Acute health effects of fine and ultrafine particles in indoor air

– Human exposure studies among vulnerable population subgroups

PhD Dissertation

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Faculty of Health Sciences

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Preface

After finishing my master studies in the summer of 2016, I started working at Department of Public Health, Research unit for Environment, Work and Health at Aarhus University. I started as a research assistant under supervision from Professor Torben Sigsgaard. To work within the field of indoor air pollution and health was new to me, however, I quickly developed an interest in the field, and after a few months Torben and I agreed that I should apply for a PhD-scholarship. During spring 2017, I was enrolled as a PhD-student having received a full-time scholarship from the Graduate School of Health. In June 2017, I began my PhD-project *Acute health effects of fine and ultrafine particles in indoor air – Human exposure studies among vulnerable population subgroups* comprising two experimental studies with human volunteers. The PhD was carried out between June 2017 and August 2021.

This thesis concludes my three-year PhD-project and is composed of three enclosed papers, based on original research and data from the experimental studies. The thesis provides a broad introduction to the field of indoor particle pollution and health, including an overview of the results of the three included papers in relation to the international state-of-the-art research within the field and a critical evaluation of the methodological approach used. The work presented in this thesis was carried out at the Climate Chambers, Department of Public Health, Aarhus University, mainly under the supervision of Professor Torben Sigsgaard. My PhD-project was an interdisciplinary project made in collaboration between the Climate Chambers, Department of Chemistry, Aarhus University, Aarhus University Hospital, and Department of Public Health, University of Copenhagen. Part of the PhD-project was funded by Realdania. It has truly been a privilege to carry out my PhD in collaboration with so many clever and engaged researchers and in such a creative and resourceful working environment.

I hope this thesis will serve as inspiration.

Karin Rosenkilde Laursen
Aarhus, August 2021

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The past couple of years as a PhD-student has been inspiring, challenging, fun, developing and hard work. Coming to an end of a more than three-year long journey, I would like to express my sincere thanks to the many people who have helped me along the way. Firstly, I will like to express my gratitude to my academic supervisors, especially my main supervisor Torben, who has believed in me from the very beginning of my research career and throughout the PhD. Thank you for your kindness, the many high-fives and good discussions. You have been good at encouraging me and tone down frustrations when needed. Thank you, Gitte, my former supervisor, who is now working at RKKP. You have given me useful advice to the PhD-process and from day one insisted on work-life balance – which I (and my family) am grateful for. Jörg, thank you for stepping in in the middle of the process helping me with the last, but hardest part of the PhD. I have always admired your knowledge and humbleness. Steffen, thank you for taking your time to listen, guide and discuss despite you're very busy schedule as head of Institute at University of Copenhagen.

I will like to thank all my colleagues at the Research Unit for Environment, Work and Health. Thank you for fun, friendship and support. Thank you to everyone being a part of our journal club "Respit" for many good scientific discussions. Especially thanks to Signe Timm, Charlotte, Grethe and Gitte for support, talks and friendship.

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Conducting an interdisciplinary PhD has meant a lot of valuable collaborators; Thank you to the clever ladies from Department of Chemistry (Marianne, Merete, Bernadette and Berit), and to all my other important collaborators – I am grateful for all of your support and valuable insights. I acknowledge the sponsors for making my PhD possible; Thank you to the Graduate School of Health for providing me with a full-time research scholarship and to Realdania for sponsoring the UltraFine Project making it possible to implement such a comprehensive study.

To my study group (Vibe, Mette Lise and Cecilie) – I am grateful for your friendship and academic discussions throughout the past 11 years. As a group of four PhD-students having known each other since the first semester of our bachelor, I have been able to take examples from you, receiving good advice and most importantly, having quality time with tapas and lots of cake. Last but not least, I am grateful to my family and friends for support and oasis. I appreciate how my family have been able to keep me busy with being a mom, a girlfriend, a housekeeper and so much more – so that work has only rarely taken up too much of my time.

Summary

The present thesis focus on health effects in humans after controlled exposure to indoor particulate air pollution. The experimental work performed in relation to the thesis consists of two controlled human exposure studies and the subsequent analyses of the exposures and the related health outcomes.

As we spend up to 90% of our time indoors, our health and well-being are affected by our indoor climate. Growing evidence suggests that particle pollution is associated with a variety of adverse health effects ranging from inflammation to cardiopulmonary disease including cancer. Cooking, candles and electronic cigarettes (e-cigarettes) emit high amounts of fine and ultrafine particles. Ultrafine particles are so small in size, that they can penetrate into the deepest regions of the lungs, potentially translocate into the blood stream, from where they can access vital organs such as the heart and brain. Furthermore, people with respiratory disease are known to be more susceptible to particulate air pollution than the background population due to chronic inflammation in the respiratory tract.
The aim of the present PhD study was to examine acute health effects from short-term exposure to cooking, candles and passive vape from e-cigarettes, respectively, among people with respiratory disease. In Project PASVAP, 16 individuals with COPD were exposed to passive vape from e-cigarettes and clean air as a control exposure. In the UltraFine Project, 36 young asthmatics were exposed to three different experimental sessions; emissions from cooking, candles, and clean air. In both studies, a randomized double-blind crossover design was applied. Participants were exposed for several hours under controlled environmental conditions in a full-scale exposure chamber. During exposures, particle characteristics including size distributions were measured, and participants reported symptoms of irritation. Objective health outcomes were evaluated at baseline and at selected time points following exposure.

The results of the two studies are reported in three papers focusing on (I) acute health effects associated with passive vape exposure from e-cigarettes, (II) the effect of cooking and candle exposure on acute inflammation in the respiratory tract and self-reported symptoms, and (III) the association of cooking and candles with airway and systemic biomarkers.
In conclusion, the present thesis demonstrates that short-term exposure to passive vape from e-cigarettes was associated with acute small responses in lungs and blood as well as throat irritation indicating inflammation in individuals with COPD. Exposure to emissions from cooking and candles were associated with lower self-reported well-being in young individuals with mild asthma, while objective markers changed in the respiratory tract and blood pointing to the existence of mild inflammation.
The findings add new perspectives of indoor particulate air pollution on health. Individual action and regulatory strategies for reducing the exposures could be a method for reducing disease related to indoor air pollution in the population – particularly among vulnerable subgroups. However, there is still a need for further research of the exposures on health in order to elucidate potential pathways of disease.

Resumé (in Danish)

Denne afhandling fokuserer på nogle af de sundhedsskadelige effekter, der kan opstå blandt mennesker efter kontrolleret eksponering for indendørs partikelforurening. Det eksperimentelle arbejde, som er udført i forbindelse med afhandlingen, omfatter to kontrollerede humane eksponeringsforsøg og efterfølgende analyser af eksponeringerne og relaterede helbredseffekter.

Vores indeklima har stor betydning for vores helbred og velvære, da vi opholder os op mod 90% af vores tid inden døre. Stigende evidens peger på, at partikelforurening er associeret med uønskede helbredseffekter fra irritation til hjerte- og lungesygdomme heriblandt kræft. Madlavning, brændende stearinlys og elektroniske cigaretter (e-cigaretter) afgiver høje koncentrationer af fine og ultrafine partikler. Ultrafine partikler er så små, at de kan nå ned i de dybeste luftveje, alveolerne, hvorfra de kan trænge over i blodbanen. Med blodet kan de ultrafine partikler nå ud til vitale organer såsom hjerte og hjerne. Personer med lungesygdomme er desuden mere sårbare over for luftforurening end baggrundsbefolkningen grundet deres kroniske betændelsestilstand i luftvejene.
Formålet med indeværende PhD-afhandling var at undersøge de akutte helbredeffekter af korttidseksponering for henholdsvis stegeos, brændende stearinlys og passiv damp fra e-cigaretter blandt personer med lungesygdom. I Projekt PASVAP blev 16 personer med kronisk obstruktiv lungesygdom (KOL) udsat for henholdsvis passiv damp fra e-cigaretter og ren luft som kontroleksponering. I UltraFine-Projektet blev 36 unge astmatikere udsat for tre forskellige eksponeringer; stegeos, brændende stearinlys og ren luft. I begge studier anvendtes et randomiseret dobbeltblindet overkrydsningsdesign. Deltagerne blev eksponeret i op til fem timer under kontrollerede forhold i et stort eksponeringskammer. Under eksponeringerne blev partikelkarakteristika, herunder størrelsesfordelinger, målt og deltagerne rapporterede symptomer relateret til irritation og generelt velbefindende. Objektive helbredsudfald blev undersøgt før og ved fastsatte tidspunkter efter eksponering.

Resultaterne fra de to studier er formidlet i tre artikler omhandlende (I) de akutte helbredseffekter associeret med passiv damp fra e-cigaretter, (II) effekterne af stegeos og stearinlys på akut inflammation i luftvejene samt selvrapporterede symptomer og (III) sammenhænge mellem stegeos og stearinlys og respiratoriske og systemiske biomarkører. Indeværende afhandling viser, at udsættelse for passiv damp fra e-cigaretter var associeret med akutte små reaktioner i lunger og blod såvel som irritation i svælget, hvilket tilsammen tyder på inflammation. Efter udsættelse for henholdsvis stegeos og stearinlys, rapporterede de unge individer med mild astma forringet velvære og målinger af biomarkører viste forandrede niveauer i luftvejene og i blodet, hvilket antyder tilstedeværende let inflammation.
Disse resultater tilføjer nye perspektiver på indendørs partikelforurenings betydning for vores helbred. Individuelle tiltag og nationale retningslinjer kan bidrage til at mindske sygdom relateret til et dårligt indeklima – ikke mindst blandt sårbare grupper i befolkningen. Det er dog nødvendigt med yderligere forskning af eksponeringernes påvirkning af vores helbred for at belyse om de påviste følger kan føre til sygdom ved daglig eksponering igennem lang tid.

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List of Original Papers

The thesis is based on three enclosed papers. In the thesis, these papers are referred to by their roman numbers:

Paper I

Karin Rosenkilde Laursen, Jakob Hjort Bønløkke, Elisabeth Bendstrup, Merete Bilde, Marianne Glasius, Vibeke Heitmann Gutzke, Shamjad Puthukkadan Moosakutty, Anna-Carin Olin, Peter Ravn, Kirsten Østergaard & Torben Sigsgaard (2021). *An RCT of acute health effects in COPD-patients after passive vape exposure from e-cigarettes*, European Clinical Respiratory Journal, 8:1, 1861580, doi: 10.1080/20018525.2020.1861580

Paper II

Karin Rosenkilde Laursen, Berit Brøndum Rasmussen, Bernadette Rosati, Vibeke Heitmann Gutzke, Kirsten Østergaard, Peter Ravn, Søren Kenneth Kjærgaard, Merete Bilde, Marianne Glasius, Torben Sigsgaard (2021). *Acute health effects from exposure to indoor ultrafine particles – A randomized controlled crossover study among young mild asthmatics*. Indoor Air. doi: 10.1111/ina.12902 (Epub ahead of print).

Paper III

Karin Rosenkilde Laursen, The Climate Chamber Group, Nichlas Vous Christensen, Frans AA Mulder, Jörg Schullehner, Hans Jürgen Hoffmann, Annie Jensen, Peter Møller, Steffen Loft, Anna-Carin Olin, Bernadette Rosati, Merete Bilde, Marianne Glasius, Torben Sigsgaard. *Airway and systemic inflammation biomarkers after short-term exposure to indoor ultrafine particles – A randomized controlled double-blind crossover study among mild asthmatic subjects* (Manuscript in preparation for *Particle and Fibre Toxicology*)

Abbreviations

CCL2: C-C motif chemokine ligand 2
CI: Confidence Interval
CO_2: Carbon dioxide
COPD: Chronic Obstructive Pulmonary Disease
CRP: C-reactive protein
E-cigarette: Electronic cigarette
E-liquid: Liquid used in electronic cigarettes (also termed “e-juice”)
EPCs: Epithelial progenitor cells
FeNO: Fractional Exhaled Nitric Oxide
FEV_1: Forced Expiratory Volume in 1 second
FVC: Forced Vital Capacity
GlycA: Glycoprotein acetylation
LABA: Long-acting $beta_2$-agonist
LAMA: Long-acting muscarinic antagonist
LDL: Low density lipoprotein
HEPA filter: High efficiency particulate air filter
IL: Interleukin
$\mu g/m^3$: Microgram per cubic meter
NO_2: Nitrogen dioxide
O_3: Ozon
PExA: Particles in Exhaled Air
PG: propylene glycol
PM: Particulate Matter
$PM_{0.1}$: Particulate Matter with aerodynamic diameter < 0.1 µm
$PM_{2.5}$: Particulate Matter with aerodynamic diameter < 2.5 µm
PM_{10}: Particulate Matter with aerodynamic diameter < 10 µm
Ppb: Parts per billion
Ppm: Parts per million
RCT: Randomized Controlled Trial
RH: Relative Humidity
SABA: Short-acting $beta_2$-agonist
SAMA: Short-acting muscarinic antagonist
SD: Standard Deviation
SMPS: Scanning Mobility Particle Sizer
SP-A: Surfactant Protein-A
T°C: Temperature in degrees Celsius
UFPs: Ultrafine particles
VAS: Visual Analogue Scale
VG: vegetable glycerine
VLDL: Very low density lipoprotein
VOC: Volatile Organic Compound
WHO: World Health Organization

Chapter 1. Introduction

A home is believed to be a good, safe and secure environment. However, a home may also be the source of various pollutants that may have significant adverse impact on health (1,2). Indoor air pollution is recognized as a significant contributor to morbidity and mortality throughout the World (3), and particles have been identified as one of the most important indoor air pollutants (4).
Particle contamination of the indoor air is suggested to have substantial negative effects on health, with cigarette smoking, cooking and candles being major contributors to indoor particulate air pollution (1,5,6). While the harmful effects of conventional cigarette smoking has been scrutinised and proven for years, there is an urgent need for research about an emerging issue in indoor air pollution: exposure to aerosol from electronic cigarettes (e-cigarettes). The use of e-cigarettes has increased worldwide and so has exposure to second-hand aerosol also termed “passive vape” (7).
Chronic low levels of exposure to indoor particles over time is an important risk factor for the health of the general population and it becomes particularly important for vulnerable individuals such as those with respiratory disease (8).
In order to reduce morbidity and mortality caused by indoor air pollution, there is a need to develop effective preventive strategies, hence, it is imperative to know if and how indoor particles affect our health. The overall aim of this thesis is to increase the understanding of the associations between indoor particulate air pollution from e-cigarettes, cooking and burning candles, and health and well-being among people with respiratory disease.

In the thesis, the **background** explains the rationale behind the study and introduces various important concepts. Additionally, it reviews the literature, finally identifying unresolved issues and knowledge gaps. The **methods** section describes the two randomized controlled exposure studies performed as a part of the PhD-project including the rationale behind the methodological choices made. The **results** section presents the main results regarding exposures and health outcomes from both studies. In the **discussion**, the findings are compared to the current literature in the field followed by a critical evaluation of the methodology. The **conclusion** summarizes the findings and in the **implications** section, further research and possible preventive measures based on the findings are suggested.

Chapter 2. Background

This chapter begins by explaining the rationale behind the study, followed by introducing various concepts of importance to the thesis. Scoping literature reviews are presented on 1) the health effects resulting from exposure to active and passive vape from electronic cigarettes, and 2) health effects related to cooking and candle emission exposure. Finally, unresolved issues and gaps in the current evidence are outlined leading to the aims and hypotheses under study in the present thesis.

2.1 Indoor air pollution

Air pollution can best be described by the fact that some substances appear in inconveniently high concentrations, often in a restricted area. By definition, air pollution "is the introduction of chemicals, particulate matter, or biological materials into the air that cause harm or discomfort to humans or other living organisms or damages the natural environment" (9). Air pollutants comprises a mixture of solid particles, liquid droplets and gases (10).
Indoor air pollution, often termed household air pollution, refers to physical, chemical and biological contamination of indoor air (10). The United States Environmental Protection Agency (EPA) defines indoor air *quality* as "the air quality within and around buildings and structures, especially as it relates to the health and comfort of building occupants" (11).

Although outdoor air pollution associated with traffic and industry first brought the issue of air pollution health effects to public attention, there is a growing recognition that indoor air pollution is of equal or even greater importance to human health (3,12). Reasons for the rising attention to indoor air quality effects on health include people spending more time indoors than outdoors; the widespread range of emission sources inside; and the increased concentrations of some pollutants indoors compared to outdoors due to insulation of buildings (2,13). The World Health Organization (WHO) calls household air pollution "the World's largest *environmental* health risk factor" for death and disability in the World and estimates that indoor air pollution is contributing to a far greater burden of disease than outdoor air pollution, both in low-income and high-income countries (3). Exposure to household air pollution is among the top ten risk factors for disease and one of the leading risk factors for death globally causing millions of deaths each year (3,14–17).
Evidence suggests that the burden of disease due to indoor air pollution is disproportionately distributed with the highest prevalence of morbidity and mortality observed in low-income countries (13,15,16). This disparity is mainly explained by inefficient combustion of solid fuels as more than one third of the World's population in low-income countries rely on biomass fuels (wood, charcoal, agricultural waste and animal dung) for cooking and heating, thereby emitting high concentrations of both gaseous and particulate pollution within households (2,15). Indoor concentrations of air pollution in high-income countries are much lower than in low-income countries, generally due to the improvement in technology for common household activities and the use of clean fuels (mainly electricity and gas) for cooking and heating (2,18). Yet, there are still significant risks to health from indoor air in high-income countries for reasons described below (2). This thesis focus on indoor air pollution in high-income countries.

In high-income countries, indoor air quality is crucial, considering the amount of time people spend indoors. Observational studies have found that people spend an estimated 80-90% of their time indoors and approximately 60% of the time in their home depending on climate, occupation, age, and health status (13,19–21).
The quality of indoor air is affected by a complex interaction of both indoor and outdoor pollutants (1,2,5,22), with outdoor sources comprising of ambient pollution from the ground, pollen, dust and air pollution emitted from traffic and industry (22). Indoor air pollutants include chemical emissions of volatile organic compounds (VOCs), radon from the ground and formaldehyde from building materials, allergens, molds, etc. Combustion products from gas heating, fireplaces, cooking, candlelight burning, and tobacco smoke affect the composition and concentration of indoor particles (1,2,5). Additionally, factors including ventilation conditions, temperature and humidity impact on the indoor air quality (1,2,22). Consequently, the way we live and behave in our homes has substantial effects on our health and well-being (23).
Although clean fuel sources of household energy predominate in high-income countries, changes in building design devised to improve energy efficiency by thermal insulation and inadequate ventilation provide indoor environments in which contaminants are readily produced and may accumulate to much higher concentrations than found in ambient air (2,24–27). High concentrations occur during cooking and candle burning with elevated pollution levels able to persist for hours (1,27). In western countries, an open kitchen-dining area is a popular choice when rebuilding old and designing new houses. However, the combination of an open kitchen and energy-efficient building construction places great demands on the impact of mechanical ventilation.

Prolonged exposure to indoor air pollution may lead to adverse health effects, even at low concentrations as accumulation of pollutants in the human body may occur (28). A poor indoor climate can cause tiredness, headache, allergic symptoms, and in the worst cases severe diseases such as respiratory disease, cardiovascular disease and cancer (2,23,24,29,30). Particle contamination is suggested to have substantial negative effects on health affecting more people than any other pollutant (4), with cigarette smoking, candlelight burning and cooking emitting the largest amount of particles, thus being the largest contributors to indoor air pollution in residential housing (1,5,6,27,31,32).
Cooking is an important aspect of everyday life and the general population is exposed to cooking emissions regardless of age, wealth and cultural food preferences (1,33). In western countries a considerable part of the population burn candles daily, while in Denmark during wintertime 39% of the population burn one or several candles daily or nearly every day (1,23,34). Indoor smoking behaviour varies across homes, but when present, tobacco smoke is the major source of indoor particulate matter (2). While the harmful effects of conventional cigarette smoking has been scrutinised and proven for years, and several preventive measures have been established (12), there is an urgent need for research related to an emerging issue in indoor air pollution, electronic cigarettes (e-cigarettes), as the aerosols have been found to be a major source of indoor ultrafine particles (27,31).
As e-cigarettes are a relative new phenomenon, and not as well-known as cooking and candles, an introduction is given below.

2.2 An introduction to electronic cigarettes

Electronic cigarettes, also known as electronic nicotine-delivery systems (ENDS), were developed in China in 2003, and since the introduction to the European and U.S markets in 2006-2007, they have become popular (35–38).

An e-cigarette is an electronic device made to simulate tobacco smoking. The device is designed to generate an aerosol – that may or may not contain nicotine – without combustion of tobacco. Different kinds of designs exist and e-cigarettes can be grouped into three basic types; 1) first-generation e-cigarettes: disposable products called cig-alikes, 2) second-generation e-cigarettes: reusable, rechargeable kits that are designed to be refilled with liquid by the user (often referred to as tank style e-cigarettes), and 3) third-generation e-cigarettes: reusable, rechargeable kits that allow the user to customize their product (see Figure 2.1) (39,40). A typical e-cigarette is composed of three integrated parts in a stainless steel shell: a battery, a coil (a heating element), and a cartridge or a tank that contains e-liquid (41). E-liquid (also known as e-juice) is the fluid that fuels the e-cigarette. The e-liquid is what provides the nicotine solution and the flavoring to the aerosol (42). Flavor options are numerous and include tobacco, menthol, candy, coffee, cake, fruits and many more (43).

Figure 2.1. Examples of different electronic cigarettes. From the left: 1) disposable style, 2) reusable tank-style and 3) reusable customizable style e-cigarette. Picture from the National Academies Press (44).

When users, often referred to as vapers, puff on an e-cigarette, the heating element is activated converting the e-liquid in the cartridge into an aerosol (commonly called vapor) that is inhaled into the lungs. In contrast to conventional cigarettes, which continuously emit smoke including particles from the combustion process itself, aerosol from e-cigarettes is only released during exhalation (see Figure 2.2) (41). One study estimated that more than 70% of inhaled aerosol is eventually exhaled to the surroundings (45).

E-cigarette aerosol contains toxic chemicals similar to tobacco products (46), hence, besides health effects among users themselves, a relevant issue related to e-cigarettes is its contamination of the ambient air causing “passive vaping” i.e. inhalation among bystanders (47). Passive vaping is considered a health problem as the concentration and number of pollutants in ambient air have been associated with potential adverse health effects (41,48–50).


Figure 2.2. Aerosol exhaled by e-cigarette users. Pictures from: Colourbox.dk (AU license).

When e-cigarettes were introduced as a market product, they entered out of thin air with the e-cigarette industry using campaign strategies seen from conventional tobacco products (51); E-cigarettes were marketed as a more appealing alternative to tobacco smoking in terms of social tolerance and health risks (51,52). Public authorities were not aware of this new product before sale – and use – exploded, why regulation has been executed ad hoc (53,54). Currently, the legislation of e-cigarettes is unclear in many countries; sale is often regulated, however, use is not (53,55). Thus, use of e-cigarettes is often permitted in otherwise smoke-free areas (46,56) causing high levels of indoor particles and consequently, passive exposure of individuals to the aerosol (48,57,58). E-cigarettes are used in many indoor places, such as restaurants, workplaces and residential homes (46). Use in indoor environments is of special concern as the emitted pollutants are not diluted as rapidly as outdoors.
Uncertainties about their impact on indoor air quality and health are causing debate among scientists and public health experts (46,59,60). Those favoring e-cigarettes (e.g. Public Health England) consider it as "harm reduction" as they perceive e-cigarettes as a long-wanted healthier alternative to conventional cigarettes (51,61,62) and as a smoking cessation tool, however, lacking supporting scientific evidence (63). Those cautioning restraint worry about the possible effects e-cigarette products may have on tobacco control measures (64). A concern is that individuals smoking conventional cigarettes, will use e-cigarettes in nonsmoking environments, while continuing their use of conventional cigarettes in areas where smoking is permitted. This is known as dual use (65). Another concern is that e-cigarettes may be an appealing starter product for young nonsmokers (66) and may subsequently initiate use of other tobacco products potentially leading to renormalization of smoking behavior (40,46,51,64). Finally, e-cigarettes are criticized for the unknown effects on health (51,64). At present millions of people are using e-cigarettes worldwide and exposure to the aerosol has become a serious public health concern (7,64,67).

2.3 Particulate matter

In the following section, particles are defined and described by subgroups and their deposition and translocation in the human body. Next, the composition and size distribution of each of the three exposures included in the thesis are outlined.

2.3.1 Definition and subgroups of particulate matter

Particulate matter (PM) is a common proxy indicator for air pollution (10). PM is a complex mixture of extremely small solid particles and liquid droplets suspended in the air (10,68,69).

These particles come in various sizes and shapes and can be made up of hundreds of different chemicals (10).
Description and sampling of particles are based on their aerodynamic diameter measured in micrometers (μm), usually referred to as particle size (69). Based on size, particulate matter is divided into three main groups: Particles that are >2.5 and ≤ 10 μm in diameter are considered "coarse". "Fine" particles are defined as ≤ 2.5 μm in diameter and are designated as $PM_{2.5}$. "Ultrafine" particles (UFPs), also referred to as nanoparticles, are ≤ 0.1 μm (100 nm) in size. They are designated as $PM_{0.1}$ (68–70).
Most of the total mass of airborne PM is usually made up of coarse and fine particles. While UFPs often contribute only very little to the total particle mass, they are the most numerous, representing more than 90% of the number of particles (31,69). Consequently, fine and coarse particles tend to dominate the particle mass size distribution, whereas UFPs tend to dominate particle number size distribution (70). Particle characteristics according to size are outlined in Table 2.1.

Table 2.1. Particle characteristics according to size

	2.5-10 μm (coarse)	**≤ 2.5 μm (fine)**	**≤ 0.1 μm (ultrafine)**
Total mass	1	1	1
Particle number	1	64	1,000,000
Surface area per particle	1	0.0625	0.0001
Total surface area per mass	1	4	100
Deposition	Filtered in proximal airways	Reaches peripheral airways	May enter systemic circulation

Table inspired from Kwon 2020 (31). Comparison of the surface area of particles with different diameters. The mass, particle number, and surface area of coarse particles are all arbitrarily designated as 1. Other numbers are relative to the coarse particle.

When reporting daily or annual mean concentrations of the air quality, measurements are usually reported in terms of PM_{10} or $PM_{2.5}$ particles per cubic meter of air volume (m^3), with PM_{10} referring to particles with an aerodynamic diameter ≤ 10 μm (10). Routine air quality measurements typically describe such PM concentrations in terms of micrograms per cubic meter ($\mu g/m^3$) (69). As UFPs reach only high concentrations in terms of their numbers, measurements of particles in the ultrafine range are often based on particle number rather than mass concentration.

2.3.2 Deposition and translocation of inhaled particles in the human body

During each inhalation, millions of particles enter the respiratory tract, where they may hit the surface of the conducting airways or the alveoli. The fate of the particles depend on several factors including the anatomical location of the deposition and the aerodynamic size of the particles in the respiratory tract during inhalation – with the latter being the most important factor in determining deposition probability (69,71,72). Toxicity and deposition of the inhaled particles further depend on exposure concentration, exposure duration, human breathing patterns (frequency and volume), proximity to the source, respiratory-tract anatomy

(including natural defenses and clearance mechanisms), and particle characteristics including hygroscopicity and shape of the particles (2,71,73).
Both toxicological and epidemiological studies indicate that small particles are more closely related to adverse health outcomes compared to larger particles (70,74). In order to understand the mechanisms behind the health responses to PM, it is vital to understand the deposition pattern of the particles, as described below.

Exposure to PM and related air pollutants can occur directly through inhalation or through the skin interface (75), with the primary exposure route in humans being through inhalation (75,76). While PM with diameters up to 100 µm is inhalable, only particles less than 10 µm in diameters (PM_{10}) are considered to enter the respiratory tract and pose a health concern (3,77). Coarse particles primarily deposit in the upper respiratory tract, in the throat and in the upper part of the trachea (see Figure 2.3), where they are subsequently removed by coughing or they are swallowed with the saliva (77,78). Such process of clearing particles from the respiratory tract might induce physiological responses such as inflammation (79).

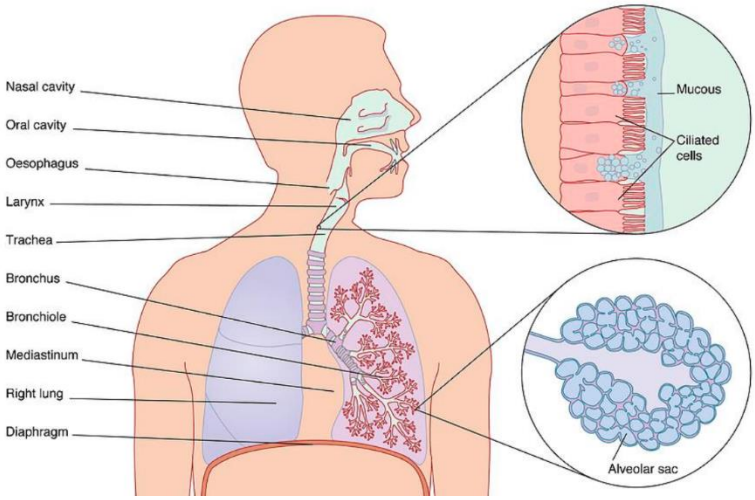

Figure 2.3. Various stages and structures of the human respiratory tract. Photo credit: Peter Gardiner / Science Photo Library.

Fine particles

Fine particles ($PM_{2.5}$) typically penetrate deep into the respiratory tract entering into the trachea and bronchi (conducting airways) where they after deposition may be removed by the cilia. In the conducting airways, the epithelium is ciliated and covered by mucus producing cells, and particles are trapped in the mucus layer and brushed up by the continuously beating cilia. Particles are moved up into the throat, from where they are subsequently swallowed. This process is called the mucociliary escalator, which is the major natural defense mechanism for deposited particles in the conducting airways (77). This process can occur within a few hours (77,78). When particles deposit in the conducting airway, they have the potential to reduce resistance to infection by damaging the ciliated epithelial cells (70). Fine particles are typically found in great numbers and due to a surface area larger than coarse

particles, fine particles have been suggested to have greater potential for interactions with biological targets and thereby cause higher inflammatory response (31,70,73).

