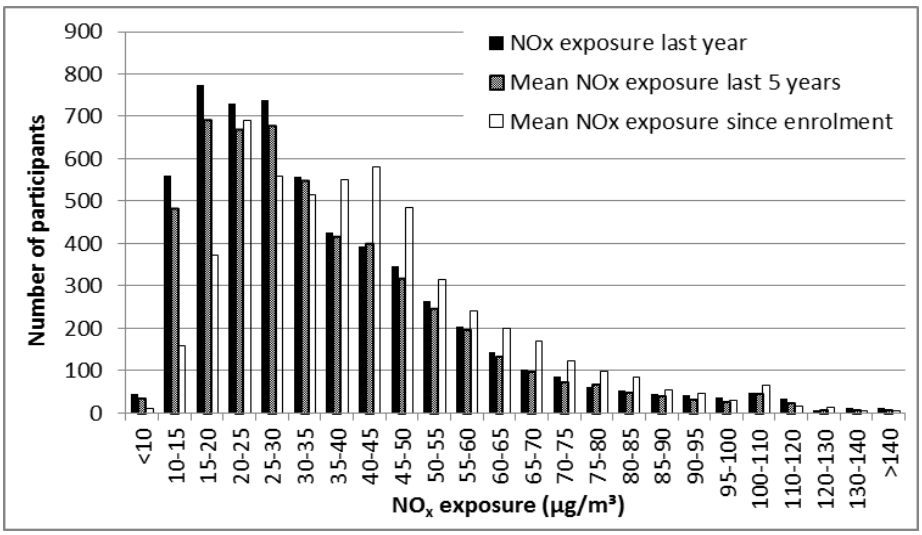

Figure 21. Distribution of NOx exposure in the cohort, as the number of participants in each interval of NOx exposure, for the three time windows. From Paper IV.

4.2.5 Model accuracy and spatial resolution

When an emission database of good quality is available, dispersion models perform well and are considered as good as or better than other commonly used methods (145). Dispersion models have an advantage over other models such as LUR models, when historical emission databases are available, because it is possible to estimate historical pollution levels with equally good accuracy over time (if the data is of equally good quality). Good historical modelling is vital for long-term epidemiological studies like paper IV, since

exposure misclassification decreases the power to discern health effects of relatively weak (on an individual level) risk factors such as air pollution. However, the spatial resolution in the dispersion model was relatively high (50-meter squares) and the coordinate for each individual entranceway used, making the precision high.

Exposure misclassification is also caused by using residential outdoor exposure as an approximation for personal exposure, but personal exposure is not possible to determine in this type of study. We lack information on how much time the participants spend at home, indoor air exposure and occupational exposure. However, indoor infiltration rates and the correlations between outdoor and indoor exposure are relatively high in Gothenburg (around 0.7) (146).

4.2.6 Relocation patterns

Relocations can also induce exposure misclassification if the participants' addresses are not followed during the study period, since the exposure will change when a person moves to a new area. Many large studies of air pollution and mortality have for practical purposes extrapolated the exposure assessment from one or a few years to a longer study period (18, 21, 32).

Around half of the participants remained at the same address during the study period, 30% relocated once, 12% twice, and 7% three times or more. 4% of the participants relocated each year on average. Almost 4/5 of all moves were within the modeled area, 1/10 moved out of the area, and 1/20 moved back into the area. Some relocations were to an area with much lower or higher exposure (24% a decrease of >10 $\mu g/m^3$, 23% an increase of >10 $\mu g/m^3$), while other relocations only changed the exposure marginally. For the whole study period relocations did not systematically change exposure over time (mean NO_x change 0.36 $\mu g/m^3$). Among the oldest participants (70-92 years old) and in the time period 1984-1993, more relocations were into areas with higher exposures.

4.2.7 Exposure extrapolation

Another issue is how far exposure from a dispersion model can be extrapolated, especially when relocations are taken into account. For this cohort and study period, back extrapolation (using a dispersion model for a recent year, adjusting for long-term trends at an urban background station) generally worked reasonably well when going back 5 to 8 years. When extrapolating further (12 to 14 years) the scattering increased, suggesting that the historical time trends at the urban background station were not valid for

all areas within our modeled area. For example, extrapolating from the dispersion model for 2009 worked well for the year 2004 (R^2=0.98, compared to using the modeled levels for 2004), but less well for 1997 when scattering increased (R^2=0.69) and he exposure was underestimated. Further analysis of backwards and forwards analysis from the different modeled years (5-8 years apart) showed varying degrees of over/underestimation (see table 9 and 10).

Table 9. R^2 for the extrapolation models compared to the base model years (forcing the intercept through the origin).

	Modelled year					
Extrapolation year	1975	1983	1990	1997	2004	2009
1975		0.93	0.90	0.80	0.57	0.60
1983	0.93		0.98	0.90	0.72	0.75
1990	0.90	0.98		0.91	0.79	0.81
1997	0.75	0.86	0.88		0.62	0.69
2004	0.71	0.83	0.87	0.82		0.98

Table 10. Slope of the linear fit between the extrapolation models compared to the base model years (forcing the intercept through the origin).

	Modelled year					
Extrapolation year	1975	1983	1990	1997	2004	2009
1975		1.28	1.24	1.45	1.19	0.98
1983	0.77		0.97	1.14	0.94	0.77
1990	0.79	1.03		1.18	0.97	0.79
1997	0.65	0.85	0.83		0.80	0.66
2004	0.78	1.02	1.00	1.18		0.82

While scattering generally increased when the modeled years were further apart, the slope did not, suggesting that factors specific to a certain year were more important. Comparisons with other studies investigating extrapolation shows that it varies between areas and time periods (see paper III).

Table 9 and 10 above are based on the assumption that the correct historical addresses and all relocations during the study period are known. If we had not known the participants' relocations during the study period the scattering would have increased over time. As an example, we compare the modelled year 2009, extrapolated backwards to the years 1997, 1990, and 1975 using the correct historical addresses, compared with a hypothetical scenario where we only had address information from the year 2004 and no knowledge of relocations (figure 22). Not knowing the correct historical address increased the scattering somewhat for 1997, more for 1990, and radically for 1975.

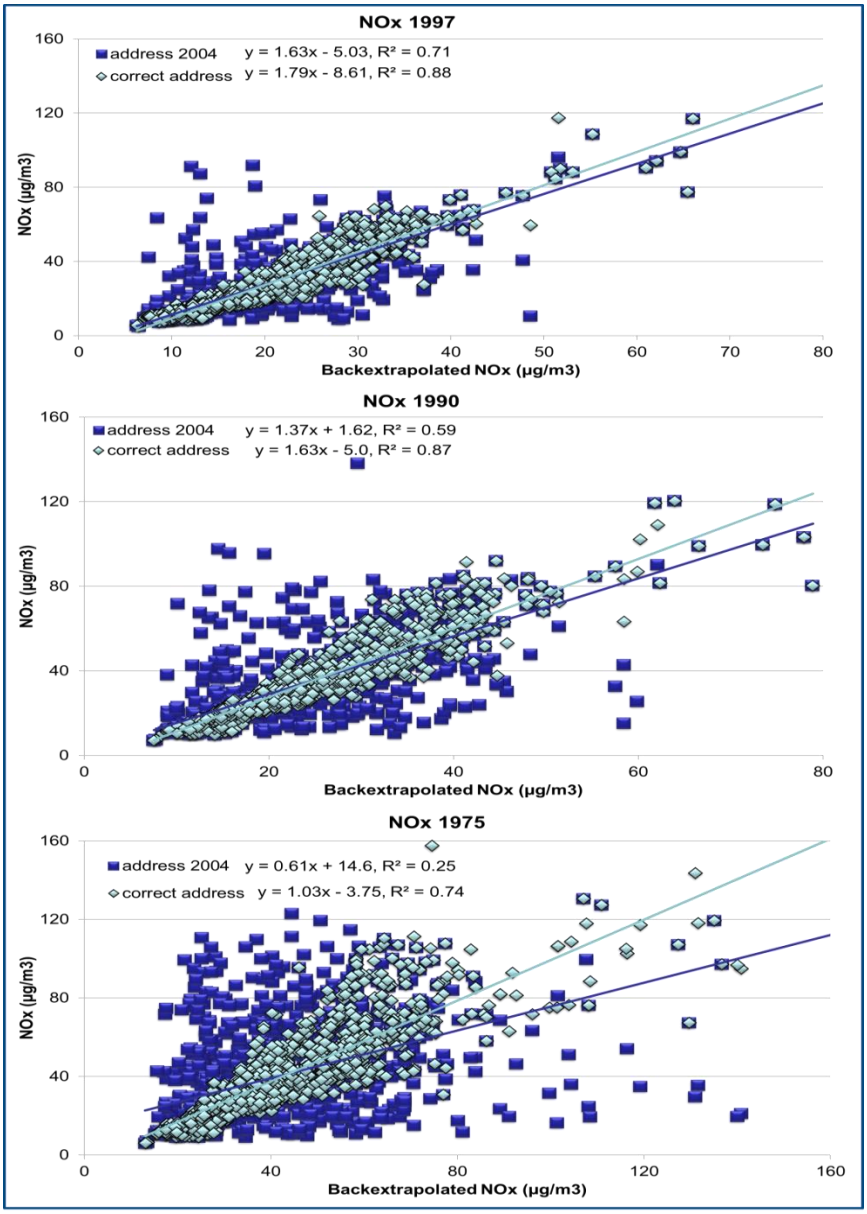

Figure 22. Comparison between estimated NO_x levels modelled 2009 and extrapolated backwards to the years 1997, 1990 and 1975, using either the correct historical addresses (green squares) or using the addresses for 2004 as if we used no information about the participants' relocations (blue squares). From Molnár et al, EAC 2015.

4.2.8 NO_x exposure and covariate distribution

The distribution of covariates in those who had a valid NO_x value at study start was similar to that of the whole cohort (Table 2, section 3.2.2). Table 11 shows the distribution of the covariates included in the main confounder model (Model 2), divided into the four quartiles of baseline NO_x exposure. All other covariates were fairly evenly distributed across the different strata of NO_x exposure. Non-smokers (never-smokers and ex-smokers at screening 1) were more frequent in the lowest NO_x exposure quartile, and heavy smokers were more frequent in the highest NO_x exposure quartile. There were some differences regarding occupational class, with a tendency for high rank white collar workers to be more frequent in both the highest and the lowest NO_x quartiles. This is probably because the areas with higher socioeconomic status and more expensive homes are located both in the central parts of Gothenburg (with high NO_x exposure), and in the affluent suburbs (with the lowest NO_x exposures).

Unfortunately, we did not have historic area-level socioeconomic variables at the time of the study, and there have been some changes in socioeconomic areas during the study period. The main residential patterns are similar however, so we can get indirect confirmation by using current socioeconomic geographical data. In Figure 23 the distribution of mean household income in Gothenburg 2012 is shown. Participants in the highest income areas in the affluent suburbs mainly in the southwestern parts of Gothenburg were to a large extent exposed to low levels of NO_x exposure. However, in central Gothenburg both the income and the NO_x exposure were relatively high. As expected, there were more participants with high-rank white collar occupations and fewer participants in manual labor, and fewer smokers, in the areas with the higher socioeconomic statuses (based on mean household incomes in 2012, participants' addresses in 1990, and covariates at baseline. Data not shown).

Table 11. The distribution of possible confounders by NO_x quartiles.

		All	NO_x Q1	NO_x Q2	NO_x Q3	NO_x Q4
NO_x, mean	µg/m³	42	20	31	44	72
Age, mean	Years	53	53	53	54	54
Smoking cat, %	Never-smokers	25	28	24	25	23
	Ex-smokers scr 1	22	24	21	22	21
	Quit before scr 2	10	8.9	11	9.0	10
	Light smokers	28	25	28	29	28
	Heavy smokers	16	14	16	15	18
Occ class, %	High rank white collar	11	12	9.4	8.6	14
	Mid rank white collar	17	20	17	14	17
	Low rank white collar	19	18	20	19	18
	Skilled manual labor	26	24	26	29	24
	Unskilled man labor	23	20	23	25	22
	Disability pension etc	5.0	5.9	4.0	5.5	4.8

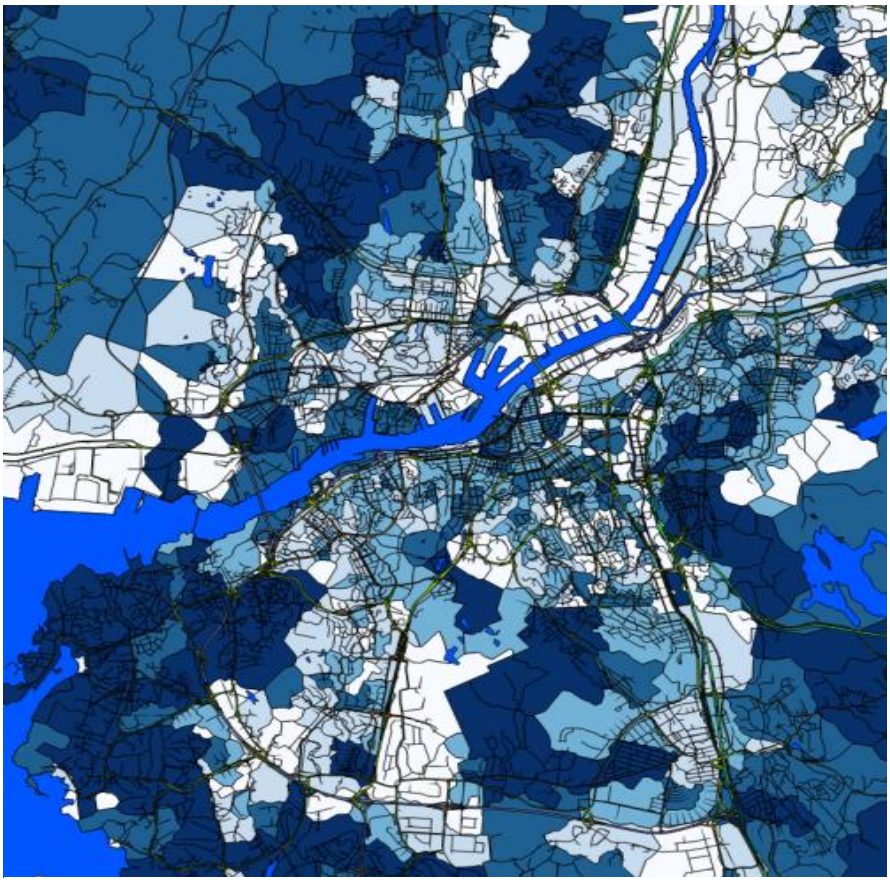

Figure 23. A map of Gothenburg showing the mean household income in each area 2012, divided into quintiles. Darker blue denotes higher incomes in the area.

4.2.9 NO_x exposure and total mortality

The risk of total non-accidental mortality was associated with NO_x exposure at participants' residential addresses (Table 12). The results did not change substantially between the crude model (Model 1), the main confounder model (Model 2), and the model adjusting for all covariates (Model 3). The effect estimate was in the expected size range; Our estimates of a 3% (95% CI for HR 1.01–1.05) increase in non-accidental mortality per 10 µg/m³ NO_x for last year's exposure is comparable with the pooled effect estimates of 5.5% (95% CI for 1.03–1.08) (35) and 4% (95% CI 1.02–1.06) (43) per 10 µg/m³ of NO_2 in two recent reviews, and the 3% (95% CI 1.00–1.05) per 20 µg/m³ NO_x in the ESCAPE study (in the model most comparable to Model 3 in the present study) (32).

Table 12. Hazard ratios (with 95% confidence intervals) per 10 µg NO_x /m³ for selected causes of mortality and three NO_x exposure metrics: The last year, the mean of the last 5 years, and the mean since enrolment. The number of events and total person-years for each model are also shown. From Paper IV.

				Mortality outcomes					
Time-window	Model	Person–years		Total	Cardiovascular	IHD	MI	Cerebrovascular	Respiratory
Last year	1	146,611	HR (95% CI)	**1.03 (1.01–1.05)**	1.02 (0.99–1.04)	1.02 (0.99–1.05)	1.03 (0.99–1.07)	1.01 (0.94–1.08)	1.02 (0.96–1.10)
			events	4,055	1,844	1,174	701	323	277
	2	146,419	HR (95% CI)	**1.03 (1.01–1.05)**	1.02 (0.99–1.04)	1.02 (0.99–1.05)	1.03 (0.99–1.07)	1.02 (0.95–1.09)	1.01 (0.94–1.09)
			events	4,047	1,840	1,171	700	322	276
	3	135,107	HR (95% CI)	**1.02 (1.01–1.04)**	1.02 (0.99–1.05)	1.02 (0.99–1.05)	1.04 (1.00–1.08)	1.01 (0.94–1.09)	1.01 (0.93–1.09)
			events	3,694	1,671	1,064	642	296	252
Last 5 years	1	126,831	HR (95% CI)	**1.03 (1.00–1.04)**	1.02 (0.99–1.04)	1.02 (0.99–1.05)	1.03 (0.99–1.07)	1.00 (0.94–1.07)	1.01 (0.92–1.08)
			events	3,974	1,819	1,152	694	321	277
	2	126,669	HR (95% CI)	**1.02 (1.01–1.04)**	1.01 (0.99–1.04)	1.02 (0.99–1.05)	1.03 (0.99–1.07)	1.01 (0.94–1.08)	1.00 (0.93–1.07)
			events	3,967	1,815	1,149	693	320	277
	3	116,980	HR (95% CI)	**1.02 (1.00–1.04)**	1.01 (1.00–1.04)	1.02 (0.99–1.05)	1.04 (1.00–1.08)	1.00 (0.94–1.08)	0.99 (0.92–1.07)
			events	3,619	1,649	1,045	635	294	254
Since enrolment	1	144,962	HR (95% CI)	**1.03 (1.01–1.05)**	**1.03 (1.00–1.05)**	1.03 (1.00–1.05)	1.03 (0.99–1.07)	1.03 (0.97–1.08)	1.02 (0.96–1.08)
			events	3,963	1,821	1,160	696	323	269
	2	144,766	HR (95% CI)	**1.02 (1.01–1.04)**	1.02 (1.00–1.04)	1.02 (0.99–1.05)	1.03 (0.99–1.06)	1.03 (0.97–1.09)	1.00 (0.94–1.07)
			events	3,956	1,818	1,157	695	322	268
	3	133,660	HR (95% CI)	**1.02 (1.01–1.04)**	1.02 (1.00–1.05)	1.02 (0.99–1.05)	1.03 (0.99–1.07)	1.03 (0.97–1.09)	1.00 (0.93–1.06)
			events	3,613	1,658	1,057	639	295	248

Model 1 includes only calendar year.

Model 2 includes calendar year, occupational class and smoking.

Model 3 includes all available potential confounders and risk factors.
Hazard ratios in **bold** represent p-values <0.05.

4.2.10 NO_x exposure and cause-specific outcomes

The effect estimates for cardiovascular mortality were similar or slightly lower compared to those for total mortality, but confidence intervals wider and usually including no effect (Table 12). Some other studies using NO_2 report stronger effects for cardiovascular compared to all-cause mortality, whereas others report weaker effects, as reviewed by Hoek et al (2013), and some report essentially null results (23, 32, 35). It is surprising that the effect estimates are not stronger for cardiovascular mortality than for all-cause mortality, since the main suggested mechanisms for increased mortality due to long-term air pollution exposure are through cardiovascular and respiratory diseases. Also, associations between air pollution and cardiopulmonary (and lung cancer) mortality, but not mortality from other causes, have been found in the largest cohort studies of air pollution (18).