As seen from Figure 2.4, fine and ultrafine particles can manage to pass through the conducting airways, penetrating deep into the lungs and, potentially depositing in the alveoli (28,77). Some fine particles, and in particular UFPs (≤ 0.1 μm) are not filtered out by the nose and bronchioles – thereby escaping the normal clearing mechanisms of the mucociliary escalator. Their size allows them to be breathed deeply into the terminal parts of the respiratory tract, into the alveolar regions, where gas exchange with the red blood cells takes place (77,80). When particles deposit this deep in the lungs, where the epithelium is extremely thin and not ciliated, clearance is accomplished by alveolar macrophages (77). However insoluble, persistent particles are not easily phagocytized (i.e. removed) by alveolar macrophages (77,80,81). Once trapped in the distal part of the lungs, these particles can cause structural and chemical changes to the lung tissue (70). Removal of insoluble particles from the alveoli may take years, and prolonged retention of particles lead to accumulation of inhaled material within the tissue, thereby increasing lung burden, which may induce chronic and severe inflammatory conditions (28,78).

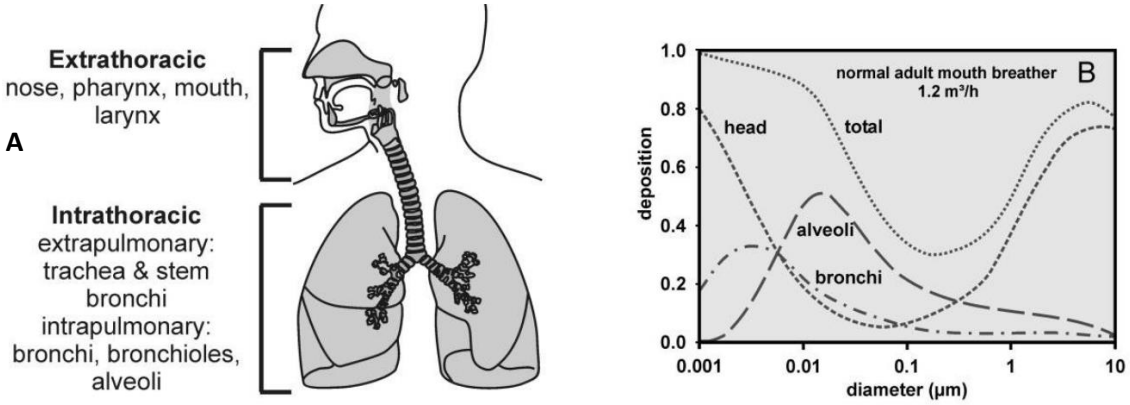

Figure 2.4. (A) The respiratory tract (B) and particle deposition efficiency of inhaled particles from 1 nm to 10 μm in a healthy adult individual assuming mouth breathing. Figure from (28).

Ultrafine particles

UFPs ($PM_{0.1}$) deposit by diffusional mechanisms (71,77). The deposition of UFPs is known to take place in the nasopharyngeal, tracheobronchial, and in the alveolar region (82), with the main part of the deposition occurring in the alveolar region (Figure 2.4) (71,77). It is noteworthy, that within the ultrafine size range there are significant differences in their deposition probabilities along the human respiratory tract as shown in Figure 2.4 (28,82). UFPs below 10 nm are able to deposit in the nasal cavity, from where they may enter the brain by the olfactory nerve (77,83). Particle translocation along the olfactory nerve is considered to be the shortest and most direct path to the brain (77).

An important characteristic of UFPs is their ability to enter circulation (31). UFPs are able to translocate across the blood-air barrier to be absorbed directly into the blood stream. From here, they are being distributed throughout the body, translocated to organs like the liver,

heart and brain (28,84). Once these toxic particles are in the tissues, they are very difficult to eliminate and significant oxidative damage can be caused (75).
Besides their ability to enter circulation, one of the most significant characteristics of UFPs, that make them more toxic than larger particles, is their large surface area (31,73). Due to a large surface area, UFPs can carry large amounts of toxic compounds on their surfaces, consequently being highly chemically reactive (70). Particles interact with or adsorb many types of toxic chemicals such as diverse polycyclic aromatic hydrocarbons (PAH), hazardous metals, and organic compounds, which significantly contribute to their toxic health effects (31,70,75). Additionally, UFPs consist of a larger number of particles than larger particles of the same mass, allowing dispersion into many more cells (28). Daily exposure to particles including adsorbed toxicants cause constant damage throughout the body and chronic exposure contributes to the risk of causing adverse effects on health (70,72).

2.3.3 Hygroscopicity

Instead of the measured dry size, it is the size of the particles *inside* the lungs, that determines deposition (71,72). For UFPs, the deposited dose and consequently, the health response are strongly influenced by their hygroscopicity (71,77). The air inside the respiratory tract has high relative humidity (RH ~99.5%). When inhaled, some particles will absorb water vapor from the humid air and grow in size. Since the deposition of particles in different compartments of the respiratory tract depends on the size of the particles (Figure 2.4), deposition will be different for the particles that grow from those that do not (71). Inhaled hygroscopic (water-absorbing) particles will grow by absorbing water vapor, which decreases their diffusion rate and hence the probability of deposition (71,77). Contrary to hygroscopic particles, hydrophobic (water-resisting) particles will not grow inside the respiratory tract. Research in ambient particles have found that particles typically range from nearly hydrophobic to very hygroscopic determined by their source, formation and transformation processes. At the relative humidity in the lungs, particles have been found to grow by a factor 1 (no growth) to 5 in diameter depending on dry size and chemical composition (71).

2.4 Chemical composition and particle size distributions of exposures

Particles in indoor air are a complex mixture and might be quite diverse in terms of size and chemical composition. Thus, particles from different emission sources may cause different biological response owing to their unique physicochemical properties as described above (75,77). The current knowledge regarding chemical composition of emissions and particle size distribution of the three exposures included in the thesis are outlined below.

Table 2.2. Chemical compounds of the exposures

E-cigarette aerosol	Cooking emissions	Candle emissions
Glycerol, propylene glycol, aldehydes (formaldehyde and acetaldehyde), PAH, carcinogenic tobacco-specific nitrosamines, silicates. Particulate matter. Metals. Nicotine. Dicarbonyls and hydroxycarbonyls.	Fatty acids and dicarboxylic acids (free fatty acids, free glycerol and mono- and di-glycerides). Alkanones (primarily 2-pentadecanone), alkanals, lactones, PAH, sterols and alkanes. Organic and elemental carbon. VOCs such as aldehydes (including formaldehyde, acetaldehyde, acrylamide and acrolein).	Elemental carbon (soot) and organic carbon. Inorganic salts such as phosphates, particularly ammonium phosphates, alkali nitrates and potassium. PAH. Low levels of VOCs such as 1-butanol and toluene.

Abbreviations: polycyclic aromatic hydrocarbon (PAH), Volatile Organic Compounds (VOCs)

2.4.1 E-cigarette aerosol

Composition of the aerosol varies within and across e-cigarettes. Inconsistency of both the device performance properties (brand and design, heating coil temperature, power voltage), factors related to the e-cigarettes user (flow rate and puff duration, inhalation, and level of user experience), and the uniqueness of the e-liquid compositions have been shown to affect particle concentrations and size distributions (42,85–88).

Generally, a high percentage of the e-liquid is composed of carrier solvents, such as vegetable glycerine (VG) and/or propylene glycol (PG), flavouring ingredients and nicotine (42,46).

Composition of the aerosol

As shown in Table 2.2, the most commonly reported chemicals in mainstream and secondhand aerosol include glycerol, propylene glycol, aldehydes, carcinogenic tobacco-specific nitrosamines (TSNA), silicate particles, and polycyclic aromatic hydrocarbon (PAH) among other components (42,57,67). PAH are organic molecules, some of which are known carcinogens (89). Moreover, the aerosol usually contains metals, volatile organic compounds (VOCs) including acetone, in addition to fine and ultrafine particles, and nicotine (41,42,61,67,86,90). The source of metals such as copper, cadmium, nickel and lead are most likely the metal-coated wires part of coil (86). Dicarbonyls and hydroxycarbonyls are also thought to be important compounds in the aerosol (61,67,91,92). Furthermore, studies of potentially toxic substances in e-cigarette aerosol have shown that known and suspected carcinogens, such as formaldehyde and acetaldehyde are present (59,67,91,93). As both glycerol and propylene glycol are hygroscopic, e-cigarette particles tend to grow by uptaking water vapor from the humid air in the human respiratory tract (94,95).

Particle size distribution

During vaping $PM_{0.1}$ and $PM_{2.5}$ is found to be elevated up to 188 times higher indoors when compared to background levels (48,67,86). Particle size distributions of emissions from e-cigarettes vary across studies, however, particles are primarily in the fine and ultrafine size

ranges (49,57,86,87,96,97). Examining aerosol generated by humans, studies have observed peaks at diameters sized 24-36 nm (57,98), while other studies found that e-cigarette aerosol is exhibiting a bimodal size distribution: one maximum has been found in the range of ~15-30 nm and one in the range of ~85-100 nm (49,86,87). One study measured a shift in the size distribution with increasing temperature; the higher the temperature of the coil, the lower the particle diameter. The size-distribution shifted from a bimodal size distribution with maximum around 60 and 100 nm to a single-mode distribution with a maximum around 45 nm (49). Fuoco et al. found main particle modes for diameters >120 nm for several e-cigarettes with varying flavours and nicotine content (97).

2.4.2 Cooking emissions

Different cooking styles emit different profiles of compounds and varying PM levels. The differences have been attributed to factors such as cooking processes (frying, roasting, grilling, boiling and broiling), ingredients, and temperature (18,99,100). In general, frying results in higher peak mass concentrations than boiling and cooking in the oven, gas stoves emit higher indoor concentrations of particles compared to electric stoves, and particle emission depend on the temperature of the stove (18,99–101). Higher emission rates are generated by foods containing more fat than foods containing less fat (18,99,101). Asian style cooking is found to emit more PM than Western cooking (18) – most likely due to frying procedures.

Composition of cooking emissions

Cooking fumes contain carbonaceous particles including organic and elemental carbon, with organic carbon being the major constituent (18,102). In a review focusing on cooking using electricity and gas, major groups of emitted chemical compounds were fatty acids and dicarboxylic acids (18). Other constituents were alkanones (primarily 2-pentadecanone), alkanals, lactones, PAHs, sterols and alkanes as reported in Table 2.2 (18,102). In general, some differences between cooking styles have been observed: grilling of meat leads to high production of aerosols made of fatty acids; cooking of meat produces greater PAH concentrations than frying vegetables; higher concentrations of organic compounds have been observed to be emitted during oil-based cooking compared to steaming and boiling which are water-based; and deep frying generates more PAH than other cooking methods due to the high temperature during cooking and the large amount of oil used (18). Meat and oils used in cooking contain saturated and unsaturated fatty acids leading to the production of free glycerol, free fatty acids, and mono- and diglycerides (18). The International Agency for Research on Cancer (IARC) has reported that cooking generates substantial amounts of known and suspected carcinogens including aldehydes such as formaldehyde (103), acetaldehyde (incl. nonanal) (104), acrylamide (105) and acrolein (106). Usually, freshly emitted cooking organic aerosols are hydrophobic (i.e. water-resistant) (107).

Particle size distribution

Particles generated from cooking are generally within the fine and ultrafine particle size ranges (18,108–110). In a comprehensive review of particulate matter from cooking, Abdullahi et al. report, that the largest amount of the measured particles in the included

studies was in the ultrafine size range, with modes in number distribution reported primarily in the range of 20-100 nm (18). In an exposure study cooking frying sausages, the majority of particles were in the size range of 50-100 nm (109). In the HOMEChem test house study (in Texas, USA), they found that for all meals examined (breakfast, lunch, dinner and Thanksgiving dinner event) ultrafine particles dominated number concentrations with number geometric mean diameters < 20 nm (110).

2.4.3 Candle emissions

In the following section emphasis is on stearin candles as candles composed of other materials (e.g. wax and paraffin) emit particles with different chemical compositions and particle size distributions (111). Stearin for candles is a mixture of mainly two fatty acids: stearic and palmitic acid from animal or vegetable fats and oils. Candlewicks are typically made of intertwined cotton threads and treated with inorganic salts acting as flame-retardants controlling the candle burn rate and thereby flame height and stability of the combustion process (111,112). Metals such as lead and nickel have been detected in candlewicks (112). The condition under which a candle burn, affects the size of particles and the chemical composition of emissions. Burning conditions can be classified into *steady burn* and *sooting burn* (111–113). During steady burning of a candle, the majority of the flame emits a yellow glowing light, which is the result of soot oxidation in the flame. Contrary, incomplete combustion, allowing soot to escape without being oxidized, is referred to as sooting burn mode. Sooting burn is caused by a flickering flame due to air approaching the flame in a horizontal manner. Air movements close to the flame, for example due to draught from a window or sudden air movements, result in a flickering flame (113).

Composition of candle emissions

There are still knowledge gaps regarding the composition of the particles emitted from candles (112,113). However, efforts to characterise particle emissions have been made in several studies. Existing literature shows that during steady burning, candles emit a relatively high number of ultrafine particles dominated by hygroscopic inorganic salts such as phosphates, particularly ammonium phosphate, alkali nitrates and to a lesser degree potassium salts (Table 2.2) (102,111–114). Most likely, the source of these particles is additives to the wick, such as ammonium phosphate added as a flame retardant (102,111,113). A flickering flame has been found to emit much higher levels of elemental carbon (i.e. soot), in an order of magnitude more, than a steady flame (113). Sooting burn experiments have shown that candles can emit high concentrations of elemental carbon, and that such burning conditions are associated with the highest mass emission factors compared to steady burning of a candle (113).

Particle size distribution

In a recent study of emissions from two types of stearin candles during steady burn conditions, the mean diameter of particles was below 10 nm (mean diameter from 7.4-8.0 nm), however, the particle number concentration above 40 nm was also significant (111). High number concentrations of ultrafine particles with a diameter below 10 nm is in agreement with studies on candles composed of paraffin, wax and beeswax (115,116). Other

studies find somewhat larger diameters for stearin candles burning in a steady mode, with particle modes being in the range 10-30 nm (109,112,113,117). In sooting burn experiments, the geometric mean diameter has been shown to increase in comparison to steady burn with mean particle diameters of candle soot being in the range of 240-300 nm (113,117).

2.5 COPD and asthma

In the studies part of this thesis, the participants suffer from chronic respiratory disease – either chronic obstructive pulmonary disease (COPD) or asthma. Chronic respiratory diseases are among the leading causes of mortality and morbidity worldwide, and among all chronic respiratory diseases, COPD and asthma are the most common (118). COPD and asthma are both categorized as "obstructive respiratory disease" where airway obstruction leads to an increased resistance during exhalation (118). Both are inflammatory diseases involving the small airways and causing airflow limitation and both are caused by gene-environment interactions (119). However, several distinctions between the two diseases exist and have implications for diagnosis, treatment, prognosis and prevention (119). An important difference between COPD and asthma is that while individuals with COPD have a fixed airway obstruction, individuals with asthma can have a normal or near-normal lung function between asthma-attacks (119). The characteristics of each of the two diseases are described below.

2.5.1 COPD

COPD is the name of a group of lung conditions that cause chronic irreversible airflow obstruction (120). COPD is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD, 2021) as *"a common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases"* (120). The most common respiratory symptoms include dyspnea, cough, mucus production and wheezing. Periods of acute worsening, called exacerbations, may happen and persist for several days (121). Often, COPD symptoms first appear when substantial lung damage exist, and for most people, symptoms worsen over time (120).

Patients with COPD can have both emphysema, chronic bronchitis or a combination of these (122). The relative contribution of chronic bronchitis and emphysema varies from person to person and so does severity (120). Depending on symptoms and clinical findings, several phenotypes have been described in the literature (120,123).
Chronic bronchitis is inflammation of the lining of the bronchi, which carry air to and from the alveoli of the lungs. The inflammation causes narrowed airways and mucus production, which can further block the narrowed airways (Figure 2.5). Individuals often develop a chronic cough trying to clear the airways (120).
Emphysema is a condition in which the walls (septum) between alveoli are destroyed as a result of damaging exposure to irritating gases and particulate matter. Emphysema leads to loss of elastic fibers of the lungs. These changes diminish the ability of the airways to remain open during exhalation and reduce exhalation air flow. As the surface area available for gas exchange is reduced due to one larger space instead of many small ones (Figure 2.6),

exhalation airflow and gas exchange are impaired. Mucociliary dysfunction (i.e. lack of mucociliary clearance of inhaled irritants) is a characteristic feature of the disease (120).

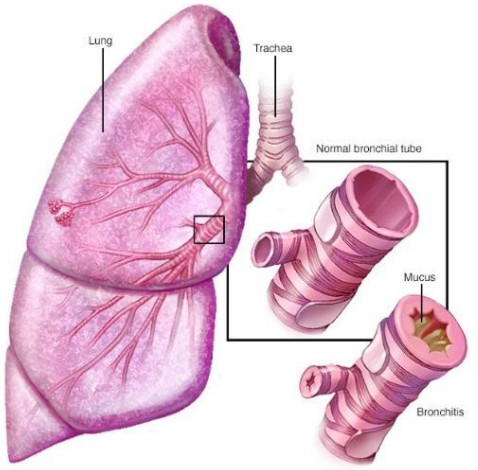

Figure 2.5. Chronic bronchitis with narrowed bronchial tubes. Picture from (124).

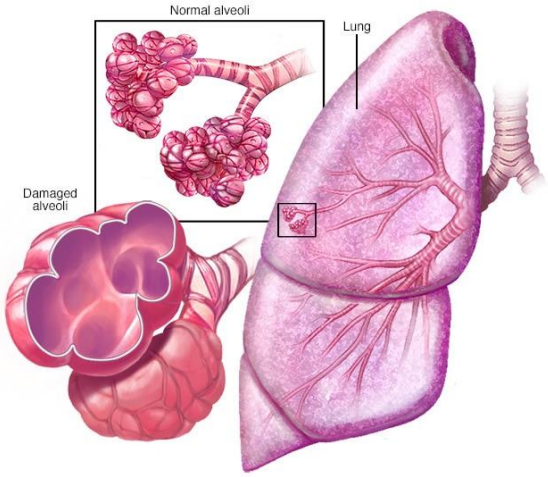

Figure 2.6. Emphysema with loss of elastic recoil. Picture from (124).

Diagnosis

Spirometry is the fundamental tool used to diagnose and stage COPD (120). While a number of different indices can be calculated from spirometry, the two central metrics are the forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC). FEV_1 is the amount of air that can be forcefully exhaled from the lungs in the first second after maximal inhalation, while FVC is the total amount of air that can be exhaled forcefully (125). Pulmonary function is strongly influenced by age, sex, height, and ethnicity and correct interpretation of spirometry results presuppose that these factors are accounted for (120). Obstruction is defined by the ratio between FEV_1 and FVC (FEV_1/FVC) below a threshold, however, there is no worldwide consensus about this threshold to define COPD (118). The two most used definitions for airflow limitation consistent with COPD are a "fixed cut-off" value of $FEV_1/FVC < 0.70$ (120) or the lower limit of normal (LLN) method of deriving a threshold as the 5^{th} percentile of values in a healthy non-smoking reference population (126). No universal LLN threshold exists as it is thought to vary between populations (118). A problem regarding the fixed cut-off value is that FEV_1/FVC decreases physiologically with age, resulting in over-diagnoses of obstruction in elderly and under-diagnosis of younger individuals (127). The European Respiratory Society (ERS) and American Thoracic Society (ATS) have agreed that the LLN is the correct diagnostic criterion for airflow limitation (128). However, other guidelines, including GOLD, recommend the use of a fixed-ratio for FEV_1/FVC for simplicity and a percent predicted FEV_1 to classify severity of impairment (120).

Risk factors

COPD results from a complex interaction between risk factors in the environment and in the individual (120). Risk factors are usually defined as determinants considered increasing the likelihood of developing a disease (129). The main risk factor for COPD is active tobacco

smoking, but other factors, such as occupational factors, infections, ambient and household air pollution, are becoming better known (118,120). Passive exposure to cigarette smoke may also be a risk factor for COPD by increasing the lungs total burden of inhaled particles and gases (118). The relative importance of risk factors varies between low-income and high-income countries (118). Furthermore, factors within the individual may predispose individuals to develop COPD. Such factors include genetic abnormalities, abnormal lung development and age (120).
Risk factors for exacerbations are respiratory infections, but they may also be triggered by environmental pollution and smoking. Up to 30% of exacerbations are of unknown etiology (121).

Prevalence
Existing COPD prevalence data vary widely due to differences in survey methods, analytical approaches and diagnostic criteria (118). Nonetheless, COPD is in the top 5 causes of morbidity and mortality globally (120). The Global Burden of Disease Study estimated that from 1990 to 2015, the prevalence of COPD increased by 44% to 174.5 million individuals having COPD worldwide (118). Population growth and ageing of the global population play a role in this increase. Globally, COPD affected 105 million males and 70 million females in 2015 (118), however, data from high-income countries show that the current prevalence of COPD is almost equal in men and women, which may be a result of the changing patterns of tobacco smoking (120). The prevalence of COPD is varying according to sex, age and country, as different risk factors are present (118,120). Despite prevalence being high in both low-, middle-, and high-income countries, 80-90% of deaths related to COPD worldwide occur in low- and middle-income countries (120,130).
COPD is one of the most common chronic diseases in Denmark (131). Between 110,000 and 130,000 people are treated medically for COPD, however, epidemiological studies indicate that approximately 400,000 Danish individuals (of a population of 5.8 million in 2020) have the disease, when including the mild cases. This is equivalent to a prevalence of 14% among adults aged 35 and above (131). Many individuals with COPD are not diagnosed or they are misdiagnosed (131).
In the coming years, COPD will likely increase due to aging populations and higher smoking prevalence (132,133).

2.5.2 Asthma
Asthma is an umbrella term for several chronic airway inflammatory diseases with similar clinical appearance but different underlying pathophysiological mechanisms (134). The Global Initiative for Asthma (2020 GINA) has defined asthma as *"a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation*" (135). The chronic airway inflammation leads to widespread but variable airflow obstruction within the lungs and airway hyper-responsiveness that is often reversible either spontaneously or after medical treatment (135). Symptoms and severity vary greatly both within and between individuals (135).

The diagnosis “asthma” covers several phenotypes, with one of the most common distinctions being allergic vs. non-allergic asthma (135). This distinction is characterized by the presence or absence of IgE-mediated airway inflammation with allergic asthma being characterized by an IgE-mediated airway inflammation involving sensitization, whereas this is absent for non-allergic asthma (135–137). Despite the heterogeneity in asthma phenotypes, they manifest themselves with similar symptoms (135).

Diagnosis

The diagnosis of asthma is primarily given after clinical examination by identification of a characteristic pattern of respiratory symptoms, and confirmed by variability in lung function measured by spirometry or peak flow – at best before treatment has begun (135,136).
As asthma is characterized by variable expiratory airflow limitation, lung function may vary between completely normal and severely obstructed in the same patient (135). When diagnosing asthma FEV_1 from spirometry is most reliable. A reduced FEV_1/FVC ratio compared with the lower limit of normal indicates expiratory airflow limitation (135). In clinical practice, variation on airflow limitation is generally assessed from variation in FEV_1 or peak expiratory flow (PEF). “Variability” refers to improvement or deterioration in symptoms and lung function over one day, from day to day, from visit to visit or from a reversibility test above the normal day-to-day variation. Using a reversibility test, one can detect rapid improvements in FEV_1 (or PEF) 15-20 minutes after inhalation of bronchodilator. A significant increase in lung function after administration of a bronchodilator indicates asthma (135). Other tests such as an exercise challenge test and a non-specific bronchial provocation test are often performed as a part of documenting variable airflow limitation (135). Asthma can be divided into stages including mild, moderate and severe asthma, depending on prescribed treatment. Mild asthma is well-controlled with low-dose inhaled corticosteroids daily or as-needed combined with short-acting β2-agonists as-needed (135).
The aim of treatment is to prevent symptoms of respiratory distress, limit exacerbations and reduce accelerated loss of lung function using pharmacological and non-pharmacological strategies such as cessation of smoking, physical activity, avoidance of triggers etc. (135). Although asthma is defined as a chronic disease, many asthmatics experience remission after appropriate treatment (136).

Risk factors

When considering risk factors for asthma, it is important to distinguish between risk factors that cause the development of asthma and those that trigger asthma symptoms and asthma attacks. Some risk factors can do both (134,135). Generally, the susceptibility to develop and express asthma is considered a complex and interactive process between the individual’s genetic predisposition and environmental factors (134,135,138,139). Genetic predisposition is an important but poorly characterized risk factor, with family history for asthma increasing the risk to a child (140). Sex affects the risk of asthma in an age-dependent manner, with asthma being more prevalent in boys until puberty, after which it is more prevalent in females (118,139,140).
Inhaled substances including allergens and particles are the strongest environmental risk

factor for developing asthma with the most well-established environmental cause of asthma in children being exposure to tobacco smoke (139); Active smoking in adulthood increases the risk of asthma and passive smoking exposure during pregnancy and early life increases the risk of asthma in the offspring (141). Outdoor air pollution such as traffic related air pollution together with indoor air pollution (mold, dampness, particles etc.) also pose a major risk factor for asthma (135,140).
Asthma triggers include changes in weather, viral infections, physical exercise, inhaled allergens and extreme emotional arousal such as anger or fear. Also, inhaled substances including allergens and particles may trigger allergic reactions or irritate the airways (135,138). Thus, ambient and indoor UFP exposures may contribute to the exacerbation of asthma symptoms (142).

Prevalence
In 2015, asthma was the most prevalent chronic respiratory disease worldwide (118), and currently, it is the most common non-communicable disease among children (138,139). The Global Burden of Disease Study estimated that the prevalence of asthma increased by 13% from 1990 to 2015 to 358 million individuals (118), however, estimates of prevalence vary considerable between studies (118,138). While asthma prevalence is highest in high-income countries, most cases of asthma-related mortality occurs in low- and middle-income countries (134,138).
In Denmark more than 400,000 individuals are prescribed anti-asthmatic medication annually. However, the number is probably underestimated, as undiagnosed cases and poor asthma management are common (143). The Danish National Database for Asthma estimates that in 2018, roughly 200,000 males and 237,000 females had asthma (144). The estimated prevalence is between 7-11%, with the highest incidence in children and adolescents, although asthma can make an appearance throughout all ages (145).

2.5.3 Vulnerability to environmental pollutants
The motivation for choosing sensitive individuals as study populations was mainly that individuals having COPD or asthma represent a considerable proportion of the general population (118). Secondly, due to their chronic inflammation in the respiratory tract, individuals suffering from COPD or asthma are more susceptible to particulate air pollution than healthy individuals, therefore they may experience symptoms and health effects at lower exposure levels (8,29,146). According to Löndahl et al., lung morphology and breathing pattern are the two most important *individual* characteristics determining deposition probability of inhaled particles in the lungs (147). In individuals suffering from COPD and asthma, the mucociliary clearance is altered (148,149). Additionally, individuals with obstructive respiratory disease have a higher minute ventilation (i.e. more frequent breathing) compared to healthy individuals, due to increased dead space ventilation (70). The combination of increased minute ventilation and impaired defense mechanisms that are characteristic of COPD and even mild asthma, enhance diffusional deposition of fine and ultrafine particles in the distal airways (28,70,77,149,150). Thus, for a given exposure, individuals with COPD or asthma have a higher total respiratory and deposited dose of particles (77,147,151).

Consequently, individuals with COPD or asthma have a considerable risk of worsening of the disease including exacerbations and attacks following exposure to particle pollution, resulting in increased disability, decreased quality of life, hospital admissions, lost earnings and costs related to the health system (118,138,139).

2.6 Indoor climate and health – Scoping review of existing evidence

In the following section, the current evidence on the health effects of emissions from e-cigarettes, cooking and burning candles is summarized. Research into health outcomes from e-cigarettes, candle and cooking emissions has involved human, animal and *in vitro* studies, nevertheless, the summaries of the existing literature are limited to encompass human studies in high-income countries with a particular focus on human exposure studies.

Epidemiological studies (including case-control, longitudinal and cross-sectional studies) are informative regarding adverse health effects both at an individual and population level as they involve real-life exposures. Yet, in these studies, cause-effect relationships of the health effects associated with the exposure are rare and exposure data are sparse. Furthermore, in observational studies one cannot be certain of what specific pollutant or mixture of pollutants are causing the observed effect (74). Controlled human exposure studies constitute a tool for separating the effects of the specific (indoor) pollution components (152,153). Also, they provide a method for establishing causality and identification of acute underlying mechanisms, thus, complementing observations from epidemiological studies on hard endpoints such as morbidity (74,152). In controlled human exposure studies, volunteer participants are exposed to pollutants in a controlled short-term set-up to provide information on measurable biological changes caused by the specific exposure (146,152). The aim is to study early, transient and reversible effects, while ensuring the safety of the participants (152,154).

2.6.1 Health effects of e-cigarettes

Due to the fact that e-cigarettes have only been on the market for a decade, current health effect studies in humans focus on acute effects using early biomarkers, while studies on long-term effects of e-cigarettes on human health are almost non-existent.
In the following review of the literature, the adverse health effects of e-cigarettes are examined. Though it is not part of the thesis objective, findings from experimental studies concerning health effects among e-cigarette users are summarized, as most evidence is found here. For passive vape exposure, evidence from observational and exposure studies are summarized, with a particular focus on the latter. Health effects of active and passive e-cigarette exposure are examined separately as the dose inhaled might be larger in active users than in passive bystanders (49,59,86), possibly affecting health differently. An outline of the included exposure studies on health effects among individuals passively exposed to e-cigarette aerosol can be found in Table 2.3.

Respiratory effects

Air pollutants including particles can damage the airway epithelia and may change the local immune balance (155,156). When encountering pathogens such as particles, inflammation, including release of pro-inflammatory mediators and recruitment of inflammatory cells, is a

natural and crucial process promoting repair of the injured tissue (156). However, excessive or persistent inflammation can contribute to tissue injury and the development and exacerbation of respiratory disease (2,70).

Active e-cigarette use

Lung function and fraction of exhaled nitric oxide (FeNO) are among of the most commonly studied endpoints in experimental studies of e-cigarette use, but results are inconsistent. Experimental studies have found reduced lung function after 5-10 minutes use of an e-cigarette (157,158), while another study found no effect on lung function (159). Exhaled NO-concentrations (FeNO) was unaffected by e-cigarette use in two experimental studies (159,160). In contrast, five experimental studies showed either increased (57) or decreased exhaled nitric oxide concentrations after using e-cigarettes (158,161–163). In the latter studies, e-cigarettes were found to have instant physiologic effects similar to those those caused by traditional tobacco cigarettes with FeNO-concentrations decreasing immediately after use of an e-cigarette (158,161–163). In a study with asthmatic and healthy e-cigarette users the observed effect on FeNO-concentrations was more prominent in users with asthma compared to healthy users (163). Several studies observed respiratory resistance, an increase in impedance, and overall peripheral airway resistance suggesting an obstructive pattern after using an e-cigarette (158,161,163,164). Brief use of e-cigarettes among healthy never-smokers have been shown to cause rapid changes in the biologic response of alveolar macrophages, the small airway epithelium, and lung capillary endothelium (165). A recent controlled study in healthy young occasional smokers and middle aged heavy smokers with chronic disease suggested that e-cigarettes induced transient lung inflammation and gas exchange disturbances thereby causing physiologically detectable injury to the small airways (166). Also, short-term e-cigarette use is found to induce symptoms such as acute cough, sore throat, and dry mouth (158).

Passive exposure

In a cross-sectional study among asthmatic non-smoking high school students, passive exposure to aerosol from e-cigarettes was associated with higher odds of reporting asthma exacerbations and attacks in the past 12 months (167).

As shown in Table 2.3, three exposure studies aimed to assess whether passive exposure is associated with respiratory symptoms, one study using machine-generated vapor (159) and two studies using human-generated vapor (98,168). Van Drooge et al. found no significant changes in the concentrations of volatile organic compounds in exhaled breath when comparing vaping and no vaping days among five healthy non-smoking volunteers being exposed to passive vape for 12 hours. Even the exhaled breath nicotine concentrations in both conditions were similar (98). Tzortzi et al. found that airway resistance as measured by impulse oscillometry increased significantly during vape exposure, while FeNO decreased significantly following exposure (168). In a chamber-study with 15 healthy never-smokers exposed to machine-generated e-cigarette aerosol, the assessment of lung function demonstrated that a one-hour passive e-cigarette smoking session did not significantly interfere with normal lung function (159).

Cardiovascular effects

Evidence on the cardiovascular effects of exposure to e-cigarette aerosols in humans is limited. Nevertheless, effects of e-cigarettes on cardiovascular biomarkers have been documented in several experimental studies (169). Such biomarkers may provide early indications of an adverse event (170). In general, particle exposure is suggested to trigger both acute cardiac events and promote the chronic development of cardiovascular disorders (170,171). Even though the biological pathways of such a correlation is not fully understood, several mechanisms such as systemic inflammation, oxidative stress and elevated blood pressure, depression of the immune system and damaging of lung cells that protect and cleanse airways have been suggested (155,172–174).

Active e-cigarette use

The existing evidence coming from experimental studies suggests that active e-cigarette use induces systemic oxidative stress and inflammation among healthy subjects, and impairs endothelial function by increasing endothelial progenitor cells (EPCs) in blood – signs of possible vascular damage prognostic of atherosclerosis (160,175–178). However, the literature is not consistent (179). Several studies measured heart rate and blood pressure following e-cigarette use with inconsistent findings (169), yet, several controlled experimental studies found increased heart rate and increased systolic and diastolic blood pressure following e-cigarette use (158,175,180,181).

Passive exposure

In a randomized experimental study examining the effect of machine-generated passive cigarette and e-cigarette smoking on complete blood count (CBC) markers, one hour of passive exposure to e-cigarettes did not affect CBC-indices among 15 healthy never-smokers. In contrast, secondhand smoke from conventional cigarettes increased the proteins of acute inflammatory load for at least one hour (182). In the same experiment, neither passive tobacco smoking nor passive exposure to e-cigarette vapor were found to alter the acute response of the anti-oxidant system (179) (see Table 2.3).

Other health effects

Active e-cigarette use

Use of e-cigarettes may suppress vital functions of the innate immune system. Nasal scrape biopsies from vapers showed extensive immunosuppression in genes with e-cigarettes having decreased expression of 358 genes in users compared with non-smokers (183).

Passive exposure

Two observational studies examining passive exposure at home by means of airborne markers and biomarkers found significantly elevated levels of salivary and urinary cotinine in individuals exposed to e-cigarette aerosol compared to volunteers from nonsmoking control homes, indicating that nonsmokers passively exposed to e-cigarette vapor absorb nicotine (184–186). In one of the studies, quantifiable levels of tobacco-specific nitrosamines (NNAL) in urine were detected among individuals exposed to secondhand aerosol from e-cigarettes (184,185).

As shown in Table 2.3, an exposure study mimicking a realistic social setting also showed that nonusers can systemically absorb nicotine following acute secondhand exposure to e-cigarette aerosol. Systemic absorption was recorded in serum, saliva and urine for several of the exposed volunteers (187). Similarly, a chamber-study with 15 never-smokers exposed to machine-generated e-cigarette aerosol for a single hour demonstrated that e-cigarette smoking lead to serum cotinine levels comparable to those for secondhand tobacco smoke (159).
In an experimental crossover study, 30 minutes exposure to aerosol from e-cigarettes lead to symptoms of sensory irritation and general complaints. The most commonly reported symptoms among the 40 healthy non-smoking individuals were burning, dryness, sore throat, cough, breathlessness and headache (188). During sessions with e-cigarettes, eye-, nose-, and throat-respiratory symptoms in addition to general complaints increased significantly compared to the control session (188).

Table 2.3. Human exposure studies on health effects in individuals exposed to passive vape from electronic cigarettes identified through literature review grouped according to health effects and listed by publication year with newest evidence presented first.

Study (reference)	Design	Study population (n=sample size)	Exposure	Health effect assessment	Findings
Respiratory effects					
van Drooge et al. (2019) (98)	Exposure study with and without vaping	Non-vaping volunteers (n=5)	E-cigarettes with five vapers vaping ad libitum during a 12-hour period. ($PM_{2.5}$: 20 µg/m^3)	Changes in the composition of volatile organic compounds (VOCs) in exhaled breath of non-smoking volunteers.	No significant changes were found when comparing the concentrations of exhaled breath between vaping and no vaping days.
Tzortzi et al. (2018) ǂ (168)	Experimental crossover study	Healthy non-smoking adults (n=40)	30 minutes of e-cigarette aerosols (at two resistance settings: 0.5 Ohm ($PM_{2.5}$: 947 µg/m^3: and 1.5 Ohm ($PM_{2.5}$: 843 µg/m^3)).	Respiratory mechanics and exhaled inflammatory biomarkers incl. FeNO.	Passive exposure to e-cigarette emissions lead to immediate alterations in respiratory mechanics and exhaled biomarkers, expressed as reduced FeNO.
Flouris et al. (2013) * (159)	Randomised, controlled study	Never-smokers (n=15)	One hour of machine-generated aerosol. PM-levels not stated.	Lung function (FEV_1, FVC, FEV_1/FVC and PEF)	Passive e-cigarette smoking did not significantly affect lung function.

Cardiovascular effects (Table 2.3. continued)					
Poulianiti et al. (2016) * (179)	Randomized single-blind crossover study	Never-smokers (n=15)	One hour of machine-generated aerosol. PM-levels not stated.	Selected redox status markers: Total antioxidant capacity (TAC), catalase activity (CAT) and reduced glutathione (GSH)	Passive e-cigarette smoking exposure did not acutely alter the response of the antioxidant system.
Flouris et al. (2012) * (182)	Randomised, controlled study	Never-smokers (n=15)	One hour of machine-generated aerosol. PM-levels not stated.	Complete blood count (CBC)	CBC indices remained unchanged during the control session and the passive e-cigarette sessions.
Other health effects					
Tzortzi et al. (2020) ǂ (188)	Experimental crossover study	Healthy non-smoking adults (n=40)	30 minutes of e-cigarette aerosols. See details above (Tzortzi et al. 2018)	Sensory irritation (eye, nose, throat-respiratory symptoms of irritation and general complaints).	A 30-minute exposure to second-hand aerosol provoked symptoms of sensory irritation and general complaints.
Melstrom et al. (2018) (187)	Exposure study mimicking a real-life setting	Never-users of combustible tobacco (n=6)	Two exposure sessions with e-cigarettes; first generation e-cigarettes and tank-style second generation e-cigarettes. (Two hours of exposure. PM levels not stated).	Systemic absorption; measuring nicotine in the serum, saliva and urine (cotinine peak levels = C_{max}).	Systemic absorption (positive change from baseline to C_{max}) was recorded in serum, saliva and urine for several of the exposed volunteers.
Flouris et al. (2013) * (159)	Randomised, controlled study	Never-smokers (n=15)	One hour of machine-generated aerosol. PM-levels not stated.	Serum cotinine	Increasing serum cotinine levels were observed after exposure to e-cigarettes. Levels were similar to those following active and passive smoking.

* Part of the same exposure study. ǂ Part of the same exposure study. *Definition of abbreviations*: E-cigarettes = electronic cigarettes. FeNO = Fractional exhaled Nitrogen Oxide. FEV_1= Forced Expiratory Volume in the first second. FVC = Forced Vital Capacity. PEF = Peak Expiratory Flow.

2.6.2 Health effects of candles and cooking

To date, the majority of studies on particulate air pollution has focused on investigating adverse health effects associated with exposure to ambient air linking particle matter, and especially $PM_{2.5}$, to cardiopulmonary disease (172,189–191). Thus, evidence on the health effects of exposures to particles from indoor sources is scarce. In the following review of the

literature, candles and cooking are reviewed together as they are often examined together, which also applies to the study in the present thesis. Observational studies and exposure studies are described apart. In Table 2.4, an overview of the reviewed exposure studies is provided.

Respiratory effects

Observational studies

Numerous studies have linked particle pollution exposure to a substantial variety of respiratory health problems (192–198), however, the literature is not consistent.

In observational studies, indoor PM concentrations have been linked to decreasing lung function and several respiratory symptoms such as cough, wheezing and asthma symptoms in general – in particular among asthmatic children (6,192,193,199). One cohort study found that especially cooking emissions may contribute to asthma symptoms (193). In a pilot study on indoor air quality at European elementary schools, nasal patency was significantly lower in children exposed to PM_{10} concentrations >50 $\mu g/m^3$ than in those exposed to concentrations PM_{10} <50 $\mu g/m^3$ (194). In another study among elementary school children, high levels of PM were associated with an increased risk of past-year asthma (200). In a cross-sectional study among middle-aged subjects, particle number concentrations in the indoor environment, mainly driven by candle burning and bio-aerosols, showed a statistically significant correlation with reduced lung function (195). In asthmatic children and elderly with asthma, indoor and outdoor air pollution measured as personal exposure (at fixed sites and on subjects) have been found to increase exhaled NO-concentrations (196,197). Changes in spirometry were only observed among children (196). Stabile et al. examined NO-concentrations in relation to normal cooking activities among 43 non-atopic non-smoking women. In their measurement campaign study, exhaled NO-concentrations decreased significantly among women using electric stoves during cooking sessions, whereas exhaled NO increased in women using gas stoves (198).

Exposure studies

In a randomized crossover sham-controlled exposure study on respiratory effects of fine and ultrafine particles from common indoor sources (candles burning, toasting bread and frying sausages) among 55 healthy volunteers, Soppa et al. found suggestive evidence that a two hour cumulative exposure to candle burning and frying sausages, respectively, was associated with small decreases in lung function (201). Toasting bread was not associated with lung function changes (201).

Cardiovascular effects

Observational studies

An observational study found significant correlations between indoor particle levels mainly driven by candle burning and higher serum levels of markers for diabetes and inflammation in middle-aged urban citizens (195). In a sub-study of the same population, decreasing levels of $PM_{2.5}$ in the bedroom due to air filtration was found to significantly improve microvascular function (202). Chuang et al. found in their randomized intervention study, that indoor air pollution exposure to $PM_{2.5}$ and total VOC was associated with systemic inflammation,

oxidative stress and elevated blood pressure (203). In a randomized double-blind crossover intervention study using air purifiers in dormitories among healthy young adults, Li et al. observed hyper-methylation in genes functioning in modulating inflammation and oxidative stress following high indoor $PM_{2.5}$ exposure ($PM_{2.5}$: 53.1 μg/m^3) (204). In another publication from the same intervention study, the authors observed changes of serum lipid metabolites after exposure to high $PM_{2.5}$, which indicated an enhancement of lipid metabolism and oxidation (205). Furthermore, they observed significantly higher blood pressure, hormones, insulin resistance, and biomarkers of oxidative stress, and inflammation among individuals exposed to higher levels of $PM_{2.5}$ (205). All of the findings are potential mechanisms in the pathophysiological pathways to cardiovascular disease (205–207).

Exposure studies

Three controlled human exposure studies, communicated in five papers, examined cardiovascular outcomes among healthy volunteers (see Table 2.4). In a double-blind chamber study, Hagerman et al. exposed 22 healthy females to candles with flickering flames. Exposures lasted four hours. Compared to filtered air sessions, candle sessions seemed to affect heart rate variability – with candle emissions significantly increasing high frequency power and tending to decrease the autonomic balance to a more parasympathetic tone (117). In their crossover sham-controlled exposure study among healthy volunteers, Soppa et al. found that particles emitted from frying sausages and candle burning did not consistently affect systolic or diastolic blood pressure, except for a significant decrease in blood pressure was observed 24 h after exposure to particles from candle burning (102). Contrary, they found an association of increasing blood pressure with short-term exposure to fine and ultrafine particles emitted from toasting bread (102). In another publication from the same study, the authors found indications of effects on systemic arterial stiffness indices that depended on the indoor source as well as on particle metric (size-specific particle number concentrations vs. particle mass concentrations) (109). Strongest associations between different particles metrics and arterial stiffness indices were observed for ultrafine particles from candle burning and frying sausages (109). In an exposure study among 17 healthy adults in a one-bedroom apartment, Naseri et al. found no significant changes in diastolic or systolic blood pressure and heart rate after short-term exposure to frying low-fat ground beef meat without ventilation (208). In an extension of the study by Naseri et al., frying ground beef significantly increased systolic, but not diastolic blood pressure two hours post-cooking exposure when exposing 50 healthy participants. Heart rate was elevated in the early phases during exposure, however, the authors explained it by physiological factors such as heat stress, physical activity, and anxiety (209).

Other health effects

Exposure studies

Ultrafine particles may reach the brain through the olfactory nerve coming from the nasal mucosa to the forebrain or through capillary transport via the lungs (84). As seen from Table 2.4, candle burning has been linked to reduced cognitive abilities in an exposure study among 30 healthy young adults (210). The results from a Mini-Mental State Examination (MMSE) test showed a statistically robust decline in cognitive function after one hour exposure to

candle burning compared to surrounding indoor conditions. Other cognitive tests showed no significant difference between high and low PM exposure conditions (210). In their exposure study of frying aerosol on human brain activity, Naseri et al. found that brain electrical activity measured by Electroencephalograph (EEG) significantly changed after exposure compared to before exposure (208).

Table 2.4. Human exposure studies on health effects of exposure to emissions from cooking and burning candles identified through literature review grouped according to health effects and listed by publication year with newest evidence presented first.

Study (reference)	Design	Study population (n=sample size)	Exposure	Health effect assessment	Findings
Respiratory effects					
Soppa et al. (2014) * (201)	Randomized crossover sham-controlled exposure study	Healthy adults (n=55)	Two levels of exposure scenarios for each exposure: 20 or 40 candles burning (CB), toasting bread (TB) on 1 or 2 toasters, frying sausages (FS) on 1 or 2 pans. (Exposure duration: 2 hours). Mean $PM_{2.5}$ mass in the range: 52.6-235.2 µg/m^3.	Lung function (FEV_1, FVC and $MEF_{25-75\%}$)	Candle burning and frying sausages may be associated with small decreases in lung function.
Cardiovascular effects					
Gabdrashova et al. (2021) ǂ (209)	Controlled experimental study	Healthy adults (n=50)	Cooking: Frying ground beef meat in sunflower oil using electric stove without ventilation. (20 minutes of cooking, but with extended stay in the room). $PM_{2.5}$ concentration up to 55 µg/m^3.	Blood pressure and heart rate	Significant increase in systolic blood pressure. No observed changes in diastolic blood pressure. Elevations in heart rate was ascribed stress and physical activity as participants cooked the food themselves.
Soppa et al. (2019) * (109)	Randomized crossover sham-controlled exposure study	Healthy adults (n=55)	Candles burning, Toasting bread, Frying sausages. See details above (Soppa et al. 2014).	Arterial stiffness; augmentation index (AIx), augmentation pressure (AP), and pulse wave velocity (PWV).	Strongest associations between different particles metrics and arterial stiffness indices was observed for UFPs from candle burning and frying sausages.

Table 2.4. continued

Naseri et al. (2019) ǂ (208)	Controlled experimental study	Non-atopic, non-smoking, and healthy adults (n=17)	Cooking. See details above (Gabdrashova et al. 2021).	Diastolic and systolic blood pressure. Heart rate.	No significant changes were observed in blood pressure or heart rate after exposure to cooking.
Soppa et al. (2017) * (102)	Randomized crossover sham-controlled exposure study	Healthy adults (n=54)	Candles burning, Toasting bread, Frying sausages. See details above (Soppa et al. 2014).	Systolic and diastolic blood pressure (BP).	BP significantly increased with increasing particle levels resulting from toasting bread. Particles emitted from frying sausages and candle burning did not consistently affect BP.
Hagerman et al. (2014) (117)	Double-blind crossover chamber study	Healthy adult females (n=22)	Indoor nano-sized particles including burning candles. 10 candles with flickering flames (4 hour exposure duration). (Mean PM concentration: 200 ± 30 µg/m^3).	Heart rate variability	Candle particle exposure affected heart rate variability to a more parasympathetic tone.
Other health effects					
Shehab et al. (2019) (210)	Crossover experimental design	Young healthy adults (n=30)	Low and high concentrations of indoor PM. (High concentrations generated by candle burning). Exposure duration: 1 hour. $PM_{2.5}$ total concentration post exposure: 41.4 ± 46.1 µg/m^3.	Cognitive performance (Mini-Mental State Examination (MMSE), the Stroop Color and Word test, and Ruff 2 & 7 test.	The results from the MMSE test showed a statistically robust decline in cognitive function after exposure to the candle burning. The other cognitive tests showed no statistically significant difference between the high and low PM exposure conditions.
Naseri et al. (2019) ǂ (208)	Controlled experimental study	Non-atopic, non-smoking, and healthy adults (n=17)	Cooking. See details above (Gabdrashova et al. 2021).	Human brain activity (electroencephalographs (EEGs))	Exposure to aerosol from frying has a significant impact on human brain particularly on the frontal and temporal lobes.

***** Part of the same exposure study. ǂ Part of the same exposure study. *Definition of abbreviations*: PM = particulate matter, UFPs = Ultrafine particles. FEV_1= Forced Expiratory Volume in the first second. FVC = Forced Vital Capacity. MEF = Maximum (mid-) Expiratory Flow.

2.7 Unresolved issues and knowledge gaps

While most of today's knowledge about the health impact of airborne particles is the result of epidemiological studies of ambient particles, health effects from exposure to indoor particle sources are poorly known. Abundant literature suggest an effect of outdoor concentrations of particles on respiratory and cardiovascular disease (172,189,190). Nevertheless, there may be considerable differences between particles of outdoor and indoor origin concerning size and chemical composition, which are important characteristics in relation to the biological effects of exposure (27).

In recent years, a great deal of research on factors that contribute to increased indoor particle concentrations has been made (1,5,6,23,27,31,32), however, knowledge on the health impacts of indoor particles is still lacking.

As seen from the reviewed literature, e-cigarettes are associated with a wide range of acute health effects, with most research focusing on health effects among users. To the best of my knowledge, only four exposure studies on the health effects of passive vape exposure have been published. One of the studies uses machine-generated vape which cannot be expected to produce the same composition of the aerosol as real users owing to the deposition of particles in the lungs (49,71,86). All of the existing studies examine health effects among healthy subjects. As the popularity and use of e-cigarettes increase there is a need for research on the health effects of passive exposure to e-cigarettes in order to inform health professionals, policy-makers, and the public (39). Thus, more research is needed to better understand potential health effects to passive bystanders, including research on individuals with existing respiratory disease, known to be susceptible to environmental exposure (8).

Health risks associated with cooking and candle emissions are poorly understood, although such awareness is necessary to ensure adequate preventive measures. Few exposure studies (four in total) have been conducted in order to investigate the potential acute health effects of cooking and candle emissions with the existing studies examining several different health outcomes with mixed results that are insufficient to draw conclusions on at the moment. To date, health effects of cooking and candle emissions have not been examined among individuals with respiratory disease in a controlled exposure study despite epidemiological studies indicating that asthmatics are particularly susceptible to ambient and indoor particulate matter (211,212).

The current scientific knowledge and basis for evaluating the underlying mechanisms and influence of indoor particle exposure on human health in susceptible groups are limited. Rising incidence of COPD and asthma in Denmark and worldwide makes it even more critical to better understand the potential adverse health effects of indoor particles among individuals with respiratory disease (118). By examining if and how individuals with respiratory disease react to indoor air pollutants, effective preventive measures can be taken to minimize progression of the disease and to improve quality of life among the people affected.

2.8 Aims of the thesis

The work compiled in this thesis aims to contribute to an increased understanding of the association between indoor particulate matter and acute health effects among individuals with respiratory disease. The association between indoor particle exposure and health is investigated in two separate controlled human exposure studies by examining:

1. Acute health effects of passive exposure to aerosol generated by e-cigarettes in a full-scale chamber under controlled conditions among individuals suffering from COPD

In study 1, the following primary hypothesis was investigated: Short-term exposure to particles generated from e-cigarettes is associated with objectively measurable effects on potential markers of small airway disease (Surfactant Protein-A (SP-A) and albumin) compared to effects of clean air exposure. As a secondary hypothesis, more subjectively reported symptoms such as mucosal irritation are expected during exposure to particles from e-cigarettes than during exposure to clean air.

2. Acute health effects of exposure to emissions from cooking and burning candles in a full-scale chamber under controlled conditions among young individuals with mild asthma

In study 2, the following hypotheses were examined:

2A. Short-term exposure to particles generated by cooking is associated with objectively measurable effects on potential markers of small airway disease (SP-A and albumin) compared to effects of clean air exposure. Secondary hypothesis: More subjectively reported symptoms including mucosal irritation are expected during exposure to particles from cooking emissions than during exposure to clean air.

2B. Short-term exposure to particles generated by burning candles is associated with objectively measurable effects on potential markers of small airway disease (SP-A and albumin) compared to effects of clean air exposure. Secondary hypothesis: More subjectively reported symptoms including mucosal irritation are expected during exposure to particles from candles than during exposure to clean air.

2C. In continuation of hypothesis 2A and 2B, the following hypothesis will be examined: Due to different size and solubility, and thus different deposition fraction of the particles, the same mass concentration of particles emitted from candles will exert a larger effect on SP-A and albumin in the small airways than particles emitted from cooking.

Chapter 3. Methods

This chapter comprises a description of the study design, study populations, data collection and statistical methods used. The two studies included in this thesis are similar with regard to experimental facilities, exposure and outcome assessments and statistical analyses, therefore these are described in combination. The combined description is followed by a specification of each of the studies encompassing design, recruitment of study participants and generation of exposures, beginning with study 1 referred to as "Project PASVAP" examining acute health effects of PASsive VAPe exposure among individuals suffering from COPD, and finishing the chapter describing study 2, the "UltraFine Project" examining acute health effects from exposure to indoor ultrafine particles from cooking and candles among young individuals with mild asthma. Project PASVAP is reported in one paper referred to as Paper I, while the UltraFine Project is reported in two papers referred to as Paper II and III. A detailed description of the methods can be found in the appertaining papers.

3.1 Common methods

3.1.1 Exposure facilities

The exposure studies were conducted at the Climate Chamber facilities at Department of Public Health, Aarhus University, Denmark. Exposure sessions took place under controlled conditions in a 72.9 m^3 (5.4 x 5.4 x 2.5 m) climate chamber referred to as "exposure chamber". In here, walls, ceiling and floor are made of welded stainless steel. Such material is optimized for experiments with gasses and particulate air pollutants as sink effects are minimized. In the centre of the chamber, a round table and several chairs were placed for the participants to sit during the exposures.

Figure 3.1. Pictures of the Climate Chamber facilities. From left: The exposure chamber between exposure sessions. The exposure chamber with two persons inside. The small adjacent chamber (no exposure inside). Photo credit: Lars Kruse, AU Foto.

Exposures were generated in a similar 30.3 m^3 (4.2 x 2.9 x 2.5 m) adjacent chamber (referred to as "small chamber" or "adjacent chamber"), and particles and gases transferred to the exposure chamber through a 10 meter stainless steel pipe connection by means of a pressure difference between the two chambers of 10 Pa. Hereby, the exposures transferred into the exposure chamber in approximately 10 seconds. The exposures were purposely mixed and diluted with clean inlet air supplied to the exposure chamber through a slot inlet along the entire length of the ceiling. Both chambers were monitored from the control room situated next to the chambers. Figure 3.1 show the Climate Chamber facilities.

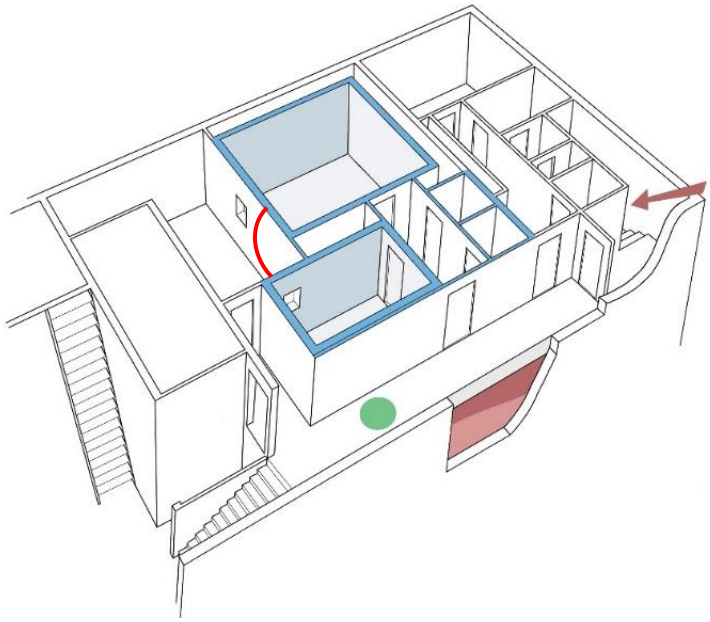

Figure 3.2. Illustration of the Climate Chamber facilities (marked with blue) and their surroundings. The red line between the two chambers illustrate the pipe connection transferring exposures from the small chamber to participants sitting in the exposure chamber. In the basement (indicated by the arrow), the air from outside is filtered through carbon and HEPA-filters.
Illustration: Hans Ole Herbst.

3.1.2 Exposure characterization

Exposure assessment in the two studies, comprising both mass and number concentrations, size distributions and chemical composition, is described below as well as in the appertaining papers.

Table 3.1. Measurements of exposures.

Measurement and instruments	Project PASVAP	UltraFine Project	Described in paper
Number concentrations in size bins			
P-Trak	+	+	-
SMPS (long DMA)	+	+	I, II, III
SMPS (nano DMA)	+	+	I, II, III
Gravimetric measures			
Dusttrak	+	+	-
PM-filters	+	+	I, II, III
SMPS	+	-	I
Optical properties of particles			
Nephelometer	-	+	II
Hygroscopicity of particles			
SMPS with humidifier	-	+	III
Chemical components			
Carbonyl compounds	+	-	I, II
NO_2	-	+	II, III
Nicotine	+	-	I
O_3	+	-	-
VOCs	-	+	II

P-Trak and DustTrak were used for exposure monitoring only, thus no data are reported.

Environmental conditions were routinely monitored and controlled by a HVAC (Heat Ventilation Air-Conditioning) system and kept as constant as possible throughout the experiments. Monitoring was done by using a logger system from Campbellsci Scientific Inc. with high quality sensors for temperature, humidity, CO_2, air flow rate, differential pressure, and ozone measurements. The exposures were monitored using a P-Trak and DustTrak and characterized by a Scanning Mobility Particle sizer (SMPS), PM-filters, and a nephelometer. Chemical information on composition of the exposures was obtained from filters, carbonyl and Tenax sampling tubes. In Table 3.1, an overview of the measures applied in the two exposures studies is provided with information on in which paper details can be found. Details on the instruments and their use can be found in appendix I.

3.1.3 Outcome assessment of clinical measurements

Ethical aspects are of great importance when humans are included in controlled experiments, therefore the outcome assessments are limited to methods that are completed easily and are as non-invasive as possible, causing only little potential inconvenience to the study participants (152,213). Evidently, the choice of health outcomes to be assessed depend on what is known and expected about the effects of the pollutant under study (146).

The health outcomes examined in the two exposure studies can be divided into respiratory outcomes (including SP-A and albumin in droplets in exhaled air, lung function, fractional exhaled NO, nasal volume and nasal lavage), systemic outcomes (blood samples analyzed for inflammatory biomarkers, gene expression and metabolomics), and general symptoms (subjectively rated symptoms).

The chosen biomarkers may indicate acute responses to the exposures or indicate that an early pathologic change has occurred, and may therefore provide early indications of adverse health outcomes.