Effect estimates for mortality due to IHD and acute MI were also similar to those for all-cause mortality, in some models stronger and some weaker but always with overlapping confidence intervals. The effect of residential NO_x exposure on mortality from cerebrovascular and respiratory diseases was weak in almost all models, but the number of events was so low that the uncertainty increased substantially.

In contrast with the (non-significant) positive associations between NO_x exposure and MI mortality, the hazard ratios for the effects of NO_x exposure on incident MI were close to null in all models for all three exposure windows (Table 5, Paper IV). Similarly weaker (or null) effects of air pollution on non-fatal compared to fatal cardiac events have been found previously (147-152). It has been suggested that air pollution affects the severity of the response rather than initiating it, or that mechanisms related to acute effects, such as arrhythmia, may be of importance (148).

4.2.11 The concentration-response relationship

Smooth functions for concentration–response showed that the relationship between NO_x exposure in the last year and non-accidental and cardiovascular

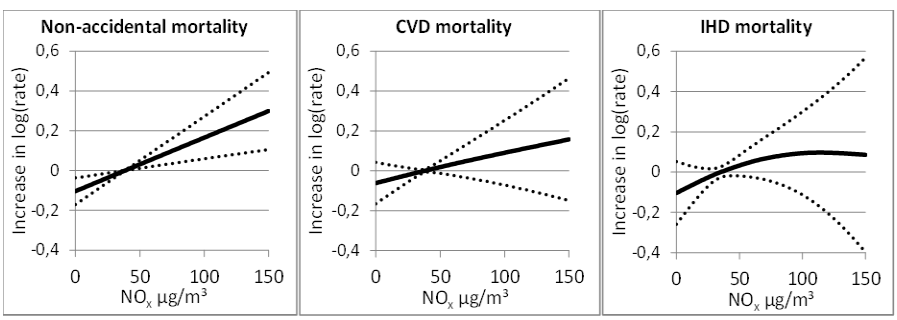

Figure 24. Smooth functions (with 95% confidence intervals) describing the impact of NO_x exposure during the last year on selected mortality outcomes. The curves represent the log(rate mortality), estimated using confounder Model 2. From paper IV.

mortality was almost linear. For IHD the effects per µg/m³ tended to be weaker at exposures >100 µg/m³ (Fig 24). The number of observations was low at high exposure levels however, increasing uncertainty. Similar results have been found in recent, large scale studies of ambient air pollution (24, 32). No signs of deviation from linearity were seen in the lowest range of exposures, where air pollution levels were lower than in most other studies and well below air quality standards. Improvements in air quality should therefore yield reductions in mortality even at low exposure levels.

4.2.12 Effects of different exposure time windows

Effect sizes for NO_x were similar for the three exposure time windows. For our main confounder model and total mortality, the HR per 10 µg/m³ NO_x was 1.03 (95% CI 1.01–1.05) for exposure in the last year; 1.02 (95% CI 1.01–1.04) for the 5-year mean exposure, and 1.02 (95% CI 1.01–1.04) for the mean NO_x exposure since enrolment (Table 12). The high correlation between shorter and longer term exposures makes separation of the time windows difficult, however.

Effects of the last year's, or the last few years', air pollution exposure have also been found in other, recent studies (31, 153, 154), and reductions in air pollution levels have been shown to decrease cardiovascular event rates as early as within months or a few years (15). This points towards comparatively rapid effects of air pollution on the cardiovascular and respiratory systems, such as the instigation of plaque instability, decreased endothelial function, inflammation, increased thrombotic potential, and increased risk of pneumonia, rather than slower processes such as the long-term progression of atherosclerosis over decades. However, a comparison of studies using different time scales of exposure (155) found similar, but slightly stronger, effects for 10 year exposures compared to 1 year exposures, indicating that chronic exposure is also important.

As discussed in section 4.2.6, several other large studies have not been able to follow participants' addresses as they relocate during the study period, which may have caused exposure misclassification over time. In a sensitivity analysis of the effect of NO_x exposure on mortality, using only the baseline exposure in 1973 (as if we had not been able to follow the participants), we found a slightly weaker association than for the analyses using the three time windows of exposure. The association was not much weaker though, and still significant for total non-accidental mortality. NO_x exposure in 1973 was highly correlated with the exposure of the other time windows.

4.2.13 Effect modification by age and time period

The effect estimates for NO_x were similar in all calendar periods except for 1983-92 when the association between NO_x exposure and mortality was weaker (Table 14). The effects were near linear for almost all outcomes and time-windows. For IHD and total mortality in 1972-1983 the effects per µg/m³ tended to be weaker at high exposures (>100 µg/m³). The results were not sensitive to the choice of degrees of freedom for the spline, nor to whether a smooth function or a third degree polynomial was used for calendar year.

When stratified on age or birth year, the effect estimates were strongest among participants below 60 years of age, though this is based on relatively few events (Table 13). The association between NO_x exposure and non-accidental mortality was also stronger among individuals born in the years 1921–1925 compared to those born in 1915–1920.

Table 13. Hazard-ratios (95% CI) per 10 µg/m³ NO_x, events and person years for non-accidental mortality by exposure to NO_x in selected time-periods. Calendar year, and smoking and occupational classes included in the model.

Time window		1973-82	1983-92	1993-2002	2003-2007
Last year	HR (95% CI)	**1.05 (1.02-1.09)**	1.00 (0.97-1.03)	**1.04 (1.01-1.07)**	1.04 (0.96-1.12)
	Events	497	1038	1659	853
	Person-years	60900	46527	30478	8514
Last	HR (95% CI)	**1.05 (1.01-1.09)**	1.00 (0.97-1.03)	**1.03 (1.00-1.06)**	1.03 (0.97-1.10)
5 years	Events	423	1049	1655	851
	Person-years	41375	46607	30222	8465
Since	HR (95% CI)	**1.06 (1.02-1.10)**	1.00 (0.97-1.03)	1.02 (1.00-1.05)	1.04 (1.00-1.08)
enrolment	Events	495	1040	1629	811
	Person-years	60532	46321	29786	8127

Since the participants were recruited only during 1915-25 it is difficult to discern whether the differences in the associations between NO_x and mortality are related to age, or something specific to the calendar period. For example, the participants were aged 60-70 years in the middle of the 1980s, when NO_x exposure levels were highest (see 4.2.2), and the association between residential NO_x and mortality was weakest, while they were youngest in the 1970s. We do not have a good biological hypothesis to explain why participants <60 years old would be more sensitive to air pollution. Possibly the composition of air pollution was different in the beginning of the study period, with a higher proportion of other harmful air

pollution components compared with NO_x. Differences in exposure misclassification due to changes in behavior as the participants age and retire, for example less outdoor physical activity, is also a possibility.

Table 14. Hazard ratios per 10 µg NO_x /m^3 (95% CI) in age groups and birth cohorts, for last year NOx exposure and total mortality. Smoking and occupational class and calendar year included in the model.

	HR (95%CI)	Events	Person-years
All	**1.03 (1.01-1.05)**	4047	146419
Born 1915-1919	1.02 (1.00-1.04)	2245	65451
born 1920-1925	**1.04 (1.01-1.07)**	1802	80968
Age <60	**1.09 (1.04-1.14)**	235	42053
Age 60-69	1.00 (0.97-1.03)	864	51855
Age 70-79	**1.04 (1.01-1.07)**	1428	36704
Age 80-92	1.02 (0.98-1.07)	1520	15807

4.2.14 On confounding and selection of covariates

As described in method section 3.5.3, covariates in the regression models were selected first through a priori theoretical assumptions about the relationships between covariates, NO_x exposure, and outcomes. These assumptions were then tested and supplemented by examining the associations between the covariates, NO_x exposure at baseline and cardiovascular mortality, and whether inclusion/exclusion of the covariates affected the association between NO_x and mortality.

Occupational class (considered a good indicator of socioeconomic class in this group of middle-aged men at that time) was assumed to be associated with residential NO_x exposure through residential area (i e, participants with a low socioeconomic standing being more likely to have a blue-collar job, to live in an area with a low mean income and a higher NO_x exposure, and to have a higher risk of cardiovascular mortality). The relationship between residential area and air pollution Gothenburg is complex though, and we did not in this study have a good area-level variable for socioeconomic status (see 4.2.8). When we included an area-level socioeconomic variable, mean household income in the area in 2012 for the participants addresses in 1990, the association between NOx and total mortality was unchanged for the period from 1990 and onwards. For the entire study period the association between NOx and mortality was slightly weaker when socioeconomy was included, but still significant. This could indicate some residual confounding by socioeconomic area. Residential patterns have changed during the last four

decades though, so current socioeconomic geographical patterns are less valid for the early part of the study period.

In the statistical analysis, smoking category was associated with both baseline NO_x exposure and mortality outcomes. The explanation is probably that participants with a lower socioeconomic status are more likely to smoke, and to live in areas with lower socioeconomic status and to some extent higher NO_x exposure. Smoking was thus also included in the main model as a "true" confounder. No other covariates were strongly associated with NO_x exposure.

The associations between NO_x exposure and non-accidental and cardiovascular mortality was slightly stronger when calendar year was not included in the model. Calendar year was considered a possible true confounder since there have been simultaneous decreasing trends in NO_x exposure (Table 3, paper IV), in coronary disease and risk factors (156) and in mortality (157) in the area during our study period. Since we have been following a closed cohort the trends in the population are not necessarily relevant, however. If the decrease in mortality was in part due to the decrease in air pollution exposure we over-adjusted for time trends, and underestimated the effects of NO_x. It seems, however, unlikely that the effect of reductions in air pollution exposure has been as important as other individual risk factors that have decreased cardiovascular risk over time.