Table 3.2. The outcomes assessed and their timing during an exposure day

Outcome	**Before exposure**	**During exposure**	**End of exposure**	**Next morning**	**Described in paper**
SP-A and albumin in exhaled air	+	-	+	+	I, III
Spirometry	+	-	+	+	I, II
Fractional Exhaled NO	+	-	+	+	I, II
Nasal Volume	UFP	-	UFP	-	II
Nasal Lavage	-	-	+	+	III
Blood sample	+	-	+	+	I, III
Cytokines in serum	UFP	-	UFP	UFP	III
C-Reactive Protein	UFP	-	UFP	UFP	III
Endothelial Progenitor Cells	UFP	-	UFP	UFP	III
Gene expression	UFP	-	UFP	UFP	III
Metabolomics	+	-	+	+	I, III
Symptom questionnaire	+	+	+	-	I, II

Definition of abbreviations: SP-A = Surfactant Protein-A, UFP = only performed during the "UltraFine Project"

The majority of the outcomes in the two studies were assessed at baseline (before exposure) and repeated immediately after exposure and the next morning (24 hours after exposure start). In Table 3.2, a simplified schedule shows when the different outcomes were assessed during an exposure day and in which paper details can be found. Several of the methods are standard methods used in the departments' previous exposure studies (214–216), however, also novel biomarkers including Surfactant Protein-A (SP-A) and albumin in droplets in exhaled air and metabolomics were examined in the two studies. Below, the measurements and the reason for the specific outcome assessment are described. When designing the studies, proteins in droplets in exhaled air, comprising SP-A and albumin, were chosen as the primary outcome. Hence, the remaining outcomes are secondary and thereby hypothesis-generating.

SP-A and albumin in droplets in exhaled air (PExA): Lower airway changes indicative of inflammation were assessed by evaluating early biomarkers from the most distal part of the lungs using the PExA® instrument set-up (217,218). The composition of the sampled microscopic droplets, including lipids and proteins, reflect the respiratory tract lining fluid in the small airways. By analysing the microscopic droplets in exhaled air, a status of the small airways are provided (217,218). PExA is a non-invasive and reproducible alternative to broncho-alveolar lavage (BAL) in assessing the lining fluid from the alveoli (219,220). Figure 3.3. summarizes how PExA works. Details on the instrument and analysis have been described previously (218,221).

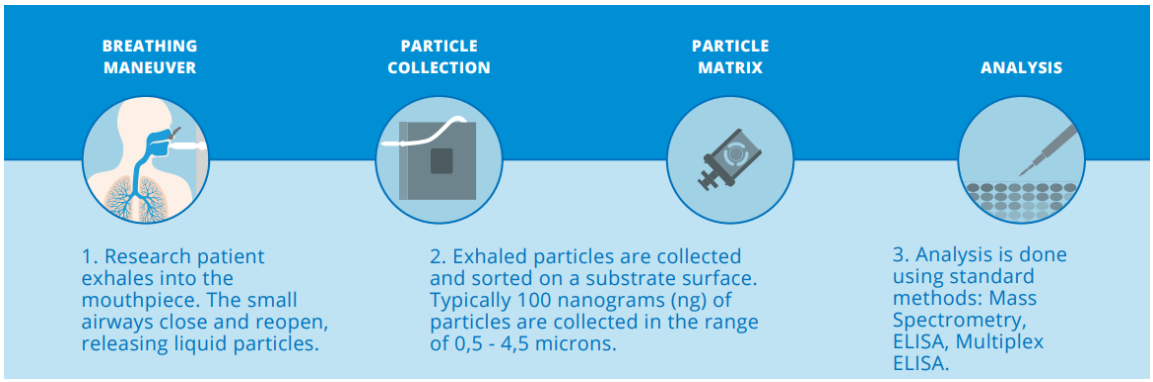

Figure 3.3. Illustration of how PExA works. Illustration from PExA AB (222).

Participants perform repeated breath maneuvers allowing for airway closure and re-opening. Exhaled droplets, also referred to as particles, are optically counted and collected on a membrane in the PExA® instrument. Samples can be analyzed for lipids and/or proteins. In the two studies included in this thesis samples were analyzed for Surfactant Protein A (SP-A) and albumin using mass spectrometry. SP-A and albumin are abundant proteins in the lung lining fluid that forms an interface between lung epithelial cells and the external environment (223). SP-A poses a number of functions that make it an interesting potential biomarker for inflammation in the small airways. Besides contributing to lowering alveolar surface tension, a major function of SP-A is participation in the respiratory innate immune system; it is capable of opsonizing or binding pathogens and other invading micro-organisms to enhance phagocytic removal from the airways (223–226). It may also act as modulator of the immune response (224). Albumin is an important blood protein and the primary determinant for colloid osmotic pressure in the blood and possibly also in the lining fluid of the small airways

(227). It is abundant in the respiratory tract lining fluid because of the leakage of plasma protein into the airways. Albumin has been extensively used as a marker of membrane permeability (227–229) and changes in albumin concentration in the respiratory tract lining fluid may be associated with small airway inflammation (221,223,230).
PExA is primarily used for early detection of various lung disease and to study pulmonary changes in people with respiratory disease (231). To date, the method has not been used in human exposure studies. However, an experimental cell study by McKenzie et al. found decreasing SP-A levels after acute exposure to nanoparticles (225), while Wang et al. suggest increasing SP-A concentrations with the production of surfactants previously shown as a defense mechanism protecting the lungs from further damage and to avoid alveolar collapsed caused by particles (226). A recent cross-sectional study found increased levels of SP-A in smokers compared to never-smokers (232).

Figure 3.4. Pictures from the outcome assessment in the two studies. Pictures to the left: A participant with asthma is performing breathing maneuvers on PExA 2.0 and later, having a blood sample taken. Picture to the right: A participant with COPD performing breathing maneuvers on PExA 1.0. Pictures: Private.

Spirometry: Spirometry is a widely used pulmonary function test to assess lung function. It provides rapid and objective information when diagnosing lung diseases and monitoring lung health (128). Spirometry is the foundation in numerous human clinical studies (146) and for decades, changes in lung function have been used for measuring health effects of exposure to PM air pollution (233,234). Evidence suggests that elevated PM-levels reduce lung function (201,233,234). To examine the respiratory response of the exposures, lung function measurements were conducted using an EasyOne^{TM} Spirometer (ndd Medical Technologies). As lung function is known to be affected by age, sex, height and ethnicity, these factors are included in the participant information on the spirometer and used when calculating predicted values (128). The following outcomes were assessed; Forced Vital Capacity (FVC) and

Forced Expired Volume in the first second (FEV_1). Subsequently, the ratio FEV_1/FVC was calculated.

Fractional exhaled NO: Fractional exhaled Nitric Oxide (FeNO) is an objective biomarker of eosinophilic airway inflammation (235). Nitric Oxide is produced by cells involved in the inflammatory response and elevated levels detected in exhaled breath may be indicative of the airway inflammation (235). Studies have reported that exposure to ambient and indoor $PM_{2.5}$ enhance NO-levels in exhaled breath (196,197,236), while exposure to cigarette smoke and vape from e-cigarettes have shown to both up- and downregulate NO-concentrations (57,158,161–163,237). Measurement of fractional concentrations of Nitric Oxide (NO) in exhaled breath is a non-invasive, simple, and safe method to determine inflammation level in the airways (235). Several factors may affect NO production including exhalation flow rate, measurement technique, diet, smoking and exercise, age and height, however, the literature is not consistent (235,238). Atopy and asthma seems to be significant factors associated with raised concentrations of NO in exhaled (235,238). In patients with asthma, NO decreases in response to treatment with corticosteroids (235). In the two studies, FeNO was measured using a chemiluminescence analyzer (NIOX VERO® Airway Inflammation Monitor; Aerocrine AB, Sweden).

Figure 3.5. Pictures from outcome assessments in the UltraFine Project. From left: Assessment of lung function using spirometry, measurement of FeNO and assessment of nasal volume by acoustic rhinometry. Pictures: Private.

Nasal volume: Nasal volume indicate the degree of patency of the nose. The nasal mucosa may be affected by inhalation of particles through the nose. Inhalation of pollutants such as particles have previously been shown to cause an inflammatory response, leading to a swelling of the nasal mucosa, thereby lowering nasal volume (194,239,240). The changes in nasal patency can be assessed by the use of Acoustic Rhinometry, which precisely locates the nasal cross-sectional area and volume of each nasal cavity by use of sound reflection (241). By determining the cross-sectional area as a function of the distance in the nasal cavity, the method provides a reliable objective measure of changes in the upper respiratory system (241). The resulting curve describes the nasal volume and gives an impression of the degree of nasal patency. Observed changes in nasal volume can be interpreted as changes in the thickness of the mucosal membrane most likely caused by inflammation (242). The details on the procedure has been described elsewhere (241).

Nasal lavage: To further assess airway inflammation in the upper respiratory system, nasal lavage samples were analysed for inflammatory cytokines interleukin-1 (IL-1) and interleukin-8 (IL-8). Nasal lavage fluid contains several markers that respond to a variety of constituents found in the environment surrounding us, and it can be used for assessment of nasal inflammatory cell influx, antioxidants and cytokines (243). IL-1 and IL-8 have shown to be released when humans are exposed to air pollution and cigarette smoke (244). However, the nasal lavage method have limitations as the recovered fluid is generally 80% of the volume originally introduced into the nose and the nasal lavage fluid is only in contact with the mucosa for 30 seconds (243). It has been proposed that flushing the nose may aggravate the inflammation (245), why no baseline measure was performed in the two studies. When interpreting results, it is important to be aware that previous studies show a clear downward shift in concentrations of all cytokines and cells from the first nasal lavage to the subsequent ones (246).

Blood sample: Blood samples were taken by venous puncture. The sampling and analysis methods are described in detail in Paper I and Paper III. Blood plasma was analyzed for several outcomes as outlined below.

• *Cytokines*: Inflammation in the airways induced by air pollution may cause formation of reactive oxidative species (ROS) and release of several cellular mediators (e.g. cytokines) into the bloodstream possibly affecting coagulation and endothelial function – potential mechanisms in the causal pathways to cardiovascular disease (206,247). The blood samples were analyzed for the cytokines interleukin-8 (IL-8), interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α), and C-C motif chemokine ligand 2 (CCL2) as these have shown to be released when exposing humans to particle pollution (70,148,172,248).

• *C-Reactive Protein (CRP)*: C-reactive protein (CRP) is an important acute-phase reactant, used clinically as a marker of the presence and intensity of inflammation. During inflammatory conditions, CRP exhibits elevated expression in serum. Traditionally, CRP has been used as an indicator of infection and cardiovascular events, however, growing evidence suggests that CRP also has important functions in inflammatory processes and host responses to infection. These include phagocytosis, production of cytokines and release of nitric oxide (NO) (249). PM-induced CRP responses have been found in several studies among children and healthy adults (250).

• *Endothelial Progenitor Cells (EPC):* Circulating endothelial progenitor cells (EPCs) are multiple different cell types that play several roles in the maintenance and repair of the endothelial tissue of the capillaries (251,252). EPCs are thought to be released from the bone marrow, with release regulated by growth factors, enzymes and surface receptors. EPCs mature in circulation and migrate into the capillary wall upon vascular injury (251). EPCs released from the bone marrow into circulation is losing markers upon maturation, why it is possible to discriminate between early and late EPCs by the presence/absence of the differential progenitor marker CD133. Early EPCs in the bone marrow are positive for the marker CD133, while circulating EPCs lose CD133 (251). Declining levels of endothelial progenitor cells within systemic circulation have been linked to increased incidence of cardiovascular events as well as related mortality (252,253). Increased levels of early EPCs

and decreased levels of late EPCs have been found following acute exposure to ambient PM in both humans and animals (254–258). The knowledge is limited regarding indoor PM, however, a recent study found declining levels of late EPCs in healthy humans after exposure to indoor UFPs (259).

• *Gene expression*: Gene expression analysis using whole blood RNA was conducted in order to better understand cellular responses to the exposures. The expression of the genes related to DNA repair and oxidative stress as well as the genes related to inflammation IL-8, TNF-α, and CCL2 were analysed. Particle exposure may disturb normal physiological pathways, activating cellular processes that mediate adverse effects (260). Gene expression changes have been shown to play an important role in the activation of toxic pathways, hence, gene signatures have the potential to act as biomarkers of $PM_{2.5}$ exposure (260). Previous studies in mice and humans have reported gene expression changes following $PM_{2.5}$ exposure (260–262).

Figure 3.6 shows different hypothetical pathways for particle-induced systemic response and subsequent potential disease endpoints.

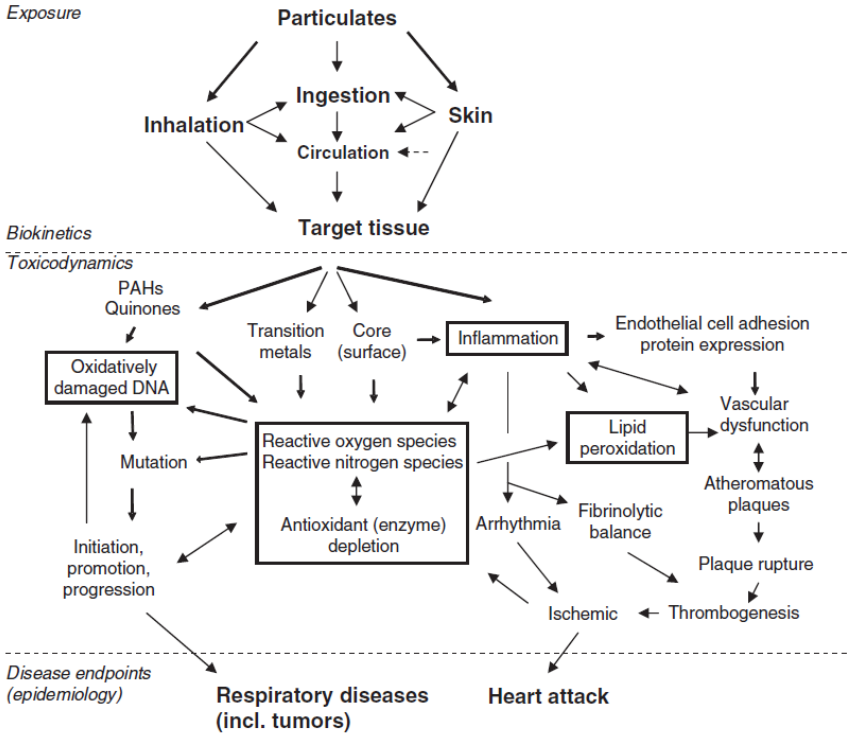

Figure 3.6. Hypothetical pathways for particle-induced oxidative stress and disease endpoints. The figure is from Møller *et al.* 2010 "Role of Oxidative damage in toxicity of particulates" (75).

• *Metabolomics*: Metabolomics enables separation and quantification of numerous groups of biomarkers using a single blood sample, for instance, routine lipids, lipoprotein subclass profiling, fatty acid composition and various metabolites (263). Several biomarkers play a critical role in many normal physiological activities, however, altered levels of serum metabolites e.g. lipids and stress hormones may be associated with inflammation (205,264,265), while cholesterols are biomarkers associated with cardiovascular

inflammation related to PM exposure (264). In the past years, metabolomics has emerged as a powerful method to understand metabolic changes in response to complex and low-dose PM exposure (205,263). Metabolomics allow suggestion of hypotheses on toxic mechanisms in order to better understand the causes of disease (263). However, a limitation is that metabolic changes remain difficult to identify due to a high number of factors potentially affecting the metabolome, in addition to the environmental exposure. Potentially confounding factors include lifestyle, diet and medicine (263). Using metabolomics in relation to air pollution is a novel approach, however, a recent intervention study found marked changes in serum metabolites, including lipids and glucose, associated to indoor $PM_{2.5}$ exposure (205).

Symptom questionnaire: To complement the many objective measures, participants' own perception of the exposures were evaluated with regard to mucosal irritation and general well-being during the exposures. Exposure to PM is known to provoke symptoms such as coughing and sneezing (199,266). Participants also evaluated the chamber with regard to light, noise, odor and temperature. Details on the 28 questions related to indoor air quality, symptoms and general well-being are found in appendix II.
The questionnaire was developed for exposure studies at the Climate Chambers and have been used in previous studies (240,267). In the time between study 1 (Project PASVAP) and study 2 (The UltraFine Project), the questionnaire was set up for computer-use, therefore it has been completed in hand during study 1, while on Surface Pro touch screen during study 2. The questions remained the same, while the scale had to be adjusted from an open Visual Analogue Scale (VAS) to a grade-scale containing numbers from 0-10.

3.1.4 Statistical analyses

Distribution of data was evaluated visually by using histograms and quantile plots. In case of non-normal distribution of residuals, analyses were performed on log-transformed outcome variables. Prior to the studies, it was decided that data should be analysed in accordance with the intended exposures. As a consequence of the crossover design participants were their own control in the statistical analyses.
When observations are repeated on individuals over time, there is independence in the data arising from a hierarchical structure. Measures sampled from different individuals are independent, however, observations from the same individual are not independent as measures within a given person are more similar, than measures between persons (268). When there are multiple levels (e.g. intra- and interpersonal levels), there is variation both *within* and *between* individuals, which is important to take into account when analysing data. Otherwise, the total variance in the outcome is affected by each of the levels separately (269). A linear mixed-effects model (also called a multi-level model) is an extension of a simple linear model allowing both fixed and random effects – hence its name mixed-effects models (270).
In the two studies, each health outcome was analyzed using a linear mixed-effects model taking into account the different design variables corresponding to the crossover design. As fixed effects the models included the particular health outcome and the exposures, time, exposure-order, day and time-exposure interaction. Participant ID was included as a random effect. The effect measure in the mixed model is expressed as the mean difference between

the outcome in the investigated category and the reference category.
Each analysis of an outcome began with a full model, where all variables and interaction between time-exposure were included. In the papers, this model is referred to as Model 1. The statistical measures of interest in Model 1 were the exposure and time-exposure interaction as an effect of any of these terms indicated a difference in the change from baseline associated with the exposure. An overall F-test for no differences between exposures was performed. In case of no statistically significant interactions, the next step was an analysis without the interaction term (referred to as Model 2), however, still including exposure and time as fixed effects. In Model 2, the statistical measure of interest was the exposure. Details regarding the models can be found in each of the papers. In the Ultrafine study, several analyses were further stratified by sex and the effects of candle exposure were compared to the effects of cooking exposure by changing the reference category.
When examining symptoms reported by participants during exposures, linear mixed-effects models were fitted followed by contrast tests to identify significant differences between the exposures at each time point. Margins plots were fitted to illustrate mean symptom development during the exposures.
For all outcomes, model fit was assessed by inspecting quantile plots for the residuals. No substantial departures from normality were observed. All analyses were performed using Stata/IC 15.0, 16.0 and 17.0 software (StataCorp, College Station, Tex). All estimates are supplemented with 95% confidence intervals (95% CI) and *p*-values, and the level of significance was assumed at a two-sided $p < 0.05$ unless stated otherwise.

3.1.5 Ethics

The Ethical Committee in Central Denmark Region approved the study protocols, both studies have been reported to the Danish Data Protection Agency and are registered at clinicaltrials.gov. The studies were conducted in accordance with The Declaration of Helsinki (213).

Table 3.3. Reference numbers for ethical registrations

	Project PASVAP	**UltraFine Project**
Ethical Committee	1-10-72-273-16	1-10-72-345-18
Danish Data Protection Agency	62908/290	2016-051-000001/780
Clinicaltrials.gov	NCT04316234	NCT04315740

3.2 Study-specific methods

3.2.1 Project PASVAP

Study design

A randomized double-blinded crossover design was applied. The experiment was carried out in groups of two or three participants. Groups were allocated to the possible exposure orders (passive vape/clean air and clean-air/passive vape) at random. All participants attended both

exposure sessions with at least two weeks between sessions to eliminate carry-over effects (271). Each session took place in the exposure chamber and lasted four hours. The study was conducted according to a double-blind protocol, hence the exposures were blinded both to the participants and to the clinical investigators. Details can be found in Paper I (supplementary files).

Study population

Individuals having a COPD-diagnosis were recruited by means of advertising at local shopping malls, general practitioners, in newspapers, and on social media. Additionally, a list of COPD-patients interested in participating in research, was obtained from the outpatient clinic at the Department of Respiratory Diseases and Allergy at Aarhus University Hospital. Prior to commencement of the study, interested individuals underwent a standard medical assessment consisting of medical history and a clinical examination including examination of COPD status. Inclusion and exclusion criteria are described in Paper I (supplementary files). A table displaying information on each included participant is provided in appendix III.
One week prior to exposure participants were asked to discontinue corticosteroids and change their long-acting bronchodilators (LABA-LAMA) to short-acting medication (SABA-SAMA) as long-acting medication could blur the possible effects of aerosols from e-cigarettes.
Eleven daily users of e-cigarettes participated in the study in order to establish the vape exposure in the adjacent chamber. They were recruited by means of advertising at local campuses, shopping malls and newspapers. To be included in the study they had to be between 20 and 65 years of age and not have any serious disease.
All participants were enrolled on "first-come, first-serve" basis. Participants with COPD received a monetary compensation of 1,000 DKK per study day, while e-cigarette users received 500 DKK per study day.

Exposure generation

Details concerning the generation of e-cigarette aerosol exposure can be found in Paper I, however, additional information is described below.
One of the most popular brands of e-cigarettes in Denmark was examined; Joyetech eGo AIO (Figure 3.7). The Joyetec eGo AIO is a reusable, rechargeable tank-style e-cigarette. The e-liquids included in the study were pre-made "Tobacco" and "Strawberry" flavour containing 6 mg/ml of nicotine. The two most commonly sold e-liquids in Denmark at the time of the study were chosen. This was examined by asking several e-cigarette retailers about the most sold liquids and examining sales statistics. Two flavors were chosen to please the vapers as not everyone liked the same flavor. Additionally, several vapers liked that they were able to vary the flavor, as they had to vape on-off for approximately three hours. Although nicotine-free liquids are available, the use of liquids containing nicotine is more common (272). The lowest possible amount of nicotine was chosen due to concern for the volunteer vapers.

Figure 3.7. Joyetech eGo AIO and e-liquids used in Project PASVAP. Pictures: Private

When generating exposures in controlled human exposure studies, one of the main issues is to decide the exposure concentration. The vape exposure levels in Project PASVAP were chosen to reflect real-life levels encountered indoors, thus, two or three vapers were chosen to generate the vape exposure. Two to three e-cigarette users represented a plausible number of users compared to public places such as restaurants, cafés, and bars or to a private space such as a living room.

The e-cigarette aerosol used for exposure was generated by individuals vaping e-cigarettes in the adjacent chamber (Figure 3.8). As an exposure day lasted up to six hours depending on the number of exposed individuals, vapers were replaced half way into the exposure. Vapers were instructed to vape e-cigarettes by turn, as pre-experimental tests showed that this would create a more even exposure. Vapers entered the adjacent chamber and began vaping just before the first participant was about to be exposed in the exposure chamber. In the exposure chamber, concentration levels build up quite fast as vapers exhaled the aerosol into a funnel just above their heads. The funnel was connected to a pipe leading aerosol into the exposure chamber by a small negative pressure of 10 Pa. In the exposure chamber, the aerosol was mixed into clean inlet air.

Before study start computer simulations made by colleagues from Department of Chemistry showed which diameter and at which air velocity the minimum loss of particles would happen during transportation in the pipe. The final set-up was implemented based on these simulations, expecting that the majority of the aerosol generated by the vapers was transferred into the exposure chamber, while only few of the largest particles might have been lost during transportation.

Figure 3.8. E-cigarette users vaping in the adjacent chamber. The aerosol was transported to the exposure chamber by a funnel in the ceiling connected to a pipe leading into the exposure chamber. Pictures: Private.

To reduce the possibility of the participants being able to distinguish between the different exposure sessions, two or three vapers occupied the small chamber at all exposure sessions. During clean air sessions the e-vapers did not use e-cigarettes. Instead, they were offered nicotine chewing gum (Nicotinell® Fruit) with 4 mg nicotine or normal chewing gum with fruit taste in order to mask the exposure by the sweet smell and to tone down urge to nicotine. The filtered clean air and e-cigarette vape sessions were identical except for the air quality.

3.2.2 The UltraFine Project

Study design

The study was designed as a three-way crossover study, with a randomized sequence of exposure to: a) air mixed with emissions from cooking, b) air mixed with emissions from candles, and c) clean filtered air. The experiment included 36 participants divided into nine groups. Thus, four subjects were exposed simultaneously. Each session lasted five hours and took place in the exposure chamber. To eliminate impact of delayed effects, the three exposure sessions were separated by 14 days. Details can be found in Paper II and III.

Study population

Participants were recruited through social media, posters, and flyers at local high schools, university campuses, dormitories, and libraries in the municipality of Aarhus, Denmark. Participants were enrolled on "first-come, first-serve" basis. Interested subjects having a diagnosis of mild asthma were invited to a pre-investigation including oral information, a health examination and skin prick testing to confirm atopy (273). Out of a sample of 80 subjects who were interested in the study, a total number of 36 young men and women with mild asthma were recruited for the study. Predefined inclusion and exclusion criteria are described in Paper II and III. A table displaying characteristics on each participant is provided in appendix IV.

Most of the included participants were treated only with short-acting β2-agonists when needed. For those participants using long-acting asthma medication, it was converted to short-acting medication two weeks prior to participation and throughout the study. Participants received a monetary compensation of 1,000 DKK per exposure day.

Exposure generation

Inspiration to the exposures came from two previous exposure studies; a double-blinded crossover study from Lund, Sweden, comprising indoor nano-sized particles including burning candles (117) and a randomized exposure study conducted in Düsseldorf, Germany (102,109,201), where they exposed healthy individuals to frying sausages and Christmas-tree candles among other indoor air pollutants. The aim was to create similar mass concentrations as Soppa and colleagues (102,109,201), as these levels had shown inflammatory responses among the exposed individuals – thus an approximate $PM_{2.5}$ mass concentration (~90 $\mu g/m^3$) was decided beforehand. In order to be able to compare cooking and candle exposures regarding health effects, similar mass concentrations across the two exposures were chosen. The emissions of fine and ultrafine particles from various cooking and candle sources were examined by their physicochemical characteristics. Candle burning using stearin candles (candlesticks and pillar candles) and cooking breast of pork in the oven showed stable increases in particle number and mass concentrations of fine and ultrafine particles that could be reproduced in an experimental setting. Subsequently, these sources were selected for the exposure of the participants and further developed in several pilot studies.

In addition to stable and reproducible exposures, the exposures had to be representative of the indoor particle sources in Denmark. In Denmark, candles of stearin are favoured over candles made of paraffin, wax etc. (112), and breast of pork is a traditional meal in many homes and has in 2014 won an online competition as Danish national dish (274). Details concerning the

generation of cooking and candle exposures can be found in Paper II, however, additional information is provided below.

Generation of candle exposure

Emissions from a burning candle can vary with time from ignition to extinction and with the conditions around the candle (111). Pilot studies showed that particle concentrations and particle sizes generally differ during the first hour (the initial phase) compared to the remaining time (stable phase). Information on this has recently been published on data from another study (111). In the present study, the stable phase with a flickering flame at slow pace was examined, corresponding to movements near the flame as caused by light draught. This can be referred to as "sooting burn" (111). Each morning, on exposure days with candles, four new taper candles, three new pillar candles and three used pillar candles (having burned previously for 7-8 hours) were lit in the small chamber two hours and 15 minutes before the first participant entered the chamber (Figure 3.9). Particles from the initial phase were aired out before participants entered the exposure chamber. The four taper candles were extinguished in water before burning down in order to avoid an uneven exposure to soot and other large particles. Four new taper candles were lit. This happened once during a candle exposure session.

Figure 3.9. Pictures to the left showing the candle exposure set-up (pictures show the initial burning phase, which was not included in the trial). Picture to the right: Polluted air dispersed into the exposure chamber through an inlet and mixed with existing air by a fan. Pictures: Private.

Generation of cooking exposure

Four ovens were placed side-by-side in the adjacent chamber (Figure 3.10). The ovens were programmed to start and stop sequentially, thus, before the first oven finished cooking meat, the next oven started and so forth. To avoid disturbances in the exposure generation, breast of pork was arranged in all four ovens before exposure. One oven at a time was cooking breast of pork at 200°C as prescribed on the packaging.

All ovens, except for oven no. 1 remained closed during the entire exposure session. Oven no. 1 had to start over cooking new meat. In total, the four ovens cooked meat five times in order for the exposure to last throughout the exposure day. Before the first participant entered the exposure chamber the cooking exposure had been activated for two hours to ensure that the particle concentration had reached equilibrium.



Figure 3.10. Pictures from the left: Ovens in the adjacent chamber. Participants in the exposure chamber. Pictures: Private.

Exposures were transferred from the small chamber to the exposure chamber through a pipe connection and a small negative pressure of 10 Pa. Different pipes were used for cooking, candle and clean air sessions and the pipes were thoroughly cleaned after each exposure. In the exposure chamber two inlets dispersed the polluted air into the chamber. Two slow-rotating fans (Lindab GTI supply air nozzle) placed on the floor, mixed the polluted air with the air in the exposure chamber (see Figure 3.9). Furthermore, there was a constant inflow of clean air from a slot inlet system in the ceiling, to secure optimal mixing of the exposure concentration added from the adjacent chamber.

Exit Poll

To examine whether the blinding of participants proved successful, an exit poll was conducted on participant's final visit. Each participant was handed a paper where they marked which exposure they thought they had been exposed to on their day one, two and three, respectively. Details concerning the exit poll can be found in Paper II.

Chapter 4. Summary of results

This chapter comprises an overview of the main results obtained from the two exposure studies. Further details of key- and sub-analyses are provided in the three papers. Additional analyses and comments have been included in the present chapter to consolidate the results in the manuscripts. Firstly, results from Project PASVAP are described followed by a description of results from the UltraFine Project.