In figure 25, "Covariate example 1" is an example of a covariate associated with the risk of cardiovascular mortality, but assumed not to be associated with NO_x exposure (heredity for cardiovascular disease might be associated with other risk factors though, such as cholesterol levels). "Covariate example 2", physical activity, is an example of a covariate assumed to be associated with both the outcome and with other covariates included in the main model, and through them possibly with NO_x exposure, but where we did not consider the association with NO_x exposure strong enough for the covariate to be considered a true confounder.

The effects of NO_x exposure on non-accidental and cardiovascular mortality, stratified by potential effect modifiers, are shown in Table 6, paper IV. There was a stronger association for both non-accidental and cardiovascular mortality in participants with hypertension compared to normotensive participants. However, the strongest association was in the group with blood pressure ranging from 140/90 to 165/100, compared to both higher and lower blood pressure, which is not biologically plausible. For cardiovascular mortality, there was a stronger association with NO_x in those with heredity for cardiovascular disease.

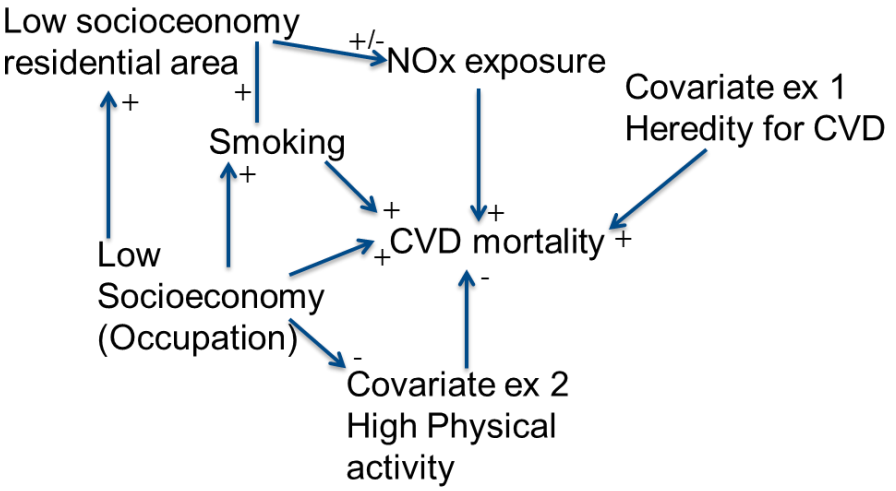

Figure 25. A depiction of the assumed relationships between selected covariates and cardiovascular mortality. A pointed arrow denotes causality in that direction, a line an observed association without a strong hypothesis of causality. + and – signs describe the direction of the relationship.

A sensitivity analysis showed that inclusion of work time physical activity and diastolic blood pressure (excluded due to high correlations with occupational class and systolic blood pressure, respectively) did not affect the association between NO_x exposure and non-accidental or cardiovascular mortality. There was no effect of changing the time scale to time in study instead of age.

4.2.15 Quantifications of health effects

So how important is the increased risk of mortality from exposure to air pollution that we found in paper IV? For some, the fact that there is an increased risk of mortality from exposure to air pollution in Gothenburg will seem very worrying, while to other a risk increase of a few percent will seem

trivial compared to other risk factors. The message that I believe is the best is that the risk is not relevant on the individual level for most people, but since every single person is exposed the risk is important on the population level. Below are a few examples to quantify the risk, and the theoretical effects of changes in exposure, based on the estimates of Paper IV and assuming a linear dose-response relationship:

Effects of relocations: In 1990, a person moving from the 80th percentile of NO_x exposure (48.5 µg/m³) to the 20th percentile of NO_x exposure (20.5 µg/m³) would reduce the risk of non-accidental mortality by 8%. By moving from the 90th percentile to the 10th the risk would be reduced by 12% (The effect was approximately 3% per 10 µg/m³ NO_x, corresponding to a parameter estimate of 0,0027. P80-P20=48,5-20,5=28 µg/m³, and 0,0027*28=8%. P90-P10=43 µg/m³, and 43*0,0027=12%). The corresponding risk reduction for a similar move from the 80th to the 20th percentile would have been 9% in 1973 and 3% in 2007, since exposure levels and contrasts decreased over time.

Improvements during the study period The mean levels of residential NO_x exposure in the cohort decreased from 41.7 in 1973 to 18.4 µg/m^3 in 2007, a reduction of 23.3 µg/m^3 during the study period. Assuming that the cohort is representative for the population, the improved air quality during the study period would thus by these estimates have led to a 6% decreased risk of non-accidental mortality, which is a substantial health benefit on the population level (the parameter estimate 0.0027*23.3 µg/m^3=6.3%.).

Gothenburg has around half a million inhabitants and approximately 5000 deaths occur each year. Consequently, using the figures above almost 300 premature deaths per year ((1.063-1)/1.063)*5000=296) may be assumed to have been avoided by the decrease in exposure to air pollution (NO_x) exposure during the study period. A similar calculation of the effect of a decrease in exposure levels to half of those in 2007 (18.4 µg/m^3 in the cohort), shows that this could decrease the number of annual premature deaths by around 120. These numbers are approximations with large uncertainties and assume that all other risk factors are controlled for, but they still indicate that considerable improvements in public health are possible through decreasing air pollution exposure in Gothenburg.

4.2.16On experimental & epidemiological studies

The level of evidence is generally considered higher in experimental studies than epidemiological studies, the gold standard in medicine being randomized controlled trials (RCT). Under experimental circumstances it is possible to carefully control for all or most other influencing factors than the one studied to a much higher extent than in epidemiological studies. On the other hand, in epidemiological studies it is possible to use larger materials, longer study periods and clinically relevant outcomes such as mortality or MI (fig 26), that might be used in experimental studies of medical treatments but are difficult to use in experimental studies of air pollution for practical or ethical reasons. While experimental studies like paper I and II can prove an effect with a higher certainty than an epidemiological study like paper IV, the connection between the effect that is proven and the clinically more relevant outcome that one wish to prevent has to be assumed (based on other studies). The association in a study like paper IV is less certain to be causal, but it is more directly an association between a real-world exposure and a clinical outcome. Often, epidemiological studies are used to find the important association between different factors, and experimental studies to study the mechanisms and provide evidence for or against the causality of the relationship.

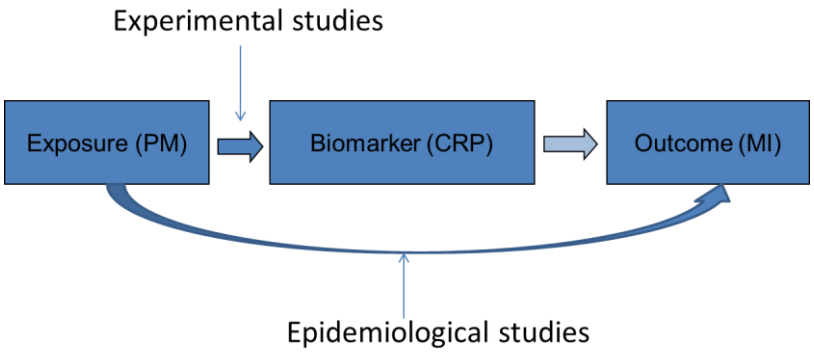

Figure 26. A schematic picture of experimental and epidemiological studies of air pollution. Experimental studies can with a higher certainty prove a causal relationship between an exposure and a biomarker or risk factor, but rarely with the clinical outcome. An epidemiological study can study the association between the exposure and the clinical outcomes (or with a biomarker/risk factor), but not prove causality.

5 CONCLUSIONS

This thesis used a combination of experimental and epidemiological studies to examine the health effects of air pollution in humans (fig 27). The main findings were:

- Short-term exposure to relatively low doses of wood smoke caused weak airway symptoms and affected biomarkers of airway effects in healthy adults, indicating increased endothelial permeability and possibly airway inflammation.
- The wood smoke exposure did not increase the levels of biomarkers of systemic inflammation and coagulation.
- Significant effects on epithelial permeability were found in the session using wood smoke from the start-up phase of the wood burning cycle but not in the session using wood smoke from the burn-out phase. However, since exposure levels were higher in the former we cannot conclude that start-up wood smoke was more toxic.
- The geographical NO_x exposure contrast in Gothenburg was considerable (3-4 times higher in the highest exposure quartile compared to the lowest).
- NO_x exposure decreased almost by half during the study period of 1973-2007.
- Exposure misclassification increased when extrapolating further than 5-7 years from an exposure model, and more if relocations were not taken into account.
- Long-term residential NO_x exposure was associated with increased non-accidental mortality in Gothenburg.
- The associations between NO_x exposure and cause-specific cardiovascular mortality were similar to that for total mortality, while there was no association with incident MI.
- The associations between NO_x exposure and mortality were near-linear, with signs of weaker effects (per unit of exposure) at higher exposure levels, and no signs of a lower threshold.
- The effect estimates were similar for shorter and longer time-windows of NO_x exposure.

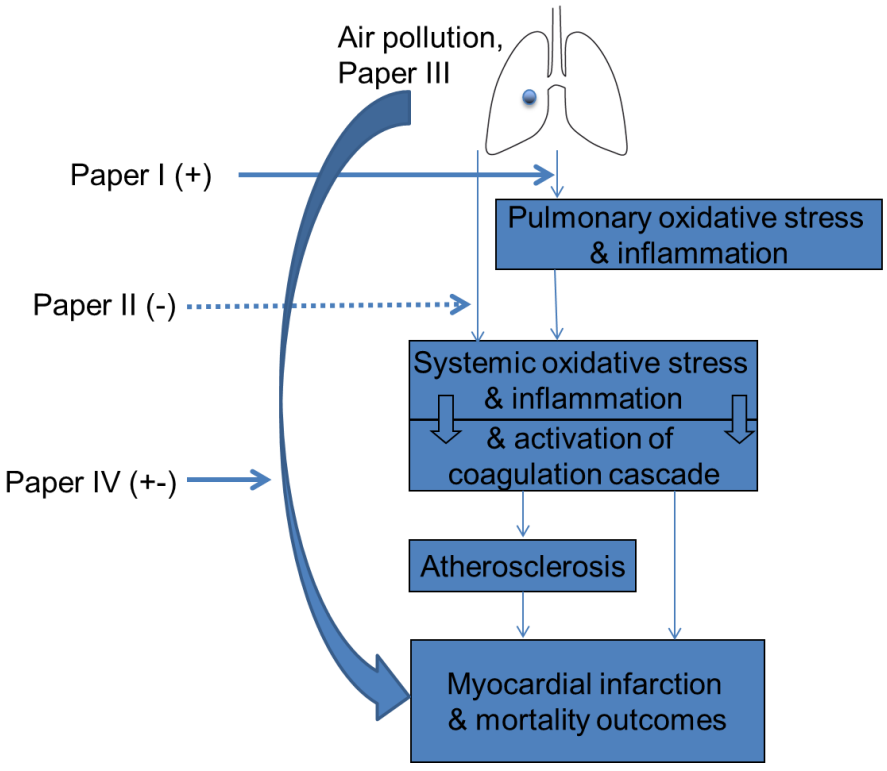

Figure 27. A schematic picture of the findings of the papers included in the thesis. A +sign indicates a positive finding whereas a –sign indicates a non-positive finding.

6 FUTURE PERSPECTIVES

This dissertation adds to the knowledge about health effects of ambient air pollution and wood smoke. Health effects of biomass combustion are important to study since this is one of the largest sources of air pollution in Sweden and the world. Current and future attempts to reduce fossil fuel use are also likely to increase biomass burning in the developing world. Wood burning is largely performed by untrained individuals using simple technology and mixed unstandardized fuels in their own homes, making implementation and enforcement of clean and efficient combustion a technical, organizational and legal challenge. Forest fires, another source of air pollution, are also likely to increase in the Nordic environment as global temperatures increases.

Regarding human wood smoke exposure studies, the effects on biomarkers reported in some studies needs to be repeated to clarify which findings are true effects of wood smoke and which findings are due to chance or particular circumstances. It is at present difficult to draw conclusions of which effects of short-term wood smoke exposure on biomarkers that are reliable. Future studies should therefore include the biomarkers for which previous studies have found effects. Sensitive and validated biomarkers of effects of wood smoke exposure would be very useful in estimating the usefulness of woodstove exchange programs, an intervention that has the potential to bring large health benefits but has been difficult to implement. Different types of wood smoke should also be tested in similar setups to increase the knowledge of which components of this complex mixture that induce the negative health effects.

Epidemiological studies of ambient air pollution should utilize high quality exposure modelling to separate the health effects of different types and sources of air pollution. Statistical modelling of different time windows of exposure can provide insight into the mechanisms through which air pollution increases morbidity and mortality. Modelling of long-term residential noise exposure can allow further investigation of this highly correlated co-exposure to air pollution, and area-level socioeconomic effects need to be taken into account. Despite several decades of intense research on the health effects of air pollution there is much we do not know about which components of air pollution that harms us, and the pathophysiological mechanisms through which we are harmed.

On the other hand, while there is a need for better knowledge we should not let that get in the way of acting on what we know now. Regarding air pollution, there is evidence enough for action. We are certain *enough* that the increased risk of morbidity and mortality by air pollution exposure is real and important, and that reductions in air pollution exposure would improve public health considerably.

ACKNOWLEDGEMENTS

Först vill jag tacka mina två handledare **Lars** och **Gerd** för att de introducerat mig till forskningsområdet och ihärdigt lotsat mig framåt. Tack för att ni alltid har tid och intresse för mig, för denna avhandling, och för andra projekt och idéer som distraherat och berikat på vägen. Nu blir er sista doktorand klar! Det är mycket jag vill säga, men det är nog dags att jag till slut lyssnar på det råd jag fått av er den senaste tiden: "Det räcker, överarbeta inte".

Tack till alla medförfattare till avhandlingens artiklar för kloka synpunkter och hjälp med delar som en enkel läkare har svårt att begripa: **Peter**, som inte bara är medförfattare till tre artiklar i avhandlingen utan också huvudförfattare till en av dem, och en Lvl 15 dwarf cleric of Moradin som känns tryggt att ha vid sin sida, **Eva**, som inte bara förstår utan dessutom kan förklara vad som försiggår bakom rutan i SAS och tillsammans med mig och Peter gläds åt att samordna stora dataset, **Pernilla**, som reder ut mätningarna och apparater när jag är förvirrad, **Annika** och **Lars W** som samlat in, vårdat och delat med sig av PPS-kohorten, och förstås **Sandra**, **Anna-Carin**, **Lena**, **Ann-Charlotte**, **Bo**, **Kerstin** och **Samar**. Utan er hade artiklarna inte blivit till, eller så hade det som blivit till inte blivit lika bra.

Tack även till alla andra kollegor i **MTox-gruppen**, och andra som korrekturläst texter, svarat på frågor, kommit med förslag eller varit med och genom diskussioner utvecklat dessa och andra forskningsprojekt. Tack till kollegor på AMM för en arbetsplats med sånt kunskapsfokus att man kan diskutera epidemiologi och vetenskapsteori över lunchen. Tack till Björn Fagerberg för kloka tankar i samtal om det lilla (nanopartiklar) och det stora (meningen med livet). Ett särskilt tack alla studiedeltagare och till vetenskapens obetalda anonyma rådgivare, reviewers.

Tack till nuvarande och tidigare samarbetspartners i andra forskningsprojekt, särskilt de inom SCAC, MKC och SCAPIS som gör att jag kan arbeta vidare med epidemiologi och luftföroreningar. Tack för finansiellt stöd från Forskarskolan Miljö och Hälsa, Sahlgrenska (forskar-AT och -ST), FORTE och GLS.

Tack till (alla) mina föräldrar för att ni förutsatt att jag kan göra vad jag vill, så att jag också gör det, och hela den utvidgade familjen. Ni frågar ibland vad jag gör egentligen, och har ni läst ända hit så vet ni något mer om det åtminstone (man får inte läsa slutet av en bok först).

Tack **Tage**, som tidigt visade intresse för luftföroreningar genom att signalera att det var dags att födas under en kvällsföreläsning om vedrök. Tack också för att du lär mig sätta saker i sitt rätta perspektiv: *”Tage, jag måste gå till jobbet nu.” ”Nej Pappa, du måste inte gå till jobbet nu. Du måste springa med mig!”* Tack också **lilla knyttet**, för att du väntar med att födas till efter disputationen (om du inte gör det, tack för att du kommer och visar vad man måste prioritera först och att det går ändå). Allra mest tack till **Marit**, min själs älskade, för att du valt att leva med mig hela den tid jag arbetat med avhandlingen och inte visar några tecken på att komma på bättre tankar. Tack både för äktenskapligt stöd och för att du läst och klokt kommenterat det mesta som står här.

REFERENCES

1. Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet. 2015;386(10010):2287-323.
2. EEA. Air quality in Europe - 2014 report. Copenhagen: European Environment Agency, 2014.
3. WHO. Burden of disease from household air pollution for 2012. Summary of results. Geneva, Switzerland: Public Health, Social and Environmental Determinants of Health Department, World Health Organisation, 2014.
4. Forsberg B, Hansson HC, Johansson C, Areskoug H, Persson K, et al. Comparative health impact assessment of local and regional particulate air pollutants in Scandinavia. Ambio. 2005;34(1):11-9.
5. Brimblecombe P. Air pollution and health history. In: Holgate S, Samet J, Koren H, R M, editors. Air Pollution and Health. San Diego: Academic Press; 1999. p. 5-18.
6. Nemery B, Hoet PHM, Nemmar A. The Meuse Valley fog of 1930: an air pollution disaster. The Lancet. 2001;357(9257):704-8.
7. Firket J. Fog along the Meuse valley. Transactions of the Faraday Society. 1936;32(0):1192-6.
8. Ciocco A, Thompson DJ. A Follow-Up of Donora Ten Years After: Methodology and Findings. American Journal of Public Health and the Nations Health. 1961;51(2):155-64.
9. Schrenk HH, Heimann H, Clayton GD, Gafafer WM, Wexler H. Air pollution in Donora, Pa.: epidemiology of the unusual smog episode of October 1948 : preliminary report. Washington, D.C.: Federal Security Agency, Public Health Service, Bureau of State Services, Division of Industrial Hygiene; 1949.
10. Logan WP. Mortality in the London fog incident, 1952. The Lancet. 1953;1(6755):336-8.
11. Ministry of Health. Mortality and Morbidity During the London Fog of December 1952. Reports on Public Health and Medical Subjects no 95. London: HSMO; 1954.
12. Wilkins ET. Air pollution aspects of the London fog of December 1952. Quarterly Journal of the Royal Meteorological Society. 1954;80(344):267-71.
13. Bell ML, Davis DL. Reassessment of the lethal London fog of 1952: novel indicators of acute and chronic consequences of acute exposure to air pollution. Environ Health Perspect. 2001;109(Suppl 3):389-94.
14. Atkinson RW, Kang S, Anderson HR, Mills IC, Walton HA. Epidemiological time series studies of PM2.5 and daily mortality and hospital admissions: a systematic review and meta-analysis. Thorax. 2014;69(7):660-5.