4.1 Project PASVAP (Paper I)

4.1.1 Exposure characteristics

In Table 4.1 characterization of the environmental exposures are listed. Temperature and relative humidity remained nearly constant throughout all exposures, due to air conditioning. Levels of CO_2 and O_3 increased during passive vape exposure.

Table 4.1. Characterization of the environmental exposures in the large exposure chamber for clean air and passive vape exposure (climate and air quality factors) described by means and standard deviations (SD)

Measurement	**Unit**	**Clean air exposure**	**Passive vape exposure**
Number of sessions, *N*		9	8
Temperature	°C	22.9 (0.3)	22.8 (0.4)
Humidity	RH%	42.8 (1.7)	42.2 (2.7)
CO_2	ppm	573 (59)	617 (49)
O_3	ppb	0.51 (1.5)	2.64 (0.9)
Sound level Leq	dB(A)	47.3 (3.1)	48.5 (3.8)
$PM_{2.5}$	µg/m^3	3.4 (2.1)	95.0 (140)
Solair (particles > 500 nm)	#/m^3	$6.8x10^4$ ($3.9x10^4$)[a]	$6.1x10^7$ ($2.1x10^7$)
P-trak (20-1000 nm)	#/cm^3	107 (43.3)[b]	$1.3x10^4$ ($2.2x10^4$)[c]
Total particle number conc. (7.37-299.6 nm)[‡]	#/cm^3	123.3 (-)[d]	$1.2x10^4$ ($8.6x10^2$)[e]
Total particle number conc. (10.6-495.8 nm)[‡]	#/cm^3	5.0 (4.2)[f]	$1.7x10^4$ ($1.1x10^3$)[g]

Definition of abbreviations: CO_2 = Carbon Dioxide, O_3 = Ozone, PM = Particulate Matter. Particles > 500 nm were measured by Lighthouse Solair 3100. Particles from 20-1000 nm were measured by TSI P-trak 8525. Total particle number concentrations are SMPS average values for the total of the measured time intervals. [a]Average of seven sessions [b] Average of five sessions [c] Average of seven sessions. [d] One session only. [e] Average of four sessions. [f] Average of two sessions. [g] Average of two sessions.

Particle number concentrations during passive vape exposures reached on average 1.2 x 10^4 (± 8.6 x 10^2) particles/cm^3 in the size range 7.37 to 299.6 nm and 1.7 x 10^4 (± 1.1 x 10^3) particles/cm^3 in the size range 10.6 to 495.8 nm. For clean air exposure, the average number of particles was low in both size ranges (<125 particles/cm^3).

Differences in mean $PM_{2.5}$ mass concentrations were observed between exposure days with passive vape ranging from 8-333 µg/m^3 (median 18 µg/m^3). On two out of eight exposure

days with passive vape, the level of particle counts were high ($PM_{2.5}$ >300 µg/m^3) compared to the remaining days ($PM_{2.5}$ <45 µg/m^3).

The peak number of particles on days with passive vape exposure was approximately in the size range of 30-40 nm. Another minor peak, was seen for larger sized particles (200-300 nm). The particle mass peaked when the particle size was around 300-500 nm (see Figure 2(a), Paper I).

4.1.2 Clinical outcomes and self-reported symptoms

To investigate acute respiratory response in relation to passive vape exposure spirometry, measurements of NO-concentrations in exhaled air (FeNO) and Surfactant Protein A (SP-A) and albumin in droplets in exhaled air were performed at several time points; I) at baseline (0 h), II) just after exposure (4 h), and III) 24 hours after exposure initiation (24 h).

Central airway response: Minor, yet, borderline-significant reductions in FEV_1 and FVC were observed for passive vape exposure compared to clean air exposure. No significant effect of passive vape exposure was found on FeNO-concentrations, although a small decline was observed on days with passive vape exposure.

Lower airway response: Only nine of 16 participants contributed to the analyses of SP-A and albumin. Figure 4.1 illustrates the adjusted mean change in concentration of SP-A and albumin for the two exposures over time. Notice the wide confidence intervals due to the scarcity of data. Differential changes in SP-A and albumin concentration of exhaled air occurred during the two exposures. A significant effect of exposure over time was observed for SP-A concentrations showing a decrease following passive vape exposure 24 hours after exposure start when compared to clean air (-1.775% (95% CI -3.35; -0.199), p=0.029). A decrease, but not significant, in mean albumin concentration was observed following vape exposure when compared to clean air (-0.814% (95% CI -2.457; 0.828), p=0.316).

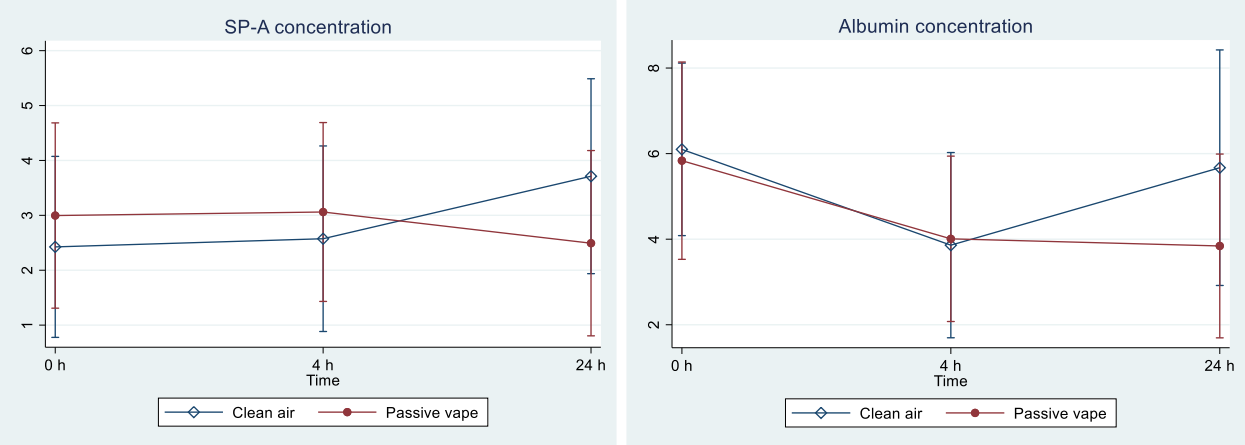

Figure 4.1. Margins plot of the adjusted mean change in biomarkers in exhaled air (SP-A and albumin) for each of the two exposures (clean air and passive vape). Biomarkers were measured before exposure (0 hours), and following exposure corresponding to 4 hours after and 24 hours after exposure start as depicted on the x-axis.

Systemic effects: To examine the acute systemic inflammation response in relation to passive vape exposure, changes in serum metabolites were examined. Blood samples at time points 0, 4 and 24 h were analyzed for routine lipids, lipoprotein subclasses, fatty acid composition and various low-molecular metabolites including amino acids. A differential change in some of the examined metabolic biomarkers occurred during the two exposure sessions as reported in Paper I. Concentrations of albumin (the main protein in blood) and acetoacetate increased significantly following vape exposure, while other markers – including cholesterol and lipoproteins – were less pronounced, however, still significantly increased compared to clean air exposure. The majority of the measured metabolites (~100) did not show any variation related to passive vape exposure.

Figure 4.2. illustrates development in concentrations of selected serum metabolites following passive vape and clean air exposure. In each of the four depicted metabolites, the concentration following exposures developed differently over time indicating interaction between exposure and time.

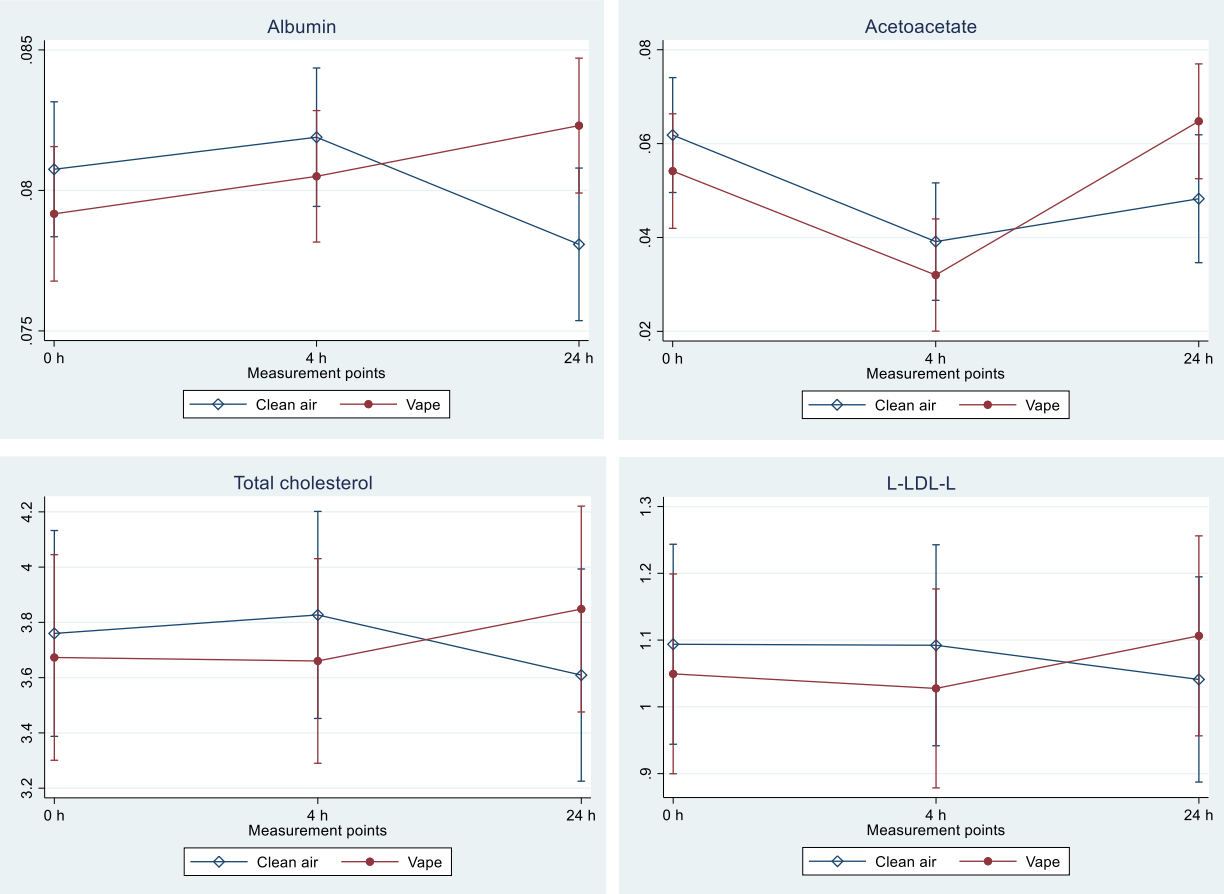

Figure 4.2. Margins plot of the adjusted mean change in selected serum metabolites for the two exposures (clean air and passive vape). Metabolites were measured before exposure (0 hours), and following exposure corresponding to 4 and 24 hours after exposure start as depicted on the x-axis. L-LDL-L is corresponding to total lipids in large LDL. Metabolites are reported in mmol/L.

Symptoms of irritation: Symptoms of mucosal irritation were rated prior to exposure (0 h), every 30 minutes during exposure, and at the end of the exposure session (4 h) – in total nine times during each exposure. In general, differences in participants' symptoms were mild when comparing days with passive vape to days with clean air ranging from 6–20% of maximum on the scale. Towards the end of exposure, throat irritation was significantly higher on days with passive vape compared to days with clean air as exposure (Figure 3, Paper I). Symptoms of relevance to COPD (shortness of breath, urge to cough, wheezing, chest tightness, and lack of general well-being) were examined following publication of Paper I. None of the examined symptoms worsened significantly during days with passive vape exposure compared to during clean air exposure. Yet, tendencies for stronger urge to cough were observed during passive vape exposure (see Figure 4.3 below).

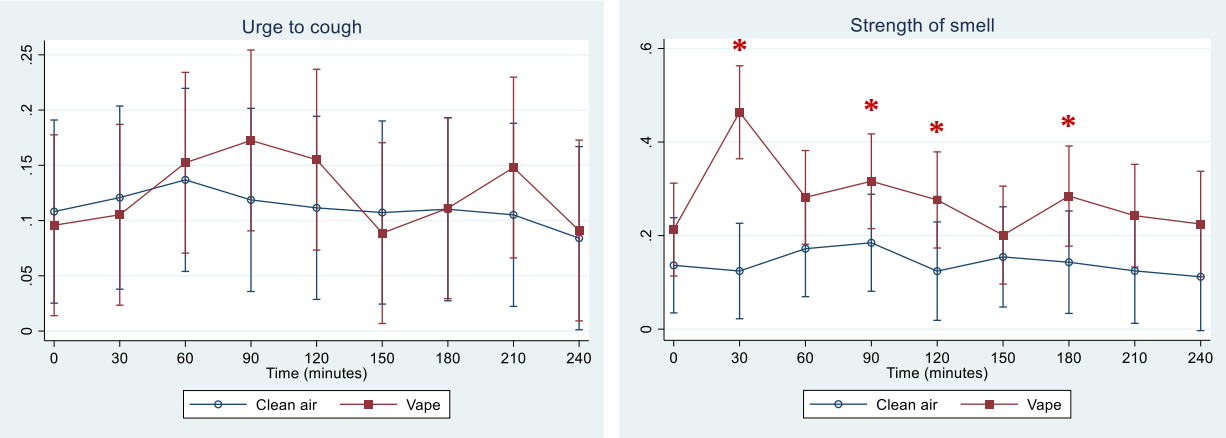

Figure 4.3. Participants' appraisal of urge to cough and strength of smell during the two exposures. Symptoms were scored by placing a cross on a 130 mm open Visual Analogue Scale (VAS). The intensity of any discomfort was registered as the length in mm from the left of the scale to the marker. The scores were rated from 0 to 100% with highest number corresponding to highest discomfort. Discomfort was evaluated as changes over time (as percentage of max). Stars indicate significant differences between exposures at given time points ($p < 0.05$).

When examining ratings of odor intensity (strength of smell), participants reported an awareness of a stronger smell during passive vape exposure compared to during clean air exposure (Figure 4.3). Participants exposed to vape on days with $PM_{2.5}$ mass concentrations >300 µg/m^3 did not deviate from the other participants in their reporting of strength of smell when inspecting individual scatter plots.

Individual response in relation to exposure: Four out of 16 individuals were exposed to $PM_{2.5}$ mass concentrations >300 µg/m^3. When examining individual scatter plots on the included health outcomes, these four individuals did not show stronger health effects than the rest of the participants. When examining biomarkers in exhaled air (SP-A and albumin) only one of four individuals exposed to high PM levels, contributed to the analyses. An example of individual scatter plots on FEV_1 is shown below (Figure 4.4).

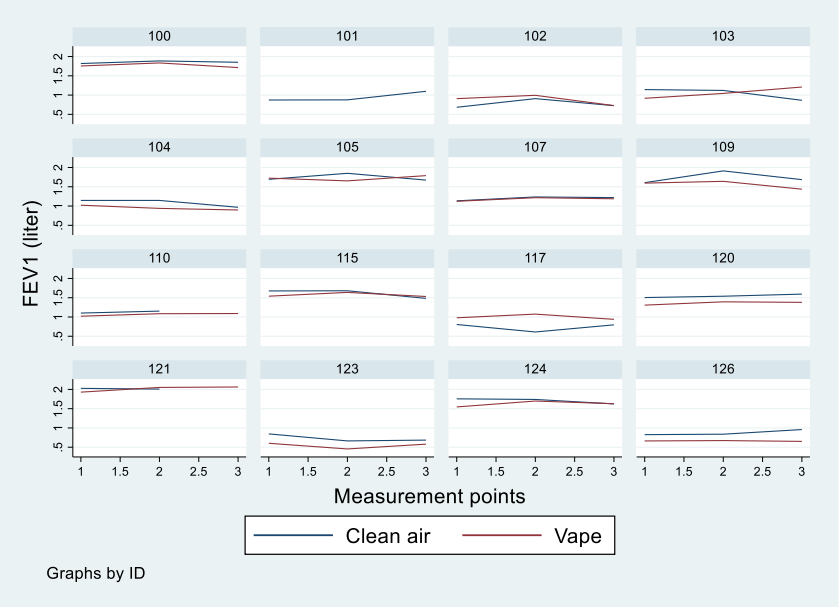

Figure 4.4. Individual scatter plots for FEV_1. Participant no. 103, 104, 107 and 123 were exposed to $PM_{2.5}$ mass concentrations > 300 µg/m^3. Missing data points occur as not all participants were able to perform the spirometry test at all time points. Measurement points 1, 2 and 3 correspond to 0, 4 and 24 hours.

4.2 The UltraFine Project (Paper II and III)

4.2.1 Exposure characteristics

Several characteristics of the exposures were examined and reported in Paper II and III, with key results reported below.

Particle number concentrations and size distributions (Paper II and III): The particle number and size distributions are given as an average over the measurement periods during each exposure day for 2.41 to 79.1 nm (nano DMA) and 14.6 to 661.2 nm (long DMA), respectively. The total particle number concentrations reached the highest mean level during candle exposure experiments with 1.7 x 10^6 particles/cm^3 (nano DMA) and 3.7 x 10^5 (long DMA), respectively. For cooking exposure, the average number of particles was highest in long DMA with a mean value of 7.2 x 10^4 particles/cm^3. For the nano DMA, particle number concentrations for cooking were 5.9 x 10^3 particles/cm^3. The average particle diameters for particles emitted from cooking were in the range 32 to 104 nm. The average mode diameter was ~ 80 nm. The average particle mode diameters derived for the candle exposure sessions were in the range 6.2 to 9.2 nm with the highest particle number concentration found for particle diameters ~7.5 nm (see Figures 1 and 2, Paper II, for average particle number size distributions for cooking and candle exposure, respectively).

Hygroscopicity of particles (Paper III): For cooking emissions, the particle distributions varied strongly with time depending on the timing of the ovens and consequently, measurements of dry and humid distributions were difficult to interpret. Candle emissions in the size range 2.4 to 79 nm (nano SMPS) showed some growth when exposed to high

humidity (~90 %) with the mode diameter changing from 7.4 to 9.5 nm (the mode of 7.4 nm was calculated from 10 dry scans before humidification, therefore deviating from the mode of 7.5 nm reported above). Particles in the larger size ranges did not seem to exert the same hygroscopic growth as observed for the smaller size ranges (see Figure 2, Paper III).

Other environmental characteristics (Paper II): Cooking resulted in high levels of VOCs especially aldehydes (Figure 3, Paper II). Concentrations of VOCs were low during sessions with clean air and candle emissions. During candle exposure, levels of CO_2 and NO_2 increased compared to during clean air exposure (see Table 2, Paper II).

4.2.2 Clinical outcomes and self-reported well-being

To examine the acute respiratory response to cooking and candle exposure, measurements of SP-A and albumin in droplets in exhaled air, spirometry, FeNO, nasal volume and nasal lavage were performed at several time points; I) at baseline (0 h), II) just after exposure (5 h), and III) 24 hours after exposure initiation (24 h). Below, key findings are described.

Upper and central airway response (Paper II and III): No significant associations were found between lung function measured by spirometry or FeNO-concentrations with cooking and candles, respectively. Nasal volume decreased on days with cooking exposure compared to clean air exposure, but not significantly. When stratifying the analysis by sex, nasal volume decreased significantly in males on days with cooking exposure. Additionally, FeNO-concentrations declined among males on days with candle exposure, though estimates were borderline significant. This decline was not observed among females.

Lower airway response (Paper III): Figure 4.5 illustrates the adjusted mean change in concentration of SP-A and albumin in the small airways for the three exposures over time. The figure shows that the concentration of SP-A following candle exposure was nearly constant over time, while concentrations of SP-A decreased five hours after exposure start following clean air and cooking exposure. Mixed models showed that compared to clean air exposure, SP-A concentrations increased following candle exposure (0.31% (95% CI -0.02; 0.63), p=0.065). The difference between candle and clean air exposure on SP-A concentrations was persistent across analyses, but with varying significance (Table 1, S4 and S5, Paper III). There was no difference between cooking and clean air exposure on SP-A when examining changes following exposures adjusted for baseline values (0.02 (95% CI -0.30; 0.35), p=0.888) (Table 1, Paper III). Exposure to cooking and candles numerically increased concentrations of albumin in the small airways compared to clean air exposure (cooking: 0.24% (95% CI -0.26; 0.74) and candles: 0.25% (95% CI -0.25; 0.75)) – however, increases were not statistically significant, although persistent across the statistical analyses (See Table 1, S4 and S5, Paper III).

Four of 324 samples were excluded from the statistical analyses, as they were contaminated with saliva, detected by extremely high levels of albumin. Sensitivity analysis including the four contaminated samples showed that differences in SP-A concentrations following the two particle exposures and clean air attenuated slightly in all models; for candles the estimated 5-24 hour change adjusted for baseline decreased from 0.31 to 0.27% (95% CI -0.05; 0.60),

p=0.099. For cooking it changed from 0.02 to -0.003 (95% CI -0.33; 0.32), *p*=0.987) (data not shown in Paper III). No sensitivity analyses including the outliers were conducted for albumin, as levels of albumin were considered too high to be true.

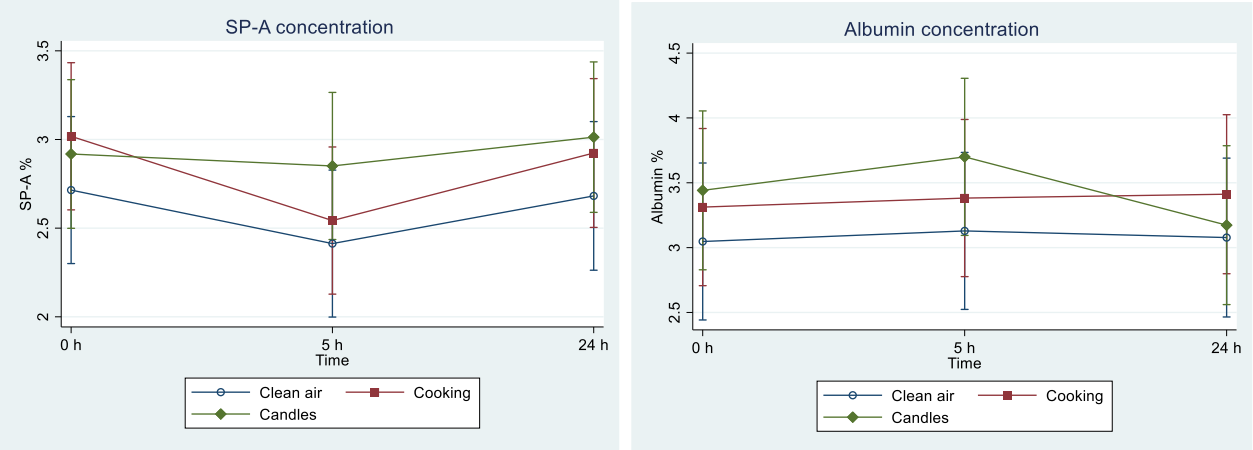

Figure 4.5. Margins plot of the adjusted mean change in biomarkers in exhaled air (SP-A and albumin) for each of the three exposures (clean air, cooking and candles). Biomarkers were measured before exposure (0 hours), and following exposure corresponding to 5 and 24 hours after exposure start as depicted on the x-axis. (Figure 4, Paper III).

Systemic inflammatory markers (Paper III): In order to examine the systemic inflammatory response, several markers of inflammation were examined; cytokines, C-reactive protein (CRP), endothelial progenitor cells (EPCs) and gene expression related to DNA repair, oxidative stress and inflammation. Serum blood was collected at selected time points (0, 5 and 24 h). Only weak, no or even reducing effects of cooking and candles were observed for systemic inflammatory biomarkers, EPC levels and gene expression. CCL2 increased significantly following candle exposure compared to when exposed to clean air and borderline significant increases in IL-8 (gene expression) following candle exposure was observed. The level of CRP declined following clean air exposure (Table 2, Paper III).

Metabolites and macromolecules (Paper III): Following cooking exposure increasing concentrations of lipids and lipoproteins were observed when compared to clean air (see example in Figure 4.6). Peaks around ~2 ppm corresponded to glycoprotein acetylation (GlycA). With a decided *p*-value of ≤0.03, no significant associations were found for candles and any of the metabolites (Table 3, Paper III).

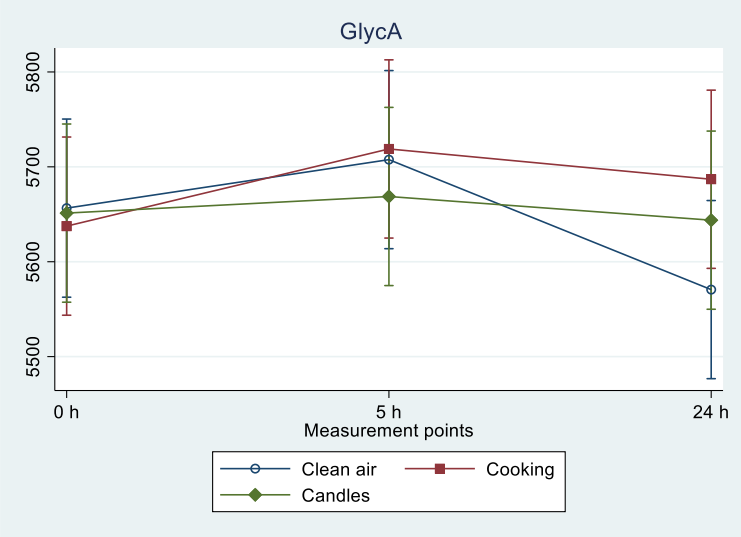

Figure 4.6. Margins plot of the adjusted mean change in GlycA for each of the three exposures (clean air, cooking and candles). Biomarkers were measured before exposure (0 hours), and following exposure corresponding to 5 and 24 hours after exposure start as depicted on the x-axis.

Self-reported symptoms and discomfort (Paper II): Symptoms of irritation and general well-being were rated prior to exposure (0 h), every 30 minutes during exposure, and at the end of each exposure session (5 h) – in total 11 times during each exposure day. During cooking and candle exposure participants reported additional and significantly more symptoms of mucosal irritation compared to during clean air exposure (See Figure 4, Paper II). During candle exposure participants reported watering eyes and blocked nose, while during cooking exposure participants experienced eye irritation (including dry eyes and watering eyes), nose irritation (including running nose and blocked nose), head ache, nausea, chest tightness and lack of general well-being. Overall, females reported more severe symptoms throughout the questions, however, differences between males and females were not statistically significant. Remarkable for the symptom rating, is that a couple of participants (five males) reported very limited or no symptoms at all throughout exposure sessions, thus seemingly not affected by the exposures.

4.2.3 Health response stratified by sex

As lung size, airway diameters, body size, and hormonal status may influence the biological transport and deposition of particles (275), analyses stratified by sex were conducted for all outcomes (however, only reported in Paper I). A brief overview of outcomes where sex responded differently to the exposures is provided below.

For several outcomes, tendencies towards sex-related differences were observed, although differences were not statistically significant; nasal volume (cooking exposure) and FeNO (candle exposure) were affected to a larger degree in males than in females. For albumin levels in the small airways, females had higher concentrations than males following cooking and candle exposure.

Overall, participants experienced a small decrease in IL-8 in nasal lavage following cooking and candle exposure, however, females experienced a larger decrease in IL-8 compared to males (Table 4.2). Contrary, males experienced higher concentrations of several metabolites

compared to females when examining the metabolites and molecules reported in Table 3, Paper III. Outcomes, where significant difference between males and females were found, are reported in Table 4.2.

Table 4.2. Outcomes with significant sex-related differences in response to exposure[†]

	Cooking exposure		
	Coefficient	95% CI	*p*-value
Cytokines in nasal lavage			
IL-8	-0.48	(-0.86; -0.10)	0.016
Metabolites and macromolecules			
Unsaturated fatty acid =CH-C<u>H</u>$_2$ (~2.04 ppm)	-108	(-199; -18.2)	0.020
Alanine (~1.45 ppm)	-156	(-246; -67.1)	0.001
Unidentified (~1.45 ppm)	-84.7	(-166; -3.46)	0.042
Lipid -CH3 (+Valine) (~1.00 ppm)	-104	(-187; -21.4)	0.015
	Candle exposure		
	Coefficient	95% CI	*p*-value
Cytokines in nasal lavage			
IL-8	-0.47	(-0.87;-0.08)	0.021

[†] Results from linear mixed models (Males are reference).

4.2.4 Candle vs. cooking exposure

Differences in particle size and chemical composition of emissions might lead to a difference in health response (70,276). Concentration of SP-A in the small airways was differently affected by candle and cooking exposures, with the difference between the two exposures being borderline significant in the main model reporting changes from 5-24 hours adjusted for baseline values (candles vs. cooking: 0.28% (-0.04; 61.0), p=0.091). There were no significant differences between candles and cooking exposure on albumin concentrations in small airways in any of the models. See Table 4.3 below.

Table 4.3. Differences in biomarker response to candle and cooking exposure (cooking = reference)

	Surfactant Protein-A		
	Coefficient	95% CI	*p*-value
mean change 5 and 24 hours adjusted for baseline	0.28	(-0.04; 0.61)	0.091
mean change 0, 5 and 24 hours	0.10	(-0.19; 0.39)	0.497
mean change 5 and 24 hours	0.21	(-0.15; 0.58)	0.247
	Albumin		
	Coefficient	95% CI	*p*-value
mean change 5 and 24 hours adjusted for baseline	0.01	(-0.49; 0.51)	0.973
mean change 0, 5 and 24 hours	0.07	(-0.37; 0.51)	0.747
mean change 5 and 24 hours	0.03	(-0.49; 0.55)	0.905

Note: Results are from linear mixed models (without interaction). Analyses are without four contaminated samples.

A significant difference was found for CCL2 in serum following candle exposure compared to cooking exposure, with candles increasing levels of CCL2 significantly more than cooking: 15.2 pg/ml (95% CI 1.12; 29.2), p=0.034. A borderline significant larger decrease in nasal volume was observed when comparing changes on days with cooking exposure to changes on days with candle exposure (-0.36 cm^3 (95% CI -0.73;0.00), p=0.052). For the majority of symptoms, cooking exerted a significantly larger response than for candles. At all time points, the unpleasantness of the chamber experience was rated significantly worse during cooking exposure compared to candle exposure. For the remaining outcomes, no noteworthy difference in the response to candle vs. cooking exposure was observed.

4.2.5 Exit Poll – effectiveness of blinding of participants

On exposure days with cooking exposure 35/36 (97.2%) participants were able to identify the exposure, thus, blinding of participants did not work as intended. Participants were not able to identify whether they had been exposed to clean air or candles in a systematic way; when exposed to candles 20/35 (57.1%) participants guessed the exposure correctly. One person did not provide a qualified guess. Chi^2-test showed no significant difference (p=0.250) whether participants thought they had been exposed to candles or clean air on days with candle exposure and vice versa. Hence, it was possible to blind exposure to candles.

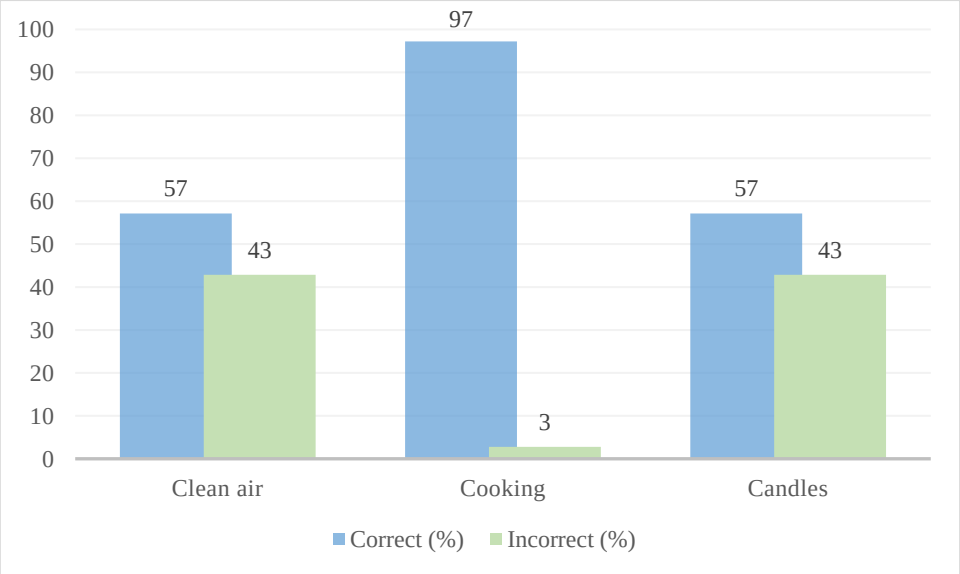

Figure 4.7. Results from exit poll with participants' appraisal of the three exposures (correct / incorrect) reported in percent. Adapted from Figure 5a, Paper II.

Chapter 5. Discussion

The present chapter provides a summary of the results and their relation to the international state-of-the-art research followed by a discussion on methodological strengths and limitations that must be kept in mind when interpreting the results. Further reflections can be found in each of the three papers.

5.1 Main findings

In the present thesis the need to study indoor particle pollution and its consequences for health in individuals with respiratory disease was addressed. The main findings from each of the two studies included in the thesis are outlined below.

Project PASVAP

In a randomized controlled double-blinded crossover trial, 16 individuals with COPD were exposed to passive vape from e-cigarettes and clean air on two different occasions. Particle concentrations on exposure days with passive vape varied depending on the performance of the e-cigarette users. On exposure days with passive vape, the peak number of particles was approximately in the size range 30 to 40 nm.

Exposure to passive vape from e-cigarettes caused systemic responses among COPD-patients, which may indicate mild inflammation. When compared to clean air exposure, a negative effect of passive vape on SP-A the morning after exposure was observed. Several metabolites increased significantly following vape exposure. FeNO and lung function decreased on days with vape exposure, though not significantly. Throat irritation was more pronounced during exposure with passive vape than during clean air exposure. Participants did not report other symptoms of mucosal irritation.

The UltraFine Project

In a randomized controlled double-blind crossover trial, 36 young individuals with mild asthma were exposed to emissions from cooking and burning candles, respectively, and clean air as control exposure. During candle exposure, the highest number concentration of particles was found below 10 nm, while for cooking the average mode diameter for particles was ~80 nm. The results suggest that exposure to cooking and candles affect the respiratory and systemic host defence and general well-being in young subjects with mild asthma. The concentration of SP-A in the respiratory tract lining fluids of the small airways was differently affected by the exposures, showing nearly stable concentrations following candle exposure, while decreasing concentrations following clean air and cooking exposure. Following cooking and candle exposure, a numerical increase albumin in the small airways was observed when compared to clean air exposure. A decline in FeNO, but not significant, was observed among males after exposure to emissions from candles. Cooking exposure was followed by increased concentrations of some lipids and lipoproteins in the blood including GlycA and decreasing nasal volume, which was significant in males. No change in FEV_1 and FVC was found for neither cooking nor candle exposure. Only weak effects were observed for systemic inflammatory biomarkers, EPC levels and gene expression. Overall, participants

reported additional and significantly more pronounced symptoms of irritation and lower well-being when exposed to cooking and candles compared to when exposed to clean air.

5.2 Results in perspective

5.2.1 Project PASVAP

Particle size distribution of e-cigarette aerosol

In Project PASVAP, the peak number of particles on days with vape exposure was in the size range ~30-40 nm. This corresponds well to comparable experiments using vaping volunteers, with particles modes around ~30 nm (49,57,98). Contrary to the size distribution observed in Project PASVAP, several studies have found a bimodal size distribution within the ultrafine size range (49,86,87). In Project PASVAP, a second, but minor peak in particles was observed in the size range 200-300 nm. Factors that may explain this difference from existing studies are the e-cigarette device, temperature of the coil, exhalation pattern by the user, and indoor environmental factors such as temperature and humidity, known to affect the generated particle size distribution (49,86).

Acute health effects

When examining respiratory outcomes, results in Project PASVAP suggest that passive vape exposure may reduce lung function in individuals with COPD. FEV_1 and FVC showed borderline significant decreases on days with vape exposure. To the best of my knowledge, only one exposure study on lung function changes following passive vape exposure has been reported (cf. Table 2.3), demonstrating that a one-hour passive vape exposure session did not interfere with normal lung function (159). Flouris et al. examined healthy volunteers and exposed them to machine-generated vape for one hour only (159), hence the more susceptible participants in Project PASVAP and the longer exposure duration may explain possible differences in results. In the same study by Flouris et al., active e-cigarette use did not affect lung function either (159). Other studies on active vape have reported decreasing lung function following brief use of an e-cigarette (157,158,277), hence, the literature is not conclusive. In their exposure study, Tzortzi et al. did not examine lung function, but reported on immediate respiratory effects including alterations in respiratory mechanics and reduced FeNO-concentrations following 30 minutes exposure to passive vape from e-cigarettes (168).

In Project PASVAP, a significant differential change in SP-A concentrations in exhaled air occurred during the two different exposure scenarios; a decrease in SP-A 24 hours following vape exposure was observed when compared to clean air exposure. The question remains whether it is a down-regulation of SP-A caused by e-cigarette emissions or an up-regulation following clean air exposure. As discussed in Paper I, it is possible that SP-A in the lining fluid of the small airways decrease following acute exposure to passive vape, as SP-A have been shown to perish after fighting pollution including micro-organisms and PM (225,278). If not restored rapidly, the concentration of SP-A in the small airways will decrease. It has been shown that the alveolar lining fluid is becoming more dysfunctional with age, with a slow alveolar lining fluid turnover (279), which may explain that decreasing levels of SP-A is not compensated for within 24 hours.

SP-A in exhaled air is a novel biomarker and no previous human exposure studies on e-cigarettes have examined SP-A in small airways, however, in vitro studies have shown negative effects of inhaled e-cigarette aerosol on lung surfactant (94,280).
Studies on effects of conventional cigarette smoking found reduced levels of SP-A in smokers with and without emphysema when compared to non-smokers (281,282), however results are not conclusive (232,283).
Dosimetry modelling has indicated that in e-cigarette users most particle deposition of the aerosol occurs in the alveolar region of the lungs (284) and several studies focusing on e-cigarette users have found adverse effects in the lower respiratory tract: Results from a recent controlled study of active e-cigarette exposure in healthy young occasional tobacco smokers and middle aged heavy smokers with chronic disease (coronary artery disease or COPD) showed that just 15 minutes of e-cigarette use caused transient lung inflammation, impaired gas exchange and reduced expiratory flow suggesting physiologically detectable injury to the small airways (166). The authors found accumulation of propylene glycol (PG) (one of the main constituents in e-liquids) within the lungs suggesting that PG interacts with the airway epithelium, triggering pulmonary clearance mechanisms (166). Proteomics analysis of airway epithelia showed that chronic e-cigarette use was associated with numerous altered proteins (~200), reflecting marked biological changes in the lung. As in the study by Chaumont et al., the authors concluded that the effects may in part be mediated by PG and vegetable glycerine (VG). Staudt et al. evaluated the biology of lung cells (by use of BAL) before and after brief use of e-cigarettes among 10 healthy never-smoking individuals and found that acute exposure to e-cigarette aerosols caused dysregulated lung homeostasis with changes in the biologic responses of alveolar macrophages, the small airway epithelium, and lung capillary endothelium (165).
In summary, aerosol from e-cigarettes may induce various disturbances in the dynamics of the host defence system in the lower respiratory tract. Thus, there are reasons to believe that a negative effect of passive vape exposure on SP-A in the lung lining fluid was observed in Project PASVAP, however, clean air exposure may have increased SP-A in an unexplainable manner. Nevertheless, the findings warrant further research as the biomarker is novel and the number of participants included in the analyses was small. The potential clinical implications of the findings are not clear, though, decreasing levels of SP-A in the alveoli may lead to impaired immune defence functions, contributing to increased susceptibility to lung inflammation including COPD exacerbations, and the ability to generate low surface tension may be impaired subsequently increasing the work of breathing (278,281,285).

Increasing concentrations of some lipids in serum including cholesterol and phospholipids (particularly LDL and VLDL) were observed in Project PASVAP following exposure to passive vape. Cholesterol is a known risk factor for cardiovascular disease, with especially higher levels of LDL known to increase the risk for atherosclerosis (286,287). Currently, there is limited information on serum metabolites following e-cigarette exposure, however, recent cross-sectional studies on e-cigarette users found similar results as in Project PASVAP indicating that e-cigarettes may influence the lipid profile (288–290). In the studies, e-cigarettes increased a number of lipid species, however, given the cross-sectional design of the studies, it is not possible to establish causality. In the study by Badea et al., the LDL-

fraction concentration was significantly higher in e-cigarette users compared to non-smokers. Also, the concentration of total cholesterol increased in e-cigarettes users, as did VLDL and albumin concentrations (288). Likewise, in Project PASVAP, vape exposure was associated with significant increases in serum albumin. Albumin might be elevated as a part of the immune system reaction to the aerosol, as serum albumin has important antioxidant functions. Albumin is considered a sensitive indicator of oxidative stress in the vasculature (288,291).
Conventional cigarette smoking is likewise known to increase total cholesterol, LDL and VLDL concentrations (287). The underlying mechanisms of the observed effects are ascribed alterations in the enzymes controlling lipid transport with a possible release of free fatty acids subsequently affecting VLDL and LDL-concentrations causing accumulation in the blood (287).

So far, examination of self-reported symptoms in relation to vape exposure have primarily focused on respiratory symptoms and observational studies have reported associations between e-cigarettes and an increase in cough, wheeze and shortness of breath among users and passive bystanders (164,167).
In Project PASVAP, only throat irritation was found to be significantly higher during vape exposure compared to during clean air exposure, although several symptoms of mucosal irritation were examined. In a recent experimental crossover study, Tzortzi et al. examined self-reported symptoms of irritation among 40 healthy non-smoking adults exposed to 30 minutes of passive vape (188). In their study, general complaints increased significantly during exposure to passive vape with the most commonly reported symptoms being burning, dryness, sore throat, cough, breathlessness and headache. Throat irritation was the most persistent symptom still significantly higher 30 minutes after vape exposure when compared to the control exposure (188). In project PASVAP, symptoms were not examined after exposure, however, throat irritation increased with time of exposure duration. A reason for Tzortzi et al. observing several symptoms of irritation during a shorter duration of time when compared to Project PASVAP, may be explained by higher $PM_{2.5}$ mass concentrations with an estimated average of 843 $\mu g/m^3$ (range: 424-1405). This is several times higher than the average and general $PM_{2.5}$ mass concentrations in Project PASVAP (mean $PM_{2.5}$: 95 $\mu g/m^3$). Tzortzi et al. even suggested that the $PM_{2.5}$ concentrations in their study were underestimated (188). In addition to PM, aerosol from e-cigarettes often contains a mixture of VOCs. In the study by Tzortzi et al. they measured high levels of VOCs such as formaldehyde, acetaldehyde and acrolein (188). Short-term exposure to VOCs has previously been shown to induce headache and symptoms of irritation including eye, nose and throat irritation (292–294). In Project PASVAP, only low levels of VOCs were measured, which – combined with low PM concentrations – might explain the few reported symptoms and the low level of irritation in general. Another, but less likely, explanation for differences in symptom reporting between the studies, is that response bias cannot be precluded in the study by Tzortzi et al., as exposures were not blinded to participants.
Exposure studies on active exposure have likewise found that short term e-cigarette use induced throat irritation including acute cough, sore throat and dry mouth (158,166). Throat irritation is claimed to be caused by PG and VG having water-absorbing properties (293).

5.2.2 The UltraFine Project

Exposure characteristics

In the UltraFine Project, the average particle mode diameter for cooking was ~ 80 nm, with average particle diameters being in the range from 32 to 104 nm. In other studies, particles generated from cooking are similarly found to be within the fine and ultrafine particle size ranges (18,108–110). In a review of particulate matter from cooking, the largest amount of the measured particles in the included studies was in the ultrafine size range, with modes in number distribution reported primarily in the range of 20-100 nm (18) with the only study of oven cooking reporting particles modes between 20-70 nm (295). The particle mode is dependent on oven temperature and the type of meal cooked, which may explain the difference in mode ranges when compared to the UltraFine Project (32-104). The observed average particle diameters during cooking in the UltraFine Project, are similar to particle concentrations in the exposure study by Soppa et al. reporting that particles between 50 and 100 nm dominated the UFP size range when frying sausages on a pan without oil (109).
In the UltraFine Project, the peak number of particles for candle emissions was found for particle diameters ~7.5 nm, with the average particle mode diameters in the range 6.2 to 9.2, thus exhibiting less variation than cooking emissions. High number concentrations of UFPs with a diameter below 10 nm is in agreement with other studies on candles (111,115,116). For candle burning, the concentration reported in Soppa et al. was dominated by particles between 10 and 30 nm (109), which is higher than the average particle mode diameters observed in the UltraFine study. In the study by Hagerman and colleagues, they observed mode of particles between 21-25 nm and 256-284 nm (117). Differences from the UltraFine Project may be explained by different ventilation rates during exposures and/or the composition of the candles used (111–113). In the UltraFine study another, but minor, peak was observed around 200-300 nm, which is in agreement with other studies including the study by Hagerman et al. reporting that particles derived from soot are having a mean diameter of ~270 nm (112,113,117). As candles in the UltraFine Project were burning with a flickering flame, the second peak observed in the study is also expected to be caused by soot particles. For candle particles, the small particle sizes showed some hygroscopic growth when exposed to high humidity, which is explained by their hydrophilic nature due to the amounts of inorganic salts contained (cf. Table 2.2) (113).

In the UltraFine Project, cooking resulted in high levels of VOCs, including aldehydes, which have also been shown in previous studies of cooking activities (18,296,297). Aldehydes may be products of unsaturated fatty acids (298). The type of ingredient cooked is key in the release of aldehyde emissions during cooking (18,297), with previous studies showing that broiling ground beef emits more aldehydes than frying vegetables (18).
In the UltraFine Project, measurements of identified VOCs showed low levels during exposure to candle emissions, with detected VOCs including benzoic acid, isopropyl alcohol, 1-butanal, toluene and benzene. Similarly, previous studies have found low concentrations of VOC during candle burning including 1-butanol, benzene and toluene (299–301). The low concentration of VOCs is probably a result of high combustion temperature and the relatively complete combustion.

Acute health effects

Cooking and candles emitted particles in the fine and ultrafine size range, which have the potential to be inhaled and subsequently deposit in the alveolar region of the respiratory system, potentially translocating into systemic circulation (28,70,71,77,81). Existing exposure studies with cooking and candle emissions have primarily focused on cardiovascular biomarkers such as arterial blood pressure, arterial stiffness, and heart rate variability (102,109,117,208,209) with inconsistent findings (cf. Table 2.4).

When examining airway response, different effects on biomarkers were observed in the UltraFine Project. Below, selected findings are discussed in relation to existing literature. Further discussions can be found in Paper II and III.

In the UltraFine Project a decrease in nasal volume on days with cooking exposure was observed – and in males this decrease was significantly different from days with clean air. The observed changes are interpreted as changes in the thickness of the mucosal membrane most likely caused by inflammation, as an inflammatory response can lead to swelling of the nasal mucosa, decreasing the overall volume of the nasal cavity (242). The observation of decreasing nasal volume is similar to results reported in two observational studies where exposure to elevated levels of indoor air pollutants (PM_{10}, dust, formaldehyde, NO_2, and molds) in class rooms lead to decreasing nasal patency (194,239). When breathing at rest, the nose and hence, the nasal mucosa is the first part of the airways in contact with the environment (28,77). Hence, it seems plausible that particles in the larger size ranges emitted from cooking, may be trapped in the nasal mucosa, consequently affecting nasal volume.

In the UltraFine Project no change in lung function (FEV_1 and FVC) was observed on days with exposure to cooking and candles, respectively. This finding is not in concordance with the findings in the exposure study performed by Soppa and colleagues (201), where they concluded that two-hour exposures to elevated indoor particles from candles and cooking may be associated with small decreases in lung function in healthy adults (201). The inconsistency in findings may be partially attributed to the different concentrations employed by the studies, differences in volunteers, as well as differences in the amount of time between exposure and the assessment of health effects. The results in Soppa et al. showed most consistent associations for $MEF_{25\%–75\%,}$ an indicator for small airways obstruction, which was not examined in the UltraFine Project. Additionally, Soppa et al. did multiple testing and stated that the small decreases in lung function following indoor particle exposure might be due to chance findings.

The observations in the UltraFine Project are also in contrast to several observational studies examining non-asthmatics and asthmatics, finding strong evidence for short-term effects of fine and ultrafine particles on lung function, especially in children (70,192,193,195,196). However, overall, effects of indoor particles on lung function remain inconclusive (302). The absence of significant associations in the UltraFine Project does not preclude that lung function in individuals with mild asthma is affected by candles or cooking emissions, however, in the UltraFine study, no such associations were observed. It may be due to the relatively low levels of PM.

Nitric Oxide (NO) is present in the exhaled breath and it has vital functions in several aspects of the lungs including being a bronchodilator, a neurotransmitter and an inflammatory mediator (303). Cells involved in the inflammatory response produce NO, hence, elevated levels may indicate airway injury and inflammation (235,303). NO production in the lungs has been reported to increase with high levels of air pollution (196,304), however, in the UltraFine Project exposure to candles seemed to reduce NO concentrations in the exhaled breath of males. Decreasing NO-concentrations from the lungs has also been observed in studies examining exposure to smoking of conventional cigarettes and e-cigarettes (161,162,237,277). The findings could be explained by high levels of NO_2 emitted from candles and cigarettes downregulating the NO synthases in the lungs (168,305). Stabile et al. suggest that alveolar macrophages are inhibited in producing regular rates of NO, as a consequence of a high dose of particles in the alveoli, as they are instead busy processing particles (198).
Whether reduced NO-concentration following candle exposure may limit the vital functions of NO in the lungs is unknown, and the results needs to be confirmed in further experimental studies. In general, the UltraFine Project was not powered to conduct analyses stratified by sex, and the outcomes discussed above are secondary outcomes of interest, why they have to be viewed as hypothesis-generating.

When examining small airway changes, results in the UltraFine Project showed different effects on SP-A concentrations following candle and clean air exposure. Whether the difference in effects are caused by a protective effect of clean air or an increasing effect of candles on SP-A is not certain. Recent research on diurnal variation in healthy individuals, showed that SP-A in the small airways increased from morning towards afternoon (306). This in combination with nearly stable levels of SP-A during candle exposure, point to an effect of clean air on SP-A concentrations in the small airways as discussed in Paper III.
The observed decrease in SP-A concentrations following cooking exposure was similar to the decrease observed following clean air. This in combination is difficult to explain, however several mechanisms may reduce levels of SP-A. Hypothetically, decreasing levels of SP-A following cooking exposure may be explained by increased consumption and/or increased leakage of SP-A into the vasculature due to increased membrane permeability not compensated for by an increased production of SP-A (225,278). In a previous study of allergen provocation in patients with asthma, the concentration of SP-A in BAL-fluid was significantly decreased due to allergic inflammation (307).
The affection of SP-A in small airways can be dependent on hydrophobic or hydrophilic surface coating (226,308), which may serve as an explanation for candles and cooking affecting SP-A differently. Also, cooking may have affected SP-A through its high levels of VOCs, however, further studies are needed to elucidate the effect of cooking and candle exposure on SP-A.

In the UltraFine Project, a numerical, but not significant, increase in albumin following cooking and candle exposure was observed in all statistical models when compared to clean air exposure. Albumin is abundant in the alveolar lining fluid because of a constant minor leakage of plasma protein into the airways (229). Lymphatic drainage and recirculation balance the continuous leak of albumin from the vascular space into the interstitial space

(227), however, increased albumin concentrations in the alveolar lining fluid may be associated to small airways inflammation, as damage to the small airways caused by inflammation can increase the permeability of the blood-air space barrier, thereby resulting in a leakage of albumin from the vasculature into the airways (228,229). Leaking of albumin into the airways may increase the colloid osmotic pressure in this space (227).

When examining systemic biomarkers of inflammation, the majority of the included biomarkers in the UltraFine Project were not indicative of inflammation. However, some markers including CCL2 and gene expression related to IL-8 increased following candle exposure, while other markers (IL-1β and TNF-α) decreased.
In several studies, enhanced levels of serum cytokines have been used to determine the systematic inflammation level in humans exposed to air pollution (70,153). Some interventional and observational studies have found that indoor particle pollution can lead to increased markers of systemic inflammation including oxidative stress, CRP and release of several cellular mediators such as cytokines (202–205) and declining levels of endothelial progenitor cells (259).
Reasons for not observing similar effects in the UltraFine study may include lack of power, low exposure levels of a single pollutant and that some compartments in the immune system may not be activated due to only weak effects. Higher particle mass concentrations or longer exposure duration may have led to similar results as found in observational studies. Observational and interventional studies represent pollution mixtures, not examined in the UltraFine Project, which may also lead to different health effects due to synergistic interaction between pollutants augmenting the individual effects. The lack of consistency in effects on inflammatory markers may also be explained by natural human defense mechanisms that may cope with relatively low-dose particle exposure for a limited period of time.

Increased levels of several lipoproteins were observed following cooking exposure when using a metabolomics approach in the UltraFine Project. Increasing levels of lipids and lipoproteins are metabolic changes commonly observed following inflammation (309,310). If the inflammatory response persists it may contribute to increased risk of atherosclerosis (310). Some of the peaks observed in unsaturated fatty acids corresponded to GlycA, which is suggested to reflect systemic inflammation at least as good as CRP as discussed in Paper III (265,309). The application of a metabolomics approach in relation to indoor air pollution is considered explorative, thus, concluding on causal relations between cooking and the observed metabolic changes, should be with caution until further confirmation of the results. Yet, a recent intervention study reported similar results, with high $PM_{2.5}$ exposure (53.1 $\mu g/m^3$) found to enhance serum lipid metabolites when compared to low $PM_{2.5}$ exposure (205), supporting the findings in the UltraFine Project.

Sex-specific response to the exposures
As a growing body of literature reports diverse responses to environmental exposures (e.g. smoking and chemical exposures) (260,275,311,312), sex-specific analyses were performed for all outcomes in the UltraFine Study. Results showed that that in some outcomes males

responded stronger (metabolites, nasal volume, FeNO), whereas in other outcomes women showed the highest response (nasal lavage biomarkers and albumin in exhaled air).
In the existing literature on sex-related response to environmental exposures, the range of plausible explanations is broad. First, the most well-characterized mediators of sex differences in immune response are genes and sex hormones (312). Second, different lung size and airway diameters may lead different deposition of fine and ultrafine particles (275,313). Third, distribution of different lifestyle factors between sexes may explain and modify the biological response (275). Generally, studies among children have found stronger associations between air pollution and respiratory health effects among boys (275,313), while most studies in adults report stronger effects among females, especially when examining innate and adaptive immune responses (275,312). However, the literature is far from consistent – like results in the UltraFine Project, and the findings warrant further research.

Candle vs. cooking exposure
In the UltraFine Project, cooking and candles induced different response in some biomarkers and self-reported symptoms, with SP-A in exhaled air being affected differently by exposure to cooking than by candle exposure, and CCL2 affected significantly more by candle exposure. A larger drop in nasal volume was observed when comparing days with cooking exposure to days with candle exposure and several symptoms of irritation were significantly more pronounced during cooking exposure compared to during candle exposure.
Differences in biomarker response may be explained by differences in particle size for the two exposures. Although both emissions were in the ultrafine size range, the different size distributions can affect the deposition in the body (cf. figure 2.4) (28). Some of the largest cooking particles may be have been caught in the nose inducing a higher inflammatory response than candles.
Additionally, the chemical composition of particles may be responsible for differences observed in biomarker response. Candle particles consist of inorganic salts, organic compounds and soot (113,114), while particles from cooking are mainly composed of organic compounds (18). In addition to fine and ultrafine particles, the emissions included gases and VOCs, which were not controlled in our study. These may also explain the observed health effects either in itself or in combination with the particles (314). Cooking induced more pronounced self-reported symptoms of irritation, which may be caused by the smell, but results are more likely explained by the fact that cooking emissions contain hundreds of pollutants and some of them are known respiratory irritants, such as aldehydes including acrolein and formaldehyde (292,294,315). Candle emission included NO_2 at ~53 ppb on average, which may have contributed to changes in exhaled NO (316,317).

5.3 Methodological considerations
In this section the internal validity of the two studies comprising issues regarding design, participants, exposures, health outcomes and statistical methods is discussed. Finally, generalizability (external validity) of the results is deliberated.

5.3.1 Design considerations

In controlled human exposure studies, randomization and blinding, among other parameters, seek to prevent systematic error (bias), and hence, increase internal validity (318,319).
The two studies included in the present thesis were controlled, randomized, double-blinded crossover studies. In a crossover study, the response of an individual to exposure A is contrasted with the same individual's response to exposure B (320). Crossover studies have at least two advantages over non-crossover studies. First, as each participant serves as his or her own control in the statistical analysis, the influence of confounding is reduced. Second, as the effect of variation between subjects is reduced, optimal crossover designs enhances statistical power to examine mechanistic pathways, therefore requiring fewer subjects than non-crossover designs (320).
In both studies, randomization was applied to minimize possible learning and other time-related effects. Participants were exposed at random to clean air, and particle exposures, with the randomization made by a statistician. A possible weakness of the design of the two studies was the time constraints; if one or several particle exposures induced long-lasting inflammatory effects, then these effects could have affected the following session. As discussed in Paper II, such late-effects of particle exposure, however, seem unlikely. If participants were somehow affected by the previous exposure session, the estimates of the exposure can be confounded by carry-over effects. Carry-over effects may be avoided with a sufficiently long "washout" period between exposures (320,321). For the two studies in the thesis, a washout period of two weeks was chosen based on previous experimental studies.
The studies were conducted according to a double-blind protocol, hence the exposures were blinded to both participants and investigators in order to prevent bias (319,321). It was possible to establish a double-blinded set-up as the exposures could be generated in an adjacent chamber. Blinding of participants minimizes perception about the exposure influencing the outcomes. This is especially important when outcomes are subjective as for the self-reported symptoms (318). Blinding of investigators, including the clinical staff, hinder that management of participants is influenced by knowledge of the exposure and that investigators reveal (unintended) clues to participants (318,321). The investigator monitoring the exposures had no contact with the participants or other investigators during exposure days, thus he kept the information about exposures to himself. In both studies, blinding was continued until basic statistical analyses had been conducted, which further eliminates the possibility of bias, as tendencies to overanalyse data for minor differences supporting the examined hypotheses are avoided.
Despite effort, blinding is not always possible. During cooking exposure, blinding of participants proved difficult due to a characteristic odor from the roasted pork (see Figure 5b, Paper II). Thus, cooking was only blinded to investigators. Blinding of candles worked as intended. In Project PASVAP, no exit poll was conducted, but from informal talk with participants, the staff got the impression that participants did not know what they had been exposed to, as they were distracted by vapers occupying and talking in the adjacent chamber both days. However, analysis of odor intensity showed that participants found days with passive vape to have a higher strength of smell than days with clean air, which leaves the question about blinding unanswered. With the benefit of hindsight, an exit poll should have been made. Yet, all exposures were blinded to investigators and objective results are not

expected to be confounded by participant's possible knowledge of the exposure. Lack of blinding of participants most likely could have affected symptom reporting and a possible effect of odor on the ratings on well-being and related symptoms cannot be excluded. Hence, for cooking (and perhaps e-cigarettes) the observed associations with reported mucosal irritation and overall comfort may be artificial associations as a result of reporting bias (322). In both studies, participants had to enter the exposure chamber with 30 minute intervals (due to performing of health examinations), hence, it was not possible to let participants enter a clean chamber, then building up the exposure, possibly making it more difficult to differentiate between the exposures because of slow and gradually odor adaptation. With the knowledge that blinding can be difficult due to odor of emissions, the possibility of including concealing compounds during clean air sessions was discussed. In Project PASVAP, vapers were chewing nicotine gum with fruit taste. However, due to a risk of studying the effect of a concealing compound instead of clean air, no further concealing compounds were used. Compared to existing controlled human exposure studies on passive vape, cooking and burning candles (cf. Tables 2.3 and 2.4), the two experiments in the present thesis used the most optimal controlled design including all possible parameters (randomization, double-blinding and crossover) to prevent bias.