15. Brook RD, Rajagopalan S, Pope CA, III, Brook JR, Bhatnagar A, et al. Particulate Matter Air Pollution and Cardiovascular Disease: An Update to the Scientific Statement From the American Heart Association. Circulation. 2010;121(21):2331-78.
16. Mills IC, Atkinson RW, Kang S, Walton H, Anderson HR. Quantitative systematic review of the associations between short-term exposure to nitrogen dioxide and mortality and hospital admissions. BMJ Open. 2015;5(5).
17. Dockery DW, Pope CA, Xu X, Spengler JD, Ware JH, et al. An Association between Air Pollution and Mortality in Six U.S. Cities. N Engl J Med. 1993;329(24):1753-9.
18. Pope CA, Thun MJ, Namboodiri MM, Dockery DW, Evans JS, et al. Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults. Am J Respir Crit Care Med. 1995;151(3):669-74.
19. Hoek G, Brunekreef B, Goldbohm S, Fischer P, van den Brandt PA. Association between mortality and indicators of traffic-related air pollution in the Netherlands: a cohort study. The Lancet. 2002;360(9341):1203-9.
20. Nafstad P, Haheim LL, Wisloff T, Gram F, Oftedal B, et al. Urban air pollution and mortality in a cohort of Norwegian men. Environ Health Perspect. 2004;112(5):610-5.
21. Filleul L, Rondeau V, Vandentorren S, Le Moual N, Cantagrel A, et al. Twenty five year mortality and air pollution: results from the French PAARC survey. J Occup Environ Med. 2005;62(7):453-60.
22. Raaschou-Nielsen O, Andersen Z, Jensen S, Ketzel M, Sorensen M, et al. Traffic air pollution and mortality from cardiovascular disease and all causes: a Danish cohort study. Environmental Health. 2012;11(1):60.
23. Carey IM, Atkinson RW, Kent AJ, van Staa T, Cook DG, et al. Mortality Associations with Long-Term Exposure to Outdoor Air Pollution in a National English Cohort. Am J Respir Crit Care Med. 2013;187(11):1226-33.
24. Cesaroni G BC, Gariazzo C, Stafoggia M, Sozzi R, Davoli M, Forastiere F. Long-Term Exposure to Urban Air Pollution and Mortality in a Cohort of More than a Million Adults in Rome. Environ Health Perspect 2013;121:324–31.
25. Health Effects Institute. Reanalysis of the Harvard Six Cities Study and the American Cancer Society Study of Particulate Air Pollution and Mortality: A Special Report of the Institute's Particle Epidemiology Reanalysis Project. Cambridge, Massachusetts: 2000.
26. Laden F, Schwartz J, Speizer FE, Dockery DW. Reduction in Fine Particulate Air Pollution and Mortality: Extended Follow-up of the Harvard Six Cities Study. Am J Respir Crit Care Med. 2006;173(6):667-72.
27. Pope CA, 3rd, Burnett RT, Thun MJ, Calle EE, Krewski D, et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. Jama. 2002;287(9):1132-41.
28. Pope CA, Burnett RT, Thurston GD, Thun MJ, Calle EE, et al. Cardiovascular Mortality and Long-Term Exposure to Particulate Air

Pollution: Epidemiological Evidence of General Pathophysiological Pathways of Disease. Circulation. 2004;109(1):71-7.
29. Krewski D, Jerrett M, Burnett RT, Ma R, Hughes E, et al. Extended follow-up and spatial analysis of the American Cancer Society study linking particulate air pollution and mortality. Res Rep Health Eff Inst. 2009(140):5-114; discussion 5-36.
30. Jerrett M, Burnett RT, Pope CA, Ito K, Thurston G, et al. Long-Term Ozone Exposure and Mortality. N Engl J Med. 2009;360(11):1085-95.
31. Schwartz J, Coull B, Laden F, Ryan L. The effect of dose and timing of dose on the association between airborne particles and survival. Environ Health Perspect. 2008;116(1):64-9.
32. Beelen R, Raaschou-Nielsen O, Stafoggia M, Andersen ZJ, Weinmayr G, et al. Effects of long-term exposure to air pollution on natural-cause mortality: an analysis of 22 European cohorts within the multicentre ESCAPE project. The Lancet. 2014;383(9919):785-95.
33. Beelen R, Stafoggia M, Raaschou-Nielsen O, Andersen ZJ, Xun WW, et al. Long-term exposure to air pollution and cardiovascular mortality: an analysis of 22 European cohorts. Epidemiology. 2014;25(3):368-78.
34. WHO. Review of evidence on health aspects of air pollution - REVIHAAP Project. Copenhagen, Denmark. 2013.
35. Hoek G, Krishnan R, Beelen R, Peters A, Ostro B, et al. Long-term air pollution exposure and cardio- respiratory mortality: a review. Environmental Health. 2013;12(1):43.
36. WHO. Health risks of air pollution in Europe – HRAPIE. Recommendations for concentration-response functions for cost-benefit analysis of particulate matter, ozone and nitrogen dioxide. Copenhagen, Denmark. 2013.
37. Brauer M, Freedman G, Frostad J, van Donkelaar A, Martin RV, et al. Ambient Air Pollution Exposure Estimation for the Global Burden of Disease 2013. Environmental Science & Technology. 2015.
38. US Embassy in Beijing. http://www.stateair.net/web/post/1/1.html2015 [cited 2015 1201].
39. Brook RD. Cardiovascular effects of air pollution. Clinical Science. 2008;115(6):175-87.
40. ICRP. Human Respiratory Tract Model for Radiological Protection. ICRP Publication 66. Ann. ICRP 24 (1-3). 1994.
41. Londahl J, Massling A, Pagels J, Swietlicki E, Vaclavik E, et al. Size-resolved respiratory-tract deposition of fine and ultrafine hydrophobic and hygroscopic aerosol particles during rest and exercise. Inhal Toxicol. 2007;19(2):109-16.
42. Brook RD, Franklin B, Cascio W, Hong Y, Howard G, et al. Air Pollution and Cardiovascular Disease: A Statement for Healthcare Professionals From the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation. 2004;109(21):2655-71.

43. Faustini A, Rapp R, Forastiere F. Nitrogen dioxide and mortality: review and meta-analysis of long-term studies. Eur Respir J. 2014.
44. WHO. WHO Guidelines for indoor air quality: selected pollutants. Copenhagen, Denmark: WHO regional office for Europe; 2010.
45. WHO. WHO air quality guidelines: global update 2005. Copenhagen, Denmark: World Health Organization, Regional Office for Europe.; 2006.
46. Rehfuess E, Mehta S, Üstün A. Assessing Household Solid Fuel Use: Multiple Implications for the Millennium Development Goals. Environ Health Perspect. 2006;114(3).
47. Naeher LP, Brauer M, Lipsett M, Zelikoff JT, Simpson CD, et al. Woodsmoke Health Effects: A Review. Inhal Toxicol. 2007;19(1):67 - 106.
48. Hedberg E, Johansson C, Johansson L, Swietlicki E, Brorstrom-Lunden E. Is levoglucosan a suitable quantitative tracer for wood burning? Comparison with receptor modeling on trace elements in Lycksele, Sweden. J Air Waste Manag Assoc. 2006;56(12):1669-78.
49. Larson TV, Koenig JQ. Wood smoke - emissions and noncancer respiratory effects. Annual Review of Public Health. 1994;15:133-56.
50. McGowan JA, Hider PN, Chacko E, Town GI. Particulate air pollution and hospital admissions in Christchurch, New Zealand. Australian and New Zealand Journal of Public Health. 2002;26(1):23-9.
51. Amann M, Bertok, I, Cofala, J, Gyarfas, F, Heyes, C, Klimont, Z, Schöpp, W, Winiwarter, W. Baseline Scenarios for the Clean Air for Europe (CAFE) Programme: Final Report, Laxenburg, Austria. 2005.
52. Youssouf H, Liousse C, Roblou L, Assamoi EM, Salonen RO, et al. Non-accidental health impacts of wildfire smoke. Int J Environ Res Public Health. 2014;11(11):11772-804.
53. Johnston FH, Henderson SB, Chen Y, Randerson JT, Marlier M, et al. Estimated global mortality attributable to smoke from landscape fires. Environ Health Perspect. 2012;120(5):695-701.
54. Bowman DMJS, Balch JK, Artaxo P, Bond WJ, Carlson JM, et al. Fire in the Earth System. Science. 2009;324(5926):481-4.
55. Kocbach Bolling A, Pagels J, Yttri K, Barregard L, Sallsten G, et al. Health effects of residential wood smoke particles: the importance of combustion conditions and physicochemical particle properties. Part Fibre Toxicol. 2009;6(1):29.
56. Londahl J, Pagels J, Boman C, Swietlicki E, Massling A, et al. Deposition of biomass combustion aerosol particles in the human respiratory tract. Inhal Toxicol. 2008;20(10):923-33.
57. Muala A, Nicklasson H, Boman C, Swietlicki E, Nystrom R, et al. Respiratory Tract Deposition of Inhaled Wood Smoke Particles in Healthy Volunteers. J Aerosol Med Pulm Drug Deliv. 2015;28(4):237-46.
58. Londahl J, Swietlicki E, Rissler J, Bengtsson A, Boman C, et al. Experimental determination of the respiratory tract deposition of diesel combustion particles in patients with chronic obstructive pulmonary disease. Part Fibre Toxicol. 2012;9:30.