Nevertheless, limitations in the two studies exist. The limited number of participants complicates representativeness of the population and the relatively short duration of exposure reveals only possible acute and transient health effects (146,152). Another challenge is that controlled human exposure studies do not reproduce neither the mixture nor temporal variation that occur in real-life exposures where possible synergistic effects between emissions may happen. Because of experimental design constraints, Project PASVAP and the UltraFine Project, investigated the health effects of exposure to only one pollutant at a time. Real-life exposures are often prolonged and repeated, consequently, health outcomes may range from acute transient effects to chronic effects with the latter often related to intense and longstanding exposure to indoor particles.
Finally, it is important to be aware that in controlled human exposure studies, the absence of significant associations between the exposure and examined outcomes do not preclude effects of the exposure (318). Thus, one cannot conclude that i.e. cooking exposure do not affect levels of EPCs in blood.

5.3.2 Participants

A potential error still remaining after bias is sought eliminated, is random error also known as chance error or statistical error (318,319). Random error can likewise threaten internal validity of the studies, affecting reliable evaluation of the exposures by masking true effects. When variation is larger than the effect, true associations can be obscured (146,319). Random error is caused by variability in the measured data arising purely by chance. To minimize random error, a sufficiently large number of participants should be recruited, as small studies often are subject to a greater degree of random error (319). In project PASVAP, we aimed for 30 participants, however, only 16 individuals participated. It proved very difficult to recruit individuals healthy enough to participate and with the possibility to cope without medication, and if healthy enough, not being busy with work or travelling. A larger

sample size might have minimized random variation (318,319). For several outcomes in the study, considerable variation, both regarding within participants and between participants was found. In the UltraFine Project, for serum cytokines and gene expression, several markers of inflammation were near detection limits, resulting in high variability.
Some of the known and presumed parameters influencing variability in human response to air pollution are health status, age, gender, atopy, airway responsiveness and exercise level (146). In both studies, variability may be increased by choosing populations with respiratory disease, as they may not be as homogenous as a population of healthy adults (146). COPD is a quite heterogeneous disease (120), and especially in Project PASVAP, participants differed with regard to health status and age (cf. Appendix III). Each parameter (age, gender, comorbidity) might have introduced random variation possibly masking effects, however, contributing to increased generalizability.

5.3.3 Exposures

Generally, control over exposure conditions including concentrations, mixtures, duration and emission source is a particular strength of controlled human exposures studies (152). However, in Project PASVAP, the concentration of exhaled aerosol from vapers proved difficult to predict and control. On days with vape exposure, low and varying levels of aerosol were generated from the vapers in the adjacent room. It proved difficult to control number and mass concentrations due to the vapers' different vaping styles and their level of experience in using e-cigarettes. The fact that the generated aerosol is, among other things, dependent on use behaviour has been shown in several other studies (85–87,187). No puffing topography protocol (i.e. a manual on puff duration and inter-puff interval) was applied in Project PASVAP, although it has been used in some experimental studies (96,168,182,188). This may have uniformed exposure concentrations, however, it would have put great demands on vapers. As a result, participants were exposed to varying levels of aerosol across exposure sessions.
In Project PASVAP, the dose administered to the majority of participants was estimated to be considerably lower than the dose encountered by people in real life; on six out of eight exposure days with passive vape, $PM_{2.5}$ was below 45 $\mu g/m^3$. It has been reported that in most cases, the indoor $PM_{2.5}$ levels during e-cigarette use are above 150 $\mu g/m^3$ (86). Consequently, real exposure to harmful substances might be underestimated. An explanation for the low exposure levels might be that some of the included vapers in Project PASVAP were e-cigarette naïve users. Naïve e-cigarette users have been shown to generate less aerosol to the surroundings compared to experienced users (61).
The chosen e-cigarette model in Project PASVAP was marketed as a beginner e-cigarette, and the health effects of e-cigarettes in general might be underestimated as a consequence of low power. Greater power in the e-cigarette increases the potential harm, as more aerosol with harmful constituents is produced and exhaled to the surroundings (88,323).

The UltraFine Project

The particle concentrations produced in the UltraFine Project are expected to occur in real life and not to be an over-estimation of indoor concentrations. In the study, average air exchange rates were high compared to ventilation in private homes. Insufficient ventilation in

private homes may provide indoor environments in which contaminants are readily produced and may build up to much higher levels than found in the UltraFine Project. When cooking using several cooktops or burning several candles at once, particle mass concentrations around 200-350 μg/m^3 have been found in real-life conditions (6,27,108,110,204). Compared to similar exposure studies, levels in the UltraFine Project were relatively low (102,109,117,201). Higher levels may have induced higher responses in the investigated health outcomes.

Studies of cooking emissions have been examined in controlled environments and in real-life kitchens. Controlled and real-life environments are different in relation to factors influencing the emissions and emission levels. In controlled experimental studies, emissions are affected mainly by cooking process, the food prepared, and the fuel used, whereas in real-life kitchens, emissions are influenced by several factors including ventilation conditions, cooking process, room arrangement, infiltration of outdoor air, and other combustion devices (18). Thus, a controlled environment is different from a real-life setting.

The choice of cooking method was pragmatic; for the exposures to last for several hours, cooking in an oven was chosen. In the HOMEChem Study the authors observed that particle concentrations below 10 nm were several times lower for oven cooking than for meals cooked on a stove (110), therefore cooking in an oven may not be similar to other types of cooking with regards to particle size distributions.

During candle exposure, a slow-rotating fan made the candles flicker at a slow pace. Steady burning of candles has been shown to emit much lower levels of elemental carbon and fewer larger sized particles than candles during sooting burn (113). The exposure could have been different if other ventilation conditions and/or candles made of other sources e.g. paraffin or wax were used. Light draught was chosen to resemble real-life scenarios, where candles burning near a window or on a table often flicker and emit soot, due to air streams caused by movement or draught in vicinity of the candle.

In both exposure studies, participants were exposed at rest, as this is presumed to correspond to a normal indoor activity level. However, exercise might modify delivery dose and hence, response, by increasing breathing rates and by causing a shift from nasal to oral breathing effectively bypassing the nose and the possible clearing of some of the inhaled particles (71,146).

5.3.4 Health assessment

The use of gold standard instrumentation including sampling equipment, sampling conditions and clinical staff is a particular strength of the controlled human exposure studies in laboratory facilities such as the Climate Chamber facilities. Human exposure studies are useful for examining multiple study endpoints within the same study, which also allows for test of explorative mechanisms as in Project PASVAP and the UltraFine Project (152). Furthermore, the possibility to combine subjective and objective measurements provides a wide range of endpoints.

To gain information on respiratory symptoms, lung function measured by spirometry, nasal volume and FeNO-concentrations were examined – methods for measuring health effects of PM air pollution, which have been used for decades (146,152). Some strengths and limitations of the measures are described in Chapter 3, however, a specific disadvantage in

Project PASVAP, was that participants were often too exhausted to complete the forced exhalations for spirometry and FeNO. Consequently, several data points were missing in the study. Contrary for the UltraFine Project, participants were able to complete all health examinations causing only few missing data.

In general, measurements of biomarkers provide an excellent way to assess the health status of an individual. Optimally, the examined biomarkers should be stable over time, and with low within individual variability (324). However, for most biomarkers variability associated with daily activities or participant-dependent factors play an important role in the assessment (324). A particular challenge when evaluating biomarkers in general and in the two exposure studies is diurnal variation, as the time of the day may influence the results obtained (325). In the two exposure studies, all clinical investigations were performed the same time of the day before and after each exposure session and at the same day of the week. Thus, diurnal variation is not believed to explain the differences observed between the exposure sessions. Participants had no intake of coffee, tea or other substances known to affect the measures. As both diurnal variation and the inflammatory activation that may be produced during exposures change the response, it is crucial to establish benchmark values for comparison against as well as for the interpretation of data. In the two studies clean air exposure was used as control exposure. Nonetheless, as the air delivered to the climate chambers was filtered through a series of filters including a final stage with HEPA- and carbon filters, the air let into the exposure chamber was very clean ($PM_{2.5} < 6$ µg/m^3) compared to everyday indoor PM concentrations (326). Thus, it is important to be aware that the clean air exposure is an exposure in itself and may affect the investigated biomarkers. In the UltraFine Project, for example CRP levels declined following exposure to clean air, possibly as a consequence of the very clean air (327,328). Knowledge of biomarkers variability is essential for causal inference, however, for novel biomarkers such information is scarce.

A potential challenge in measuring health effects in human exposure studies, is an unfavorable signal-to-noise ratio. For safety reasons, the particle concentration to which the participants are exposed, are usually kept relatively low. Additionally, the exposure duration is short (a few hours), and consequently, biological effects may be weak and the signal-to-noise ratio unfavorable. Thus, if inter- and intra-individual variability (noise) is high, it will be difficult to distinguish real change (the signal) from natural changes and diurnal variation (324). An unfavorable signal-to-noise ratio has been stated as one of the reasons that metabolomics is not well developed within environmental research as the metabolic changes thought to occur following exposure are expected to be minor in comparison with the inter-individual variability that can be observed in a human population (263).

The majority of the selected types of biomarkers used in the two exposure studies are standard methods previously used, they have been validated in previous studies and clinical utility is known. However, some of the key biomarkers showing possible effects of exposure are new, and interpretation towards actual health effects is difficult. Nevertheless, inclusion of new biomarkers is also considered a strength, with the possibility of suggesting new mechanistic pathways. The new biomarkers in the two studies were chosen to become better at detecting the often very weak effects of exposure in controlled studies.

5.3.5 Statistics

When using linear mixed-effects models, models including interaction between exposure and time (referred to as Model 1) are preferred over models without interaction, as it is evident when a change in the health outcome occurs. However, when interaction terms are not statistically significant, models without interaction can be fitted. Such models can be fitted in several ways depending on the results one wish to report. In Paper I and II, results from the models without interaction (cf. Model 2) reported the mean change for each exposure for all included time points, corresponding to a summary measure of the difference between exposure days. When clear deviations between baseline values for the exposures are present, the baseline values should instead be adjusted for in the model as in Paper III. When baseline values for the examined exposures are similar, results will be the same regardless of which model is used.

One advantage in human exposure studies is the possibility to examine several different health outcomes, however, multiple comparisons increase the risk of chance findings i.e. the risk of finding false-positive results (318). Multiple comparisons require caution when interpreting data, and in the two studies it is therefore underlined that secondary outcomes are merely hypothesis-generating, and should not be seen as conclusive.

5.3.6 Generalizability

In the following, generalizability is considered in regard to the included study populations, exposures and health outcomes. On a global basis, the problem regarding indoor air pollution in high-income countries cannot be compared to extreme indoor exposure levels in low-income countries. Therefore, results from the present study are limited to encompass high-income countries with similar living conditions as in Denmark. The thesis only comprise particulate air pollution, hence, health effects of other factors of importance to the indoor climate e.g. dampness, chemistry, mold, radon etc., were not examined.

In the present thesis, the study populations in the two studies consisted of two small groups of individuals with respiratory disease; elderly with COPD and young adults with mild asthma. As the characteristics of their respiratory disease make the included individuals more vulnerable to PM pollution compared to healthy individuals, the results of the studies may not apply to individuals in the general population. However, results may be generalized to the target populations as discussed below.

As individuals volunteered to participate, one thing to consider is whether there exist differences between those volunteering and those not wishing to participate. Participants with COPD were between 56 and 77 years of age, having either mild or moderate COPD with several participants suffering from comorbidity. Their educational level and lifestyle was quite diverse and both men and women were included. Thus, they may be representative of the target population i.e. adults with COPD. Participants with mild asthma were between 18 and 25 years of age and most of them were studying at a higher educational institution. Hence, participants in the UltraFine Project may be more well-educated than the general population of young asthmatics, but otherwise representative of the target population. The results of thesis may be generalized to other vulnerable subgroups of the population such as children, the elderly and individuals with other respiratory disease.

A challenge in evaluating health effects from the three included exposures is the diversity in exposure sources in real life. In addition to the behaviour of e-cigarette users there is great heterogeneity in e-cigarette devices and e-liquids, which made it challenging to select representative users and products when designing the study. In 2014, more than 460 brands of e-cigarettes existed with new brands continuously being brought into the market. Each brand may provide a different result (43). Furthermore, e-liquid including flavour and nicotine content affects the aerosol and complicates research on potential health effects. In Project PASVAP, two out of approximately 17,000 flavours worldwide were used (329). Consequently, results may be difficult to apply to the entire landscape of e-cigarette devices and e-liquids.

Different cooking styles emit different profiles of compounds and varying PM levels (18), therefore, cooking emissions generated from breast of pork in an oven as in the UltraFine Project is likely not representative of all cooking styles. Candles are produced from a variety of materials including wick material, and it is not clear how the individual components of the candle affect particle emissions when burning (111). Thus, it is uncertain to which extent candle emissions from the UltraFine Project are representative for other candles of different composition and design. For all three exposure scenarios, the participating subjects were only studied after one single exposure, whereas in real life diverse, prolonged and repeated exposures could potentially enhance the health response.

In the two studies, new biomarkers showed interesting results, however the results need to be confirmed by other studies before reaching final conclusions on inflammatory effects of the exposures. As the outcomes observed in the two studies are transient mechanistic effects it is difficult to extrapolate the observed changes to endpoints frequently applied in epidemiological studies such as morbidity and mortality. However, the scientific relevance of human exposure studies is to provide evidence of causal effects or suggest biological pathways of inflammation in the development of clinical effects, thus, the outcomes from the two studies should be seen as complementing evidence obtained from epidemiological studies (152,153).

Chapter 6. Conclusions

This chapter summarize the main conclusions of the two studies part of the thesis. An experimental approach was used to examine the acute health effects of indoor particulate air pollution among individuals with respiratory disease. In Project PASVAP, the association between exposure to passive vape from e-cigarettes and acute health effects was explored in individuals with COPD. In the UltraFine Project, the association between emissions from cooking and burning candles and acute health effects among young individuals with mild asthma was examined. Overall, the results of the present thesis indicate that indoor air polluted with fine and ultrafine particles from e-cigarettes, cooking or candles can induce mild inflammatory responses and decrease well-being among individuals with respiratory disease. The main conclusions from each study are outlined below.

6.1 Project PASVAP

• Different vaping styles among e-cigarette users affected mass and number concentrations of the exhaled aerosol. Consequently, large variations in exposures was observed throughout the study.

• The peak number of particles on days with vape exposure was in the size range of ~30-40 nm.

• Passive exposure to aerosol from e-cigarettes was associated with decreasing levels of SP-A in exhaled air and increased levels of several plasma metabolites including cholesterol and LDL when compared to clean air exposure (cf. hypothesis 1).

• Negative changes – but not statistically significant – were observed in FEV_1 and FVC on days with passive vape exposure compared to days with clean air exposure. No significant effect of passive vape exposure was found on FeNO-concentrations.

• Passive vape exposure was associated with participants reporting more throat irritation – although at low levels – when compared to clean air exposure. Other symptoms related to COPD and mucosal irritation did not show significant associations with passive vape exposure (cf. secondary hypothesis 1).

In summary, Project PASVAP suggest that exposure to passive vape from e-cigarettes is capable of exerting acute responses in lungs and blood as well as throat irritation indicating mild inflammatory effects among individuals with COPD. Further studies are needed to confirm the results.

6.2 The UltraFine Project

• Burning candles and cooking emit particles within the ultrafine size range. Candles were found to emit particles with modes for diameters ~7.5 nm, while particles from cooking were found to have a mode for diameters ~80 nm. During exposure to high humidity, candle particles in the smaller size range showed some hygroscopic growth, most likely explained by the hydrophilic nature of inorganic salts.

• Exposure to cooking emissions was associated with increasing lipids and lipoproteins including GlycA, and decreasing nasal volume – particularly among males. Although not significant there appeared to be a trend for albumin concentration in the small airways to increase following cooking exposure. SP-A appeared to decrease like clean air (cf. hypothesis 2A).

• Exposure to emissions from cooking decreased participants' general well-being and exerted more serious reported symptoms including mucosal irritation in the eyes and nose during exposure compared to when exposed to clean air (cf. secondary hypothesis 2A).

• Following exposure to emissions from burning candles, CCL2 in serum increased significantly. Candles seemed to reduce NO concentrations in the exhaled breath of males. SP-A in the small airways remained nearly stable following candle exposure, yet, SP-A was affected differently by candles and clean air. A numerical, but not significant, increase in albumin in the small airways was observed (cf. hypothesis 2B).

• Exposure to burning candles exerted more subjectively reported symptoms, reported as eye and nose irritation during exposure compared to clean air exposure (cf. secondary hypothesis 2B).

• Only weak and inconsistent associations were found between candle and cooking exposure, respectively, and systemic inflammation biomarkers, EPCs and gene expression for oxidative stress, DNA repair and inflammation.

• In some outcomes, males and females responded differently to the exposures. Whether this is a random finding or caused by biological differences is unknown.

• Candles and cooking affected SP-A in the small airways differently, however, only borderline statistical significant differences between the two exposures was observed in one of the statistical models. No significant differences between the two exposures were observed for albumin in the small airways (cf. hypothesis 2C).

• Candles affected CCL2 in serum significantly more than cooking did, while cooking affected several symptoms of irritation significantly more than candles. Nasal volume was affected to a higher degree during cooking exposure compared to candle exposure. Differences in health effects caused by cooking and candle exposure may be explained by differences in particle size and chemical composition of the two different emission sources.

In summary, short-term exposure to cooking and candle emissions at levels found in residential homes can induce mild transient inflammatory responses in airways and blood. Emissions from candles, and in particular cooking, decreased wellbeing among asthmatic individuals. Candles and cooking affected biomarkers differently, but no conclusion can be drawn on the hypothesis that candles induce a larger effect on SP-A and albumin in the small airways, than cooking, although a borderline significant difference between candle and cooking exposure on SP-A concentration was observed. Several of the findings are exploratory and warrant further research.

Chapter 7. Implications and future research

In the present chapter, strategies for prevention of indoor particulate air pollution and challenges for future research are reflected upon.

Public health is concerned with preventing disease and promoting health in populations (330). Nowadays, ambient and indoor air pollution are of concern to the health of populations worldwide. Globally, there exist limited information about indoor exposure to particle pollution to understand and support the health effects observed in epidemiological studies, thus, the present thesis adds to the existing knowledge. In particular, the thesis adds new perspectives on indoor particulate air pollution among vulnerable population subgroups. It has been demonstrated that emissions from e-cigarettes, cooking and burning candles can induce mild inflammatory response in airways and blood as well as cause symptoms of irritation among people with respiratory disease. The results in the present thesis therefore support recommendations to reduce indoor air pollution, including taking actions toward exposure to passive vape from e-cigarettes and emissions from cooking and burning candles in order to protect the public from potential adverse health effects.

The most effective approach to improve indoor air quality is to prevent or limit the emissions at the source. As cooking, and in particular, e-cigarettes and candle burning are modifiable exposures, reducing such exposures could be a method for reducing disease related to indoor air pollution in the population – and especially in vulnerable population subgroups, who may be inconveniently affected by the pollutants.

Reduction of indoor air pollution in residential homes is the responsibility of the resident, however, comprehensive public health policies and strategies that aim to reduce indoor air pollution in the population can be developed and implemented to promote individual action. At a national level, guidelines on how to reduce indoor particle pollution can be made to inform individuals. At present, several Danish non-governmental companies and institutions have established advice to the public on how to improve their indoor climate including the need for natural ventilation (331–333). Moreover, public policies may encourage or mandate engineering solutions that increase ventilation thereby reducing air pollution concentrations inside.

Regarding passive vape exposure, national strategies including effective public policies to reduce the emission source, i.e. e-cigarettes, are clearly preferable. During the last couple of years, several efforts have been implemented in order to regulate and hence, reduce use of e-cigarettes. In 2016, “The law on e-cigarettes” was implemented in Denmark based on “Tobacco Products Directive (EU)” (334). Included in the law is restriction of sales to minors and a prohibition on commercials. The law on e-cigarettes is protecting the public against vaping indoors in schools, in day care facilities, and in public transportation. However, other public places including work places, shopping malls and hospitals, should decide for themselves whether it is allowed to use e-cigarettes indoor. In Denmark, as of April 1st 2022 it is prohibited to sell e-liquids with flavors other than tobacco and menthol (335). The coming regulation may address some concerns regarding prevention of e-cigarette uptake and consequently, prevention of passive vape exposure (66,336), as sweet flavors are a critical motivator for e-cigarette use especially among youth and young adults (66,337).

Some countries such as Argentina, Brazil, and Jordan have prohibited production and sale of e-cigarettes (336). It is unlikely that e-cigarettes will be banned in most countries including Denmark in the near future, thus, it is necessary to keep reducing use and hence, exposure to emissions from e-cigarettes. In line with recommendations from WHO Tobacco Free Initiative and the Danish Cancer Society (38,338), it is suggested that regulations to protect bystanders from passive vape exposure should be enacted, similar to that of the smoke-free legislation (cf. the Danish "Law on tobacco products"), thereby prohibiting use of e-cigarettes anywhere that use of conventional cigarettes is prohibited. Previously, smoke-free legislation has provided examples of successful interventions associated with health benefits at a population level (339,340).

Additionally, individual actions in private homes can reduce indoor particulate air pollution. Use of e-cigarettes should be avoided indoor – especially in the presence of children, people with respiratory disease and other vulnerable subgroups. Cooking is for most people an inevitable part of everyday life, however, interventions to reduce air pollution caused by cooking can be established. Evidence show that ventilation (whether natural or mechanical) is key in lowering indoor concentrations of PM (99,341). Simple, but effective actions are to use a cooker hood and air out several times a day. Similar actions are needed to reduce emissions from burning candles. Individuals can reduce the number of candles burned at once, but most importantly they should remember to air out when candles are extinguished. At the societal level, an innovation project called "CANdle Development for Low Emissions" with partners from several universities and industry, is currently working on producing low-emission candles, with the aim of reducing indoor air pollution. Over the past couple of years, battery-operated candles have become popular. They are often sold in order to reduce fire hazards (342), but an important side benefit is that they are emission-free, and hence, a healthier alternative.
Additionally, evidence is pointing towards air filtration using HEPA- and carbon filters as a possible solution to effectively reduce household air pollution in high-income countries (2,30,202,205,328). The suggested preventive measures could potentially benefit the whole population, but would be of particular importance for subgroups in the population susceptible to PM pollution.

7.1 Future research

To better characterize possible adverse health effects, there is a need for further investigation of indoor PM exposure. This may ideally provide essential information on how to create effective preventive strategies for reducing exposure to emissions from e-cigarettes, cooking and burning candles and thereby benefit public health.
Future investigations are needed to clearly identify targetable mechanisms by which indoor pollutants may influence morbidity including more controlled human exposure studies to replicate the limited existing findings of adverse health effects caused by indoor fine and ultrafine particles. Further, interventions with long-term effects, and susceptible populations who are most likely to benefit from these interventions are warranted.
As e-cigarettes have only been on the market for a little more than a decade with the industry evolving constantly, there are many unknowns regarding their impact on health. There is a

strong need for independent research, i.e. research not sponsored by the industry, in e-cigarette toxicity clarifying the specific problematic compounds in the aerosols and the related health effects (343). Current studies on health effect in humans focus on the acute effects and early biomarkers of exposure. The overall evidence suggests potential respiratory and cardiovascular effects from passive vape exposure. However, results are inconsistent, and large cohort studies with long-term exposures are needed to better understand the health effects of active and passive exposure to e-cigarette aerosol. Importantly, future studies should choose the comparator to e-cigarettes wisely. Comparing e-cigarettes to conventional cigarettes makes sense when research is about long-term adult smokers who want to switch to e-cigarettes, but otherwise conventional cigarettes are not a useful comparison. Thus, more research should be conducted comparing effects of e-cigarettes to background exposure. Concerning cooking and candle exposure, there is still a need for detailed characterization for different emission scenarios; candles composed of other materials and different cooking styles. Health effects among other groups in the population should be examined in controlled exposure studies, with further identification of the mechanisms causing the effects observed in epidemiological studies.
For the three exposures included in this thesis, it would be interesting to determine deposition patterns of particles and constituents that are emitted and encountered through the exposures for better understanding their impact on health. Additionally, deposition patterns and health effects across sex warrant further research.

As individuals spend most of their time indoors and most of that time is in the home environment, indoor air pollution continues to be an important target for improving health. Whether long-term exposure over years to indoor pollutants and mixtures of pollutants can lead to long-term health effects, is a question that remains to be convincingly answered. From a public health perspective, it may be the most important question.

Chapter 8. Bibliography

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Appendices

Appendices

The following appendices contain some selected additional material for the thesis – in the text referred to by their roman numbers: Appendix I-IV.

I. Description and use of instruments characterizing the exposures

II. Details on symptom questionnaire

III. Project PASVAP. Participant characteristics

IV. The UltraFine Project. Participant characteristics

Appendix I – Description and use of instruments characterizing the exposures

P-Trak: A P-TRAK Ultrafine Particle Counter (TSI Inc.) is used to detect and count the total number of particles (#/cm³) in the size range of 20-1000 nm (1). The portable instrument was placed in the control room to sample air through tubes one meter into the exposure chamber, and used to monitor the exposures.

SMPS: A Scanning Mobility Particle sizer (SMPS) is an instrument capable of measuring particle size distributions and number concentrations with accuracy and precision (2). The SMPS was placed in the control room to sample air through a one meter copper tubing into the exposure chamber. It was used with either a nano Differential Mobility Analyzer (DMA) or a long DMA connected to an Ultrafine Condensation Particle Counter (TSI UCPC, 3776). The DMA size distributes the particles in size fractions. Subsequently, the particles are counted in the UCPC, so it is possible to read the number concentrations (#/cm³) as well as the particle size distribution. To measure hygroscopic growth of particles, a humidifier was placed in front of the SMPS for short periods of time. It was then possible to measure the particle size distribution before, during, and after exposure to a relative humidity of 90%.

DustTrak: A DustTrak Aerosol Monitor equipped with a $PM_{2.5}$ inlet (TSI, St Paul, Minnesota) was used to monitor the particle mass emissions (µg/m^3) (3). With the logging interval set to one minute, it was possible to follow the development of the particle mass emission over time during exposure sessions.

PM-filters: PM_{10} and $PM_{2.5}$ were sampled during exposures using SKC PTFE filters with PMP Support by means of Personal Environmental Monitor (PEM)-samplers and later, analysed for gravimetric measurements of particle mass concentrations (µg/m^3). Filters were placed in the exposure chamber at the center table in the height of participants' faces. A PEM-sampler is a device with a sampling pump, drawing particulate matter of either 2.5 or 10 µm in aerodynamic diameter through the impactor. The sampling pumps were set to operate at 2 L/min. Larger particles are captured on a disposable pre-oiled impaction disc which fits into the top of the cassette while smaller particles passing the impactor, thereby being collected on a 37 mm filter. The PM-sampler is ideal for environmental PM sampling and in indoor air studies (4). Following exposure sessions, each filter was weighed three times. The average mass from the three weighing was used in subsequent analyses.

Nephelometer: A nephelometer is an instrument able to measure the concentration of suspended particles. It measures suspended particles by applying a light beam and a light detector. Particle density is then a function of the light reflected intro the detector from the particles (5). The particles' potential to scatter visible light was measured with a polar nephelometer (Ecotech Pty Ltd., Aurora 4000) at different wavelengths. The nephelometer was placed in the control room and a one meter of conductive tubing was connected to the exposure chamber. The scattering coefficient has dimensions of cross-sectional area per unit

volume, i.e., m^{-2} or m^{-3}, which is often reported as inverse meters (m^{-1}) or inverse Megameters (Mm^{-1}).

Volatile Organic Compounds and carbonyl compounds: Chemical components including carbonyl compounds and Volatile Organic Compounds (VOCs) were sampled using carbonyl cartridges and Tenax TA adsorbent tubes, respectively. The sampling tubes were situated close to the exhaust ventilator of the chamber. Carbonyl cartridges were connected to a sampling pump set to operate at 2 L/min through tubes into the control room. Tenax TA tubes were connected to a sampling pump system operating at 12 mL/min by tubes leading into the control room.

NO_2: Nitrogen dioxide (NO_2) was measured using API Chemiluminescent NO_2 analyser model 200 A. It uses a chemiluminescence detection principle coupled with electronics to allow accurate and low level measurements of the reaction between nitric oxide and ozone to determine the nitric oxide concentration in gas. NO_2 was measured in the range of 0-500 ppb.

Nicotine filters: Nicotine was sampled during the exposure using SKC PTFE filters with PMP Support Ring 37 mm. Nicotine was collected on the same filters as for $PM_{2.5}$ and PM_{10} mass estimation, thus, they were placed at the center table in the height of participants' faces. After weighting of the mass, filters were analyzed for nicotine.

O_3: Ozone (O_3) was measured by API photometric O_3 analyzer model 400. The model measures low ranges of ozone in the ambient air, by using a method that relates the absorption of ultra-violet (UV) light in proportion to the ozone present (6).