59. Boman BC, Forsberg AB, Jarvholm BG. Adverse health effects from ambient air pollution in relation to residential wood combustion in modern society. Scandinavian Journal of Work Environment & Health. 2003;29(4):251-60.
60. Sigsgaard T, Forsberg B, Annesi-Maesano I, Blomberg A, Bolling A, et al. Health impacts of anthropogenic biomass burning in the developed world. Eur Respir J. 2015.
61. Kocbach Bolling A, Totlandsdal A, Sallsten G, Braun A, Westerholm R, et al. Wood smoke particles from different combustion phases induce similar pro-inflammatory effects in a co-culture of monocyte and pneumocyte cell lines. Part Fibre Toxicol. 2012;9(1):45.
62. Vedal S. Ambient Particles and Health: Lines that Divide. J Air Waste Manag Assoc. 1997;47(5):551-81.
63. Mills NL, Donaldson K, Hadoke PW, Boon NA, MacNee W, et al. Adverse cardiovascular effects of air pollution. Nat Clin Pract Cardiovasc Med. 2009;6(1):36-44.
64. Newby DE, Mannucci PM, Tell GS, Baccarelli AA, Brook RD, et al. Expert position paper on air pollution and cardiovascular disease. European Heart Journal. 2015;36(2):83-93.
65. Kelly FJ, Fussell JC. Air pollution and airway disease. Clin Exp Allergy. 2011;41(8):1059-71.
66. Eisner MD, Anthonisen N, Coultas D, Kuenzli N, Perez-Padilla R, et al. An official American Thoracic Society public policy statement: Novel risk factors and the global burden of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2010;182(5):693-718.
67. Nel A. Atmosphere. Air pollution-related illness: effects of particles. Science. 2005;308(5723):804- 6
68. IARC. Household Use of Solid Fuels and High-Temperature Frying. Lyon, France: International Agency for Research on Cancer, 2010.
69. IARC. Diesel and gasoline engine exhaust and some nitroarenes. Lyon, France: International Agency for Research on Cancer, 2013.
70. IARC. Outdoor air pollution. Lyon, France: International Agency for Research on Cancer, 2013.
71. Wilhelmsen L, Tibblin G, Werkö L. A primary preventive study in Gothenburg, Sweden. Preventive Medicine. 1972;1(1-2):153-60.
72. Rosengren A, Wedel H, Wilhelmsen L. Coronary Heart Disease And Mortality In Middle Aged Men From Different Occupational Classes In Sweden. BMJ. 1988:1497-500.
73. Wilhelmsen L, Berglund G, Elmfeldt D, Tibblin G, Wedel H, et al. The multifactor primary prevention trial in Göteborg, Sweden. European Heart Journal. 1986;7(4):279-88.
74. Olin AC, Aldenbratt A, Ekman A, Ljungkvist G, Jungersten L, et al. Increased nitric oxide in exhaled air after intake of a nitrate-rich meal. Respiratory Medicine. 2001;95(2):153-8.

75. Molnar P, Gustafson P, Johannesson S, Boman J, Barregard L, et al. Domestic wood burning and PM2.5 trace elements: personal exposures, indoor and outdoor levels. Atmospheric Environment. 2005;39(14):2643-53.
76. Strandberg B, Sunesson AL, Olsson K, Levin JO, Ljungqvist G, et al. Evaluation of two types of diffusive samplers and adsorbents for measuring 1,3-butadiene and benzene in air. Atmospheric Environment. 2005;39(22):4101-10.
77. Levin JO, Lindahl R, Andersson K. High-Performance Liquid-Chromatographic Determination of Formaldehyde in Air in the Ppb to Ppm Range Using Diffusive Sampling and Hydrazone Formation. Environmental Technology Letters. 1988;9(12):1423-30.
78. Egeghy PP, Hauf-Cabalo L, Gibson R, Rappaport SM. Benzene and naphthalene in air and breath as indicators of exposure to jet fuel. J Occup Environ Med. 2003;60(12):969-76.
79. Borg GAV. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982;14:377–81.
80. Andersson L, Lundberg PA, Barregard L. Methodological aspects on measurement of Clara cell protein in urine as a biomarker for airway toxicity, compared with serum levels. J Appl Toxicol. 2007;27(1):60-6.
81. Lärstad M, Ljungkvist G, Olin A-C, Torén K. Determination of malondialdehyde in breath condensate by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography B. 2002;766(1):107-14.
82. Ellingsen DG, Ulvestad B, Andersson L, Barregard L. Pneumoproteins and inflammatory biomarkers in asphalt pavers. Biomarkers. 2010;15(6):498-507.
83. Basu S. Radioimmunoassay of 15-keto-13,14-dihydro-prostaglandin F2[alpha]: an index for inflammation via cyclooxygenase catalysed lipid peroxidation. Prostaglandins, Leukotrienes and Essential Fatty Acids. 1998;58(5):347-52.
84. Hornung RW, Reed LD. Estimation of average concentration in the presence of non-detectable values. Applied Occupational and Environmental Hygiene. 1990;5:48–51.
85. Quantum GIS Development Team. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. 2014.
86. R Development Core Team. The R Foundation for Statistical Computing, Vienna, Austria [cited 2015 0324]. Available from: http://www.r-project.org.
87. ATS/ERS. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912-30.
88. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, et al. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FeNO) for Clinical Applications. Am J Respir Crit Care Med. 2011;184(5):602-15.

89. Barregard L, Sallsten G, Andersson L, Almstrand AC, Gustafson P, et al. Experimental exposure to wood smoke: effects on airway inflammation and oxidative stress. J Occup Environ Med. 2008;65(5):319-24.
90. Sehlstedt M, Dove R, Boman C, Pagels J, Swietlicki E, et al. Antioxidant airway responses following experimental exposure to wood smoke in man. Part Fibre Toxicol. 2010;7(1):21.
91. Riddervold I, Bonlokke J, Olin A-C, Gronborg T, Schlunssen V, et al. Effects of wood smoke particles from wood-burning stoves on the respiratory health of atopic humans. Particle and Fibre Toxicology. 2012;9(1):12.
92. Muala A, Rankin G, Sehlstedt M, Unosson J, Bosson JA, et al. Acute exposure to wood smoke from incomplete combustion - indications of cytotoxicity. Part Fibre Toxicol. 2015;12(1):33.
93. Olin AC, Stenfors N, Torén K, Blomberg A, Helleday R, et al. Nitric oxide (NO) in exhaled air after experimental ozone exposure in humans. Respiratory Medicine. 2001;95(6):491-5.
94. Pietropaoli AP, Frampton MW, Hyde RW, Morrow PE, Oberdörster G, et al. Pulmonary Function, Diffusing Capacity, and Inflammation in Healthy and Asthmatic Subjects Exposed to Ultrafine Particles. Inhal Toxicol. 2004;16(s1):59-72.
95. Langrish JP, Lundbäck M, Barath S, Söderberg S, Mills NL, et al. Exposure to nitrogen dioxide is not associated with vascular dysfunction in man. Inhal Toxicol. 2010;22(3):192-8.
96. Steerenberg PA, Snelder JB, Fischer PH, Vos JG, van Loveren H, et al. Increased exhaled nitric oxide on days with high outdoor air pollution is of endogenous origin. Eur Respir J. 1999;13(2):334-7.
97. Mar TF, Jansen K, Shepherd K, Lumley T, Larson TV, et al. Exhaled nitric oxide in children with asthma and short-term PM2.5 exposure in Seattle. Environ Health Perspect. 2005;113(12):1791-4.
98. Jansen KL, Larson TV, Koenig JQ, Mar TF, Fields C, et al. Associations between health effects and particulate matter and black carbon in subjects with respiratory disease. Environ Health Perspect. 2005;113(12):1741-6.
99. Delfino RJ, Staimer N, Gillen D, Tjoa T, Sioutas C, et al. Personal and ambient air pollution is associated with increased exhaled nitric oxide in children with asthma. Environ Health Perspect. 2006;114(11):1736-43.
100. Barraza-Villarreal A, Sunyer J, Hernandez-Cadena L, Escamilla-Nuñez MC, Sienra-Monge JJ, et al. Air pollution, airway inflammation, and lung function in a cohort study of Mexico City schoolchildren. Environ Health Perspect. 2008;116(6):832-8.
101. Berhane K, Zhang Y, Linn WS, Rappaport EB, Bastain TM, et al. The Effect of Ambient Air Pollution on Exhaled Nitric Oxide in the Children's Health Study. Eur Respir J. 2010.
102. Broeckaert F, Clippe A, Knoops B, Hermans C, Bernard A. Clara Cell Secretory Protein (CC16): Features as a Peripheral Lung Biomarker. Annals of the New York Academy of Sciences. 2000;923:68-77.

103. Hermans C, Knoops B, Wiedig M, Arsalane K, Toubeau G, et al. Clara cell protein as a marker of Clara cell damage and bronchoalveolar blood barrier permeability. Eur Respir J. 1999;13(5):1014-21.
104. Kishore U, Greenhough TJ, Waters P, Shrive AK, Ghai R, et al. Surfactant proteins SP-A and SP-D: Structure, function and receptors. Molecular Immunology. 2006;43(9):1293-315.
105. Hermans C, Bernard A. Lung Epithelium-specific Proteins. Characteristics and Potential Applications as Markers. Am J Respir Crit Care Med. 1999;159(2):646-78.
106. Bonlokke JH, Riddervold IS, Gronborg TK, Skogstrand K, Hougaard DM, et al. Systemic effects of wood smoke in a short-term experimental exposure study of atopic volunteers. J Occup Environ Med. 2014;56(2):177-83.
107. Bernard A, Hermans C, Van Houte G. Transient increase of serum Clara cell protein (CC16) after exposure to smoke. J Occup Environ Med. 1997;54(1):63-5.
108. Broeckaert F, Arsalane K, Hermans C, Bergamaschi E, Brustolin A, et al. Serum Clara Cell Protein: A Sensitive Biomarker of Increased Lung Epithelium Permeability Caused by Ambient Ozone. Environ Health Perspect. 2000;108(6):533-7.
109. Blomberg A, Mudway I, Svensson M, Hagenbjork-Gustafsson A, Thomasson L, et al. Clara cell protein as a biomarker for ozone-induced lung injury in humans. Eur Respir J. 2003;22(6):883-8.
110. Provost EB, Chaumont A, Kicinski M, Cox B, Fierens F, et al. Serum levels of club cell secretory protein (Clara) and short- and long-term exposure to particulate air pollution in adolescents. Environment International. 2014;68:66-70.
111. Bernard AM, Roels HA, Buchet JP, Lauwerys RR. Serum Clara Cell Protein: An Indicator of Bronchial Cell Dysfunction Caused by Tobacco Smoking. Environmental Research. 1994;66(1):96-104.
112. Shijubo N, Itoh Y, Yamaguchi T, Shibuya Y, Morita Y, et al. Serum and BAL Clara cell 10 kDa protein (CC10) levels and CC10-positive bronchiolar cells are decreased in smokers. Eur Respir J. 1997;10(5):1108-14.
113. Hermans C, Aly O, Nyberg B-I, Peterson C, Bernard A. Determinants of Clara cell protein (CC16) concentration in serum: a reassessment with two different immunoassays. Clinica Chimica Acta. 1998;272(2):101-10.
114. Van Miert E, Sardella A, Nickmilder M, Bernard A. Respiratory effects associated with wood fuel use: a cross-sectional biomarker study among adolescents. Pediatr Pulmonol. 2012;47(4):358-66.
115. Freberg BI, Olsen R, Thorud S, Daae HL, Hersson M, et al. Pulmonary function and serum pneumoproteins in professional ski waxers. Inhal Toxicol. 2016;28(1):7-13.
116. Salvador-Morales C, Townsend P, Flahaut E, Vénien-Bryan C, Vlandas A, et al. Binding of pulmonary surfactant proteins to carbon nanotubes;

potential for damage to lung immune defense mechanisms. Carbon. 2007;45(3):607-17.
117. Niu W, Liu Y, Qi Y, Wu Z, Zhu D, et al. Association of interleukin-6 circulating levels with coronary artery disease: a meta-analysis implementing mendelian randomization approach. Int J Cardiol. 2012;157(2):243-52.
118. The Emerging Risk Factors Collaboration: Kaptoge S DAE, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. The Lancet. 2010;375(9709):132-40.
119. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, et al. Inflammatory Markers and the Risk of Coronary Heart Disease in Men and Women. N Engl J Med. 2004;351(25):2599-610.
120. Libby P. Inflammation in atherosclerosis. Nature. 2002;420(6917):868-74.
121. Barregard L, Sallsten G, Gustafson P, Andersson L, Johansson L, et al. Experimental Exposure to Wood-Smoke Particles in Healthy Humans: Effects on Markers of Inflammation, Coagulation, and Lipid Peroxidation. Inhal Toxicol. 2006;18(11):845-53.
122. Allen RW, Carlsten C, Karlen B, Leckie S, Eeden Sv, et al. An Air Filter Intervention Study of Endothelial Function among Healthy Adults in a Woodsmoke-impacted Community. Am J Respir Crit Care Med. 2011;183(9):1222-30.
123. Ghio AJ, Soukup JM, Case M, Dailey LA, Richards J, et al. Exposure to wood smoke particles produces inflammation in healthy volunteers. J Occup Environ Med. 2011;69 (3)(3):170-5.
124. Jensen A, Karottki DG, Christensen JM, Bonlokke JH, Sigsgaard T, et al. Biomarkers of oxidative stress and inflammation after wood smoke exposure in a reconstructed Viking Age house. Environ Mol Mutagen. 2014;55(8):652-61.
125. Fibrinogen Studies Collaboration: Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, et al. Plasma Fibrinogen Level and the Risk of Major Cardiovascular Diseases and Nonvascular Mortality. Jama. 2005;294(14):1799-809.
126. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, et al. Markers of Inflammation and Cardiovascular Disease. Circulation. 2003;107(3):499-511.
127. Stec JJ, Silbershatz H, Tofler GH, Matheney TH, Sutherland P, et al. Association of Fibrinogen With Cardiovascular Risk Factors and Cardiovascular Disease in the Framingham Offspring Population. Circulation. 2000;102(14):1634-8.
128. The Emerging Risk Factors Collaboration: Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR ea. C-Reactive Protein, Fibrinogen, and Cardiovascular Disease Prediction. N Engl J Med. 2012;367(14):1310-20.
129. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. The Journal of Clinical Investigation. 2005;115(12):3378-84.

130. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, et al. Fibrin D-Dimer and Coronary Heart Disease. Circulation. 2001;103(19):2323-7.
131. Lacroix-Desmazes S, Navarrete A-M, André S, Bayry J, Kaveri SV, et al. Dynamics of factor VIII interactions determine its immunologic fate in hemophilia A. Blood. 2008;112(2):240-9.
132. Haverkate F. Levels of haemostatic factors, arteriosclerosis and cardiovascular disease. Vascular Pharmacology. 2002;39(3):109-12.
133. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective Study of Hemostatic Factors and Incidence of Coronary Heart Disease : The Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 1997;96(4):1102-8.
134. Seaton A, Soutar A, Crawford V, Elton R, McNerlan S, et al. Particulate air pollution and the blood. Thorax. 1999;54(11):1027-32.
135. Basu S. F2-Isoprostanes in Human Health and Diseases: From Molecular Mechanisms to Clinical Implications. Antioxidants & Redox Signaling. 2008;10(8):1405-34.
136. Risom L, Møller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2005;592(1-2):119-37.
137. Soyer OU, Dizdar EA, Keskin O, Lilly C, Kalayci O. Comparison of two methods for exhaled breath condensate collection. Allergy. 2006;61(8):1016-8.
138. Helleday R, Segerstedt B, Forsberg B, Mudway I, Nordberg G, et al. Exploring the Time Dependence of Serum Clara Cell Protein as a Biomarker of Pulmonary Injury in Humans. Chest. 2006;130(3):672-5.
139. Sallsten G, Gustafson P, Johansson L, Johannesson S, Molnar P, et al. Experimental Wood Smoke Exposure in Humans. Inhal Toxicol. 2006;18(11):855-64.
140. Riddervold IS, Bønløkke JH, Mølhave L, Massling A, Jensen B, et al. Wood smoke in a controlled exposure experiment with human volunteers. Inhal Toxicol. 2011;23(5):277-88.
141. Danielsen PH, Bräuner EV, Barregard L, Sällsten G, Wallin M, et al. Oxidatively damaged DNA and its repair after experimental exposure to wood smoke in healthy humans. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2008;642(1-2):37-42.
142. Unosson J, Blomberg A, Sandstrom T, Muala A, Boman C, et al. Exposure to wood smoke increases arterial stiffness and decreases heart rate variability in humans. Part Fibre Toxicol. 2013;10:20.
143. Forchhammer L, Moller P, Riddervold I, Bonlokke J, Massling A, et al. Controlled human wood smoke exposure: oxidative stress, inflammation and microvascular function. Part Fibre Toxicol. 2012;9(1):7.
144. Pope CA, Hansen JC, Kuprov R, Sanders MD, Anderson MN, et al. Vascular Function and Short-Term Exposure to Fine Particulate Air Pollution. J Air Waste Manag Assoc. 2011;61(8):858-63.

145. Beelen R, Voogt M, Duyzer J, Zandveld P, Hoek G. Comparison of the performances of land use regression modelling and dispersion modelling in estimating small-scale variations in long-term air pollution concentrations in a Dutch urban area. Atmospheric Environment. 2010;44(36):4614-21.
146. Molnár P, Johannesson S, Boman J, Barregard L, Sallsten G. Personal exposures and indoor, residential outdoor, and urban background levels of fine particle trace elements in the general population. J Environ Monit 2006;8(5):543-51.
147. Rosenlund M, Berglind N, Pershagen G, Hallqvist J, Jonson T, et al. Long-term exposure to urban air pollution and myocardial infarction. Epidemiology. 2006;17(4):383-90.
148. Rosenlund M, Bellander T, Nordquist T, Alfredsson L. Traffic-generated air pollution and myocardial infarction. Epidemiology. 2009;20(2):265-71.
149. Rosenlund M, Picciotto S, Forastiere F, Stafoggia M, Perucci CA. Traffic-related air pollution in relation to incidence and prognosis of coronary heart disease. Epidemiology. 2008;19(1):121-8.
150. Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, et al. Long-Term Exposure to Air Pollution and Incidence of Cardiovascular Events in Women. N Engl J Med. 2007;356(5):447-58.
151. Gan WQ, Koehoorn M, Davies HW, Demers PA, Tamburic L, et al. Long-term exposure to traffic-related air pollution and the risk of coronary heart disease hospitalization and mortality. Environ Health Perspect. 2011;119(4):501-7.
152. Puett RC, Hart JE, Yanosky JD, Paciorek C, Schwartz J, et al. Chronic fine and coarse particulate exposure, mortality, and coronary heart disease in the Nurses' Health Study. Environ Health Perspect. 2009;117(11):1697-701.
153. Elliott P, Shaddick G, Wakefield JC, Hoogh Cd, Briggs DJ. Long-term associations of outdoor air pollution with mortality in Great Britain. Thorax. 2007;62(12):1088-94.
154. Lepeule J, Laden F, Dockery D, Schwartz J. Chronic Exposure to Fine Particles and Mortality: An Extended Follow-Up of the Harvard Six Cities Study from 1974 to 2009. Environ Health Perspect. 2012.
155. Pope CA, 3rd. Mortality effects of longer term exposures to fine particulate air pollution: review of recent epidemiological evidence. Inhal Toxicol. 2007;19 Suppl 1:33-8.
156. Wilhelmsen L, Welin L, Svärdsudd K, Wedel H, Eriksson H, et al. Secular changes in cardiovascular risk factors and attack rate of myocardial infarction among men aged 50 in Gothenburg, Sweden. Accurate prediction using risk models. Journal of Internal Medicine. 2008;263(6):636-43.
157. Berg J, Bjorck L, Lappas G, O'Flaherty M, Capewell S, et al. Continuing decrease in coronary heart disease mortality in Sweden. BMC Cardiovascular Disorders. 2014;14(1):9.