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Appendix II – Details on symptom questionnaire

Questions asked including their endpoints

		Endpoint	
Question no.	Rated variable	0	10
1	Illumination	Annoyingly weak	Annoyingly strong
2	Glare due to reflexes	Not at all	Annoyingly strong
3	Sound level (noise level)	Annoyingly sound dead	Annoyingly noisy
4	Air temperature	Annoyingly cold	Annoyingly hot
5	Air humidity	Annoyingly dry	Annoyingly humid
6	Air movements	Annoyingly stale	Annoyingly draft
7	Air quality	Pleasant	Annoyingly unpleasant
8	Odor intensity	None	Very strong
9	Need more ventilation	No	Yes, very much
10	Eye irritation	Not at all	Very strong
11	Dry eyes	Not at all	Very strong
12	Watering eyes	Not at all	Very strong
13	Irritation of throat	Not at all	Much
14	Irritation of the nose	Not at all	Much
15	Irritation of the skin	Not at all	Much
16	Runny nose	Not at all	Very much
17	Blocked nose	Not at all	Completely blocked
18	Sweating	Not at all	Much
19	Sleepiness or drowsiness	Not at all	Much
20	Headache	Not at all	Very strong
21	Concentration difficulties	Not at all	Much
22	Nausea	Not at all	Very strong
23	Urge to cough	Not at all	Annoyingly strong
24	Shortness of breath	Not at all	Very strong
25	Wheezing	Not at all	Very strong
26	Tightness of chest	Not at all	Very strong
27	General well-being	Very good	Very bad
28	Stressed chamber experience	Not at all	Ongoing strong

Note that definition of the left endpoint of the scale for question no. 1-6 was different from the other questions. Q1-Q6 have 5 as most positive point, while Q7-Q28 have 0 as most positive endpoint

Appendix III – Project PASVAP. Participant characteristics

Descriptive statistics of participants in Project PASVAP (N=16)

No.	Sex	Age	Height (cm)	Weight (kg)	BMI	Comorbidity
1	F	67	169	83	29.1	Osteoporosis, dyspnoea
2	F	69	179	100	31.2	Metabolic disorder
3	M	58	171	85	29.1	Epilepsy, migraine
4	M	64	179	123	38.4	Other lung disease, hypertension
5	M	64	186	84	24.3	Bronchitis, allergy, skin disease
6	F	68	162	103	39.2	Type 2 diabetes
7	M	76	168	76	26.9	Skin disease (rosacea)
8	M	66	190	98	27.1	Bronchitis, metabolic disorder, eczema, phantom pain (fingers amputated), osteoarthritis
9	F	77	166	68	24.8	Hypertension, metabolic disorder
10	M	68	174	97	31.9	Eczema
11	F	69	153	86	36.7	Hypertension, asthma, allergy, psychological disorder (anxiety/depression)
12	M	67	170	67	23.1	-
13	M	77	180	106	32.7	Type 2 diabetes, gout, cardiovascular disease
14	M	67	172	65	21.9	Hypertension
15	F	56	165	62	22.7	-
16	M	69	167	112	40.2	Cardiovascular disease, bronchitis, type 2 diabetes, psychological disorder (anxiety)

Abbreviations: F = Female, M = Male, cm = centimetre, kg = kilogram, BMI = Body Mass Index

Appendix IV – The UltraFine Project. Participant characteristics

Descriptive statistics of participants in the UltraFine Project (N=36)

No.	Sex	Age	Height (cm)	Weight (kg)	BMI
1	F	24	174	70	23.1
2	F	22	172	60	20.3
3	M	25	185	72	21.0
4	M	25	174	77	25.4
5	F	24	173	62	20.7
6	F	22	173	61	20.4
7	M	24	172	70	23.7
8	M	20	184	75	22.2
9	F	23	170	70	24.2
10	M	23	182	94	28.4
11	F	23	176	65	21.0
12	M	22	190	77	21.3
13	F	23	161	81	31.2
14	M	22	173	66	22.1
15	F	21	173	74	24.7
16	M	23	171	68	23.3
17	M	22	183	73	21.8
18	F	21	167	62	22.2
19	M	23	186	62	17.9
20	F	21	175	78	25.5
21	F	20	165	63	23.1
22	M	21	173	69	23.1
23	M	22	181	83	25.3
24	M	23	179	71	22.2
25	F	21	165	64	23.5
26	F	21	163	60	22.6
27	F	23	170	78	27.0
28	F	25	179	69	21.5
29	F	22	166	56	20.3
30	F	22	169	90	31.5
31	M	23	172	56	18.9
32	M	22	183	77	23.0
33	F	21	164	56	20.8
34	M	21	183	87	26.0
35	F	23	168	79	28.0
36	F	18	175	52	17.0

Abbreviations: F = Female, M = Male, cm = centimetre, kg = kilogram, BMI = Body Mass Index

Papers

Paper I

An RCT of acute health effects in COPD-patients after passive vape exposure from e-cigarettes

Laursen, KR., Bønløkke, JH., Bendstrup, E., Bilde, M., Glasius, M., Gutzke, VH., Moosakutty, SP., Olin, AC., Ravn, P., Østergaard, K., & Sigsgaard, T. (2021)

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An RCT of acute health effects in COPD-patients after passive vape exposure from e-cigarettes

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ABSTRACT

Background: E-cigarette use has been shown to have short-term acute effects among active users but less is known of the acute passive effects, particularly among individuals with existing respiratory diseases.

Objective: To investigate local and systemic effects of short-term passive vape exposure among patients with mild or moderate chronic obstructive pulmonary disease (COPD).

Methods: In a double-blinded crossover study 16 non-smoking COPD-patients (mean age 68) were randomly exposed for 4 h to passive vape (median $PM_{2.5}$: 18 µg/m^3 (range: 8–333)) and clean air ($PM_{2.5}$ < 6 µg/m^3) separated by 14 days. Particles were measured using an ultrafine particle counter (P-TRAK) and a scanning mobility particle sizer (SMPS). Health effects including Surfactant Protein-A (SP-A) and albumin in exhaled air, spirometry, FeNO, and plasma proteins were evaluated before, right after, and 24 hours after exposure. Participants reported symptoms throughout exposure sessions. Data were analyzed using mixed models.

Results: SP-A in exhaled air was negatively affected by exposure to vape and several plasma proteins increased significantly. Throat irritation was more pronounced during passive vape exposure, while FVC and FEV_1 decreased, however, not significantly.

Conclusions: SP-A in exhaled air and some plasma proteins were affected by passive vape in patients with COPD indicating inflammation, showing that passive vape exposure is potentially harmful.

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Electronic nicotine delivery systems; electronic cigarettes; human exposure; RCT; particulate matter; spirometry; COPD; secondhand vape

Introduction

As e-cigarettes are by some parties considered a healthier alternative to conventional cigarettes, they are promoted as an aid to decrease or quit tobacco smoking [1]. However, their use as an effective smoking cessation device is questionable [2,3] and so is the idea that they are safe to use [4,5]. There is concern that e-cigarettes may have adverse long-term health effects and serve as a gateway product to cigarettes [6,7]. Several studies focusing on short-term health effects among e-cigarette users indicate that e-cigarettes are not harmless. Evidence show that e-cigarettes are affecting the cardiovascular as well as the respiratory system including respiratory symptoms such as cough, sore throat and dry mouth, reduced lung function, increase in impedance, increased oxidative stress biomarkers, decrease in exhaled NO (FeNO), signs of possible vascular damage, and increased blood pressure and heart rate in users [4,8–11].

E-cigarettes produce an aerosol often referred to as 'vapor' that is inhaled by the user. Unlike conventional cigarettes, e-cigarettes produce no secondary or side-stream emissions; therefore, passive exposure (i.e. exposure to air exhaled by vapers) consists only of what the user exhales. As the number of e-cigarette users is increasing, so is exposure to passive vape. Also, the use of e-cigarettes is often permitted in otherwise smoke-free areas causing passive vape exposure for individuals present [12]. Passive vape exposure remain a concern as previous studies have demonstrated that vape from e-cigarettes can contain toxic chemicals that are harmful to health [13]. A WHO-

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commissioned review found that while there are a limited number of studies in this area, it can be concluded that e-cigarette aerosol is a new air contamination source for particulate matter, which includes fine and ultrafine particles, as well as 1,2-propanediol, VOCs, heavy metals, and nicotine [14]. It is reasonable to assume that the increased concentrations of toxicants from passive vape over background levels poses an increased risk for the health of all bystanders [14] – and, bystanders with respiratory disease might be even more sensitive.

More research is needed to better understand potential health effects to passive bystanders, especially among vulnerable populations, including individuals with existing respiratory disease, known to be frail to environmental exposure [15]. Thus, the objective of the present study was to investigate acute local and systemic effects of short-term passive exposure to vape from e-cigarettes among individuals with mild or moderate Chronic Obstructive Lung Disease (COPD). The hypothesis was that passive vape exposure would lead to inflammation in lungs and blood.

Materials and methods

Details on recruitment, exposures, clinical testing, and statistical analyses are provided in supplemental online material.

Design

In a randomized double-blind, crossover trial participants were exposed to two exposure sessions (Figure 1). Each session lasted 4 h; either air mixed with aerosol from e-cigarette users (median $PM_{2.5}$: 18 μg/m^3 (range: 8–333)) or clean filtered air ($PM_{2.5}$ < 6 μg/m^3) at least two weeks apart in order to eliminate carry-over effects. The filtered clean air and e-cigarette vape sessions were identical except for the air quality.

Participants

Non-smoking patients with mild or moderate COPD were recruited. Patients had a known diagnosis of COPD determined by symptoms and spirometry using FEV_1/FVC below lower limit of normal and MRC score≥2 and CAT (COPD Assessment Test) score≥10. If patients were on long-acting bronchodilators and inhaled corticosteroids, it was converted to short-acting bronchodilators one week prior to participation. Before any exposure session, participants were required to be without signs of infections or airway symptoms for at least one week, and not to have taken any medicine during at least 48 hours. Additionally, participants were asked by a doctor whether they had been abstaining from smoking/vaping, which had to be affirmed. We aimed to include 30 participants according to our power calculation.

Exposure

Exposure sessions took place in an exposure chamber, while exposure generation took place in an adjacent chamber. Three participants were exposed at a time. The aerosol was generated by two or three vapers who, in turn, vaped on e-cigarettes. The most popular brand of e-cigarettes in Denmark was examined; Joyetech eGo AIO (with a 2 ml tank, standard battery capacity of 1500 mAh and a standard Cubis BF coil (0.6 ohm)). We chose the two most commonly sold e-juices in Denmark at the time of the study; The included e-juices were pre-made 'Tobacco' and 'Strawberry' flavour (70% propylene glycol/30% vegetable glycerine) from 'InSano' containing 6 mg of nicotine acquired in containers of 10 ml. All participants were exposed to both flavours in combination. A controlled flow of aerosol was transferred from the vaping chamber to the exposure chamber by a pipe connection using a negative pressure of 10 Pascal. During clean air sessions, vapers were present in the vaping chamber; however, they did not use e-cigarettes. Instead, they were chewing gum (Nicotinell® Fruit) with 4 mg nicotine and fruit taste in order to mask the exposure. The specific vape exposure levels used in this study were chosen to obtain levels comparable with real-life scenarios.

Data collection

Particle size range (7–500 nm) as well as gravimetric measurements were determined using particle counters and filters. Prior to, right after, and 24 hours following exposure each participant underwent a series of health examinations including sampling of Particles in Exhaled Air (PExA), spirometry, Fraction of Exhaled Nitrogen Oxide (FeNO), and a blood sample. During each exposure session, a questionnaire assessing symptoms was completed prior to exposure, every 30 min during exposure, and at the end of the exposure session.

Statistics

Mixed models based on the univariate repeated measurements ANOVA were performed, taking into account the different design variables corresponding to the crossover design [16]. Model 1 included a time-exposure interaction. If the interaction was non-significant, model 2 without interaction was performed. Surfactant Protein-A (SP-A) and albumin in

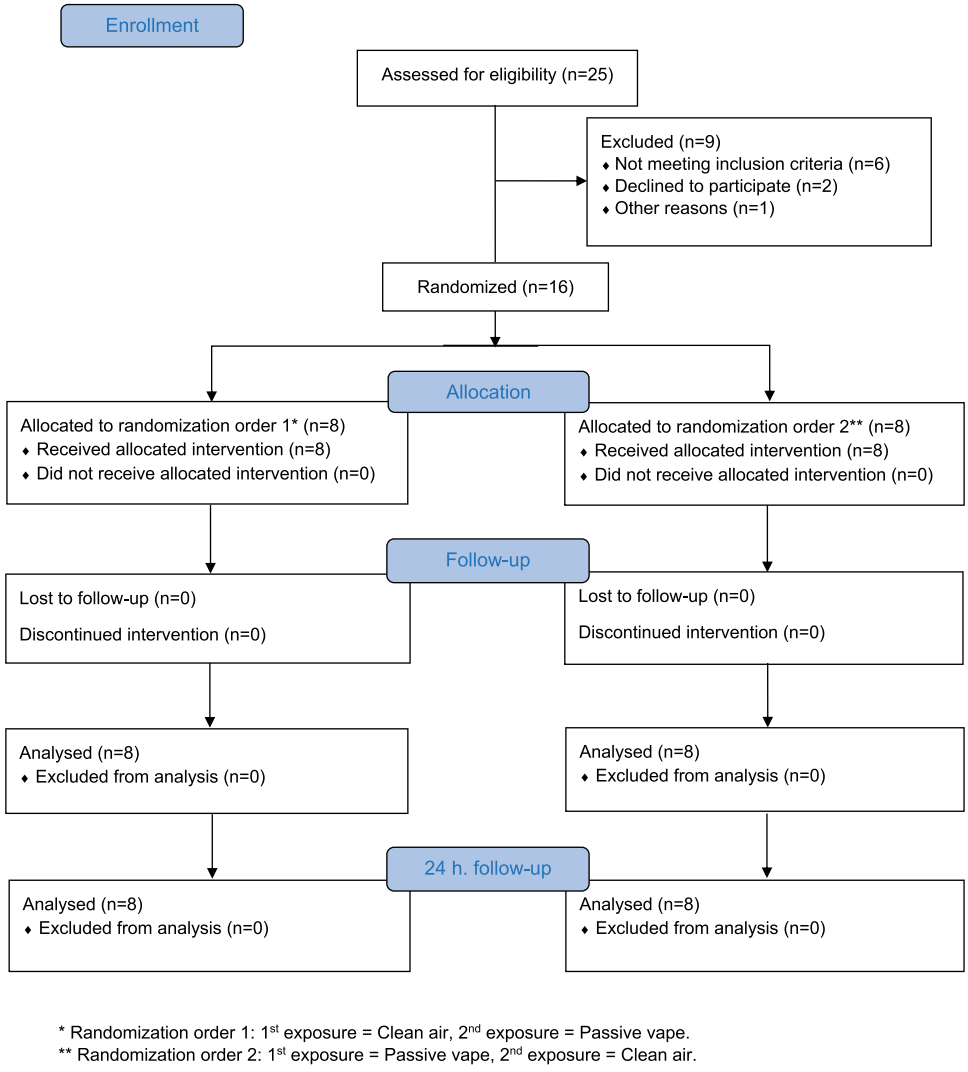

Figure 1. CONSORT (consolidated standards of reporting trials) flow diagram: Number of participants from enrollment to analysis.

exhaled air were the primary outcomes of interest. Secondary outcomes were spirometry, FeNO, plasma proteins, and self-reported symptoms.

Ethics

The Ethical Committee in Central Denmark Region approved the study protocol (ref.no. 1–10-72-273-16) and the project was conducted in accordance with The Declaration of Helsinki. Participants had received written and oral information about the project and provided written consent prior to participation.

Results

Sixteen patients (6 female; 10 male) with moderate severe COPD and a mean age of 67.6 years participated (See Table 1).

Exposures

As shown in Figure 2(a), the peak number of particles on days with vape exposure was approximately in the size range of 30–40 nm. Another peak,

Table 1. Participant characteristics for the study population.

Characteristic	Measure
Participants, *N (%)*	16 (100.0)
Female, *n (%)*	6 (37.5)
Male, *n (%)*	10 (62.5)
Ex-smoker, *n (%)*	16 (100.0)
Age in years, *mean (min-max)*	67.6 (56–77)
Height (cm), *mean (SD)*	171.9 (9.03)
FEV_1* (liter), *mean (SD)*	1.52 (0.57)
FEV_1* (% predicted), *mean (SD)*	0.57 (0.23)
FVC* (liter), *mean (SD)*	2.47 (0.57)
FVC* (% predicted), *mean (SD)*	0.73 (0.19)
FEV_1/FVC*, *mean (SD)*	0.64 (0.33)

Definition of abbreviations: FEV_1 = Forced Expiratory Volume in the first second; FVC = forced vital capacity. * The reported FEV_1 and FVC were measured at participant's pre-examination, which was held before final inclusion in the trial.

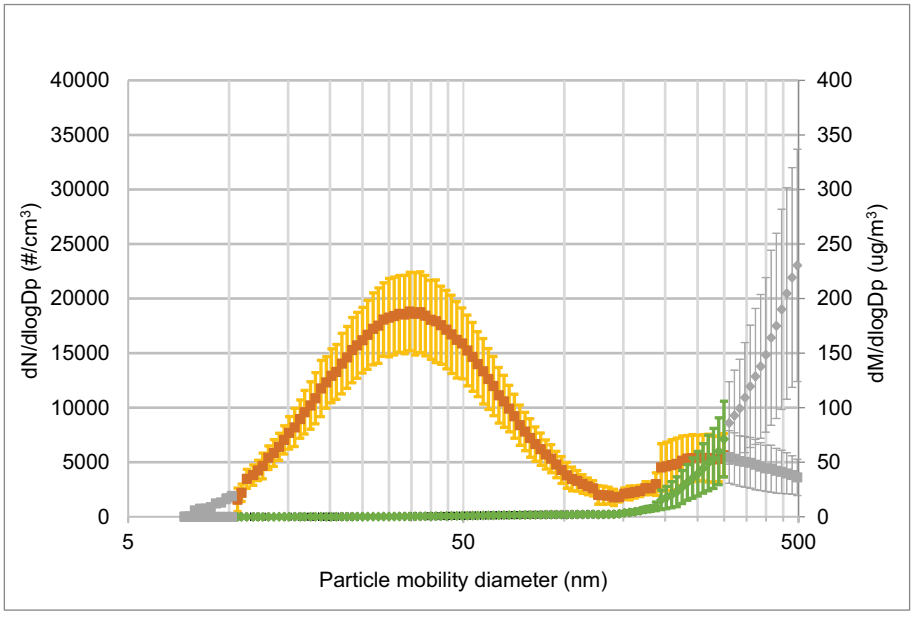

Figure 2(a). Average SMPS size distribution of particles and corresponding mass distributions during a) days with passive vape, b) days with clean air. Mean particle size distribution (orange curve (dN/dlogDp (particle number (#)/cm^3))) and particle mass (green curve (dM/dlogDp (µg/m^3))) ± SD. In Figure 2(a) colored symbols are an average of six measurement series, grey symbols from at least two measurement series. In Figure 2(b) colored symbols are an average of four measurement series, grey symbols from at least one measurement series.

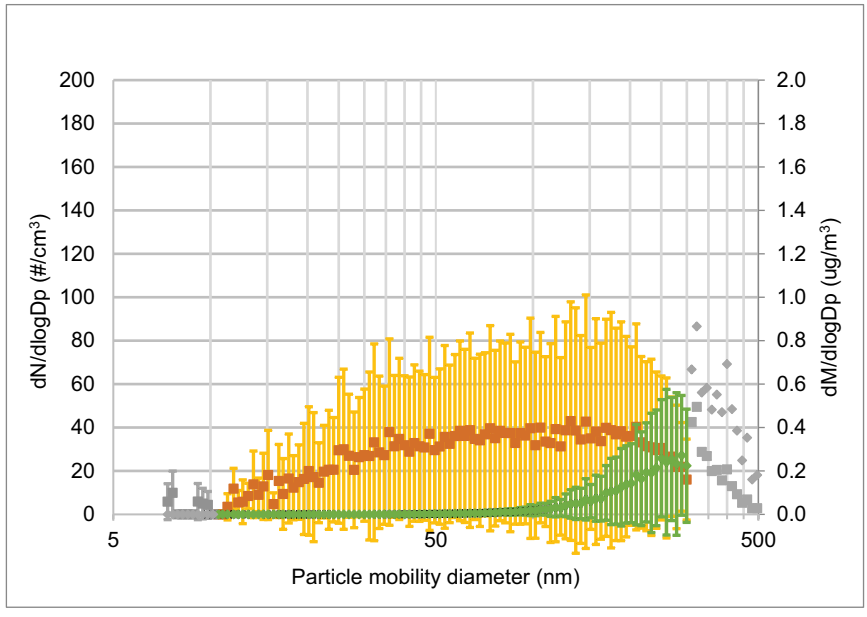

Figure 2(b) (Continued).

although minor, was seen for larger particles (200–500 nm), which is coinciding with information from the vapers on the coil overheating. Hence, these particles could derive from combustion. The particle mass peaked when the particle size was around 300–500 nm. On exposure days with clean air particle concentrations were very low (see Figure 2(b) for average concentrations). Figure E1 in the online data supplement shows the individual measurements during each exposure day.

Formaldehyde, acetone, and acetaldehyde

Average concentrations of formaldehyde, acetaldehyde and acetone in the exposure chamber were similar between experiments with or without vaping (see Figure E2 in the online data supplement). Measurements in the adjacent room with vapers and at the outlet during one exposure session, showed higher concentrations of acetaldehyde (mean ± SD) (3.2 ± 0.2 μg m^{-3}) and acetone (15.2 ± 1.2 μg m^{-3}) in the air from the adjacent room than in the exposure chamber (2.0 ± 0.2 μg m^{-3} and 7.2 ± 0.2 μg m^{-3}, respectively), while formaldehyde was observed at similar levels (3.3 ± 0.1 μg m^{-3} in adjacent room and 4.1 ± 0.2 μg m^{-3} in exposure chamber). In addition, an analysis method for nicotine in particle filter samples was developed, but, unfortunately, the method was not sensitive enough to detect nicotine in all but one of the analyzed samples.

Health outcomes

PExA

All participants performed PExA-samples, however, data are only analyzed for nine out of 16 participants as samples from seven participants were 'below limit of detection' (<40 ng). Among those nine participants having enough sample material, three participants contributed with only one measure and one person only contributed to measures on one of the exposure days – therefore only five participants are included in the mixed effect analyses. There was a significant increase in percent of SP-A in PExA-samples 24 hours after exposure start compared to before exposure start (p = 0.021), also the time-exposure interaction was significant for percent SP-A (p = 0.029) showing a decrease after passive vape exposure taking into account the diurnal effect (Table 2). Percent of SP-A in the PExA-sample increased after exposure to passive vape (0.579 (95% CI −0.483; 1.641)), however insignificant. Exposure to passive vape did not affect percent of Albumin in PExA-samples or the Albumin/SP-A ratio.

Spirometry

Minor, however, borderline-significant reductions in FEV_1 and FVC were observed for passive vape compared to clean air (−0.046 l and −0.071 l, respectively) (see Table 3).

FeNO

As seen from Table 3, exposure to passive vape did not significantly affect FeNO when compared to exposure to clean air (−1.60 (95% CI −5.14; 1.94)).

Table 2. Change in particles in exhaled air comparing passive vape exposure to clean air exposure (reference).

	Coefficient	95% CI	*p*-value
SP-A % Model 1			
Passive vape exposure	0.579	(−0.483;1.641)	0.271
Measured after exposure	0.166	(−0.901;1.232)	0.751
Measured 24 h after exposure	1.437	(0.242;2.632)	0.021
Passive vape x after exposure	−0.097	(−1.590;1.397)	0.895
Passive vape x 24 h after exposure	−1.775	(−3.350;-0.199)	0.029
Albumin % Model 2			
Passive vape exposure	−0.814	(−2.457;0.828)	0.316
Measured after exposure	−1.961	(−3.795;-0.127)	0.037
Measured 24 h after exposure	−0.787	(−2.795;1.220)	0.427
Albumin/SP-A Model 2			
Passive vape exposure	−0.165	(−1.322;0.991)	0.771
Measured after exposure	−1.156	(−2.436;0.124)	0.075
Measured 24 h after exposure	−1.361	(−2.768;0.045)	0.057

Definition of abbreviations: SP-A = Surfactant Protein-A. In model 1 for Albumin % and Albumin/SP-A, the interaction term was not significant, why there is no results shown.

Table 3. Change in lung outcomes comparing passive vape exposure to clean air exposure (reference).

Model 2	n	Coefficient	95% CI	*p*-value
Spirometry				
FEV_1	16	−0.045	(−0.096;0.004)	0.073
FVC	16	−0.071	(−0.146;0.004)	0.065
FEV_1/FVC	16	0.002	(−0.014;0.010)	0.683
FeNO	14	−1.599	(−5.137;1.939)	0.370

Definition of abbreviations: FEV_1 = Forced Expiratory Volume in the first second (liter); FVC = Forced Vital Capacity (liter); FeNO: Fractional Exhaled Nitric Oxide (NO) (ppb). * Two participants were unable to perform the exhaled nitric oxide test at all time points.

Blood plasma

The time-exposure interaction was significant for several of the analyzed proteins (see Table 4), indicating that a differential change in the plasma occurred during the two exposure sessions. Albumin (p = 0.006) and Acetoacetate (p = 0.014) were highly significant, while other markers – in particular cholesterol and lipoproteins – were less pronounced, however, still significant. Citrate, free cholesterol in very large HDL plus triglycerides in medium HDL, in plasma were positively associated by exposure to vape alone, though the majority of the measured proteins did not show any variations related to exposure (model 2). Time also had a significant influence on several of the proteins indicating diurnal or post-prandial variation (results not shown).

Symptoms from eyes, nose, and throat

Data on symptoms were registered by all participants every 30 minutes during the 4-h exposure sessions

Table 4. Significant change in plasma proteins comparing passive vape exposure to clean air exposure (ref.).

Model 1	Exposure x time 24 h. coefficient	95% CI	*p*-value
Total cholesterol	0.326	(0.031;0.621)	0.031
Total esterified cholesterol	0.236	(0.0177;0.454)	0.035
Total free cholesterol	0.090	(0.011;0.169)	0.026
Acetoacetate	0.024	(0.005;0.043)	0.014
Beta-hydroxybutyrate (bOHbutyrate)	0.026	(0.002;0.050)	0.031
Albumin	0.006	(0.002;0.010)	0.006
Cholesteryl esters in small VLDL (S-VLDL-CE)	0.013	(0.001;0.026)	0.042
Conc. of very small VLDL particles (XS-VLDL-P)	2.30E-09	(4.13E-11;4.56E-09)	0.046
Total lipids in very small VLDL (XS-VLDL-L)	0.031	(0.000;0.061)	0.048
Conc. of large LDL particles (L-LDL-P)	1.51E-08	(4.09E-10;2.99E-08)	0.044
Total lipids in large LDL (L-LDL-L)	0.110	(0.002;0.218)	0.047
Phospholipids in large LDL (L-LDL-PL)	0.023	(0.001;0.046)	0.044
Cholesteryl esters in large LDL (L-LDL-CE)	0.063	(0.002;0.124)	0.044
Conc. of medium LDL particles (M-LDL-P)	1.35E-08	(6.36E-10;2.65E-08)	0.040
Total lipids in medium LDL (M-LDL-L)	0.069	(0.002;0.136)	0.042
Phospholipids in medium LDL (M-LDL-PL)	0.012	(0.001;0.024)	0.041
Conc. of small LDL particles (S-LDL-P)	1.49E-08	(1.17E-09;2.86E-08)	0.034
Total lipids in small LDL (S-LDL-L)	0.042	(0.002;0.082)	0.037
Phospholipids in small LDL (S-LDL-PL)	0.008	(0.000;0.016)	0.047
Model 2	Exposure coefficient	95% CI	*p*-value
Citrate	0.005	(0.001;0.010)	0.041
Conc. of very large HDL particle (XL-HDL-PL)	0.011	(0.002;0.021)	0.021
L-VLDL-PL %	−0.160	(−0.317;-0.002)	0.047
Triglycerides in medium HDL (M-HDL-TG)	0.002	(0.001;0.004)	0.026

Plasma proteins are measured as mmol/L except from XS-VLDL-P, L-LDL-P, M-LDL-P, S-LDL-P, and XL-HDL-PL, which are measured as mol/L.

including 0 min of exposure, resulting in nine time points. As seen from Figure 3, differences in participants' symptoms were mild when comparing days with passive vape to days with clean air ranging from 6–20% of maximum on the scale. However, throat irritation was significantly higher on days with passive vape compared to days with clean air at time 180 and 210 min, showing that throat irritation worsened over time on days with passive vape. There were no differences on eye and nose irritation when comparing passive vape to clean air, except for eye irritation that became significantly higher as time went by when exposed to passive vape.

Discussion

In the present study, we have shown that exposure to passive vape from e-cigarettes resulted in symptoms and systemic health effects among COPD-patients. We found that SP-A in exhaled air was affected by time and exposure to vape concentrations of median 18 μg/m^3 (range: 8–333) indicating a negative effect of passive vape on SP-A the morning after exposure. Furthermore, several plasma proteins increased significantly indicating inflammation caused by vape exposure. We found a borderline significant decline in lung function, but no effect on FeNO among the 16 COPD-patients exposed to passive vape.

Average concentrations of formaldehyde, acetaldehyde and acetone were similar between days with and without vaping, which is in contrast to previous studies where emission of carbonyl compounds from thermal degradation of e-liquid constituents has been observed [17–19]. In our study, the air exchange was high (2.1 ± 0.2), and it seems that levels of carbonyl compounds were also affected by other factors than vaping, such as emission of carbonyl compounds from participants. This might explain why no particular difference in carbonyls between exposure days was seen [20]. We measured particles along their way from the small chamber through the pipe connection and into the large chamber using a P-TRAK. We found that we only lost very few particles during the transportation. However, the exposure was purposely mixed with clean inlet air and diluted when entering the large chamber.

Our results regarding SP-A in the lungs after passive vape exposure are novel. The significant time-exposure interaction showed a differential change in SP-A in exhaled particles during the two different exposure scenarios; a decrease was observed after passive vape exposure compared to clean air. One reason for the low 24-h level might be depletion of SP-A due to inflammation caused be the passive vape exposure. Similarly, a study by McKenzie et al. found decreasing SP-A levels after acute exposure to nanoparticles [21]. SP-A is a pivotal part of the respiratory immune system; it has the ability to opsonize or bind pathogens and other invading micro-organisms to enhance phagocytic removal from the airways [22]. It seems likely that SP-A decrease after acute exposure to particles, as they perish after fighting invading micro-organisms. The clinical implications of decreasing SP-A levels are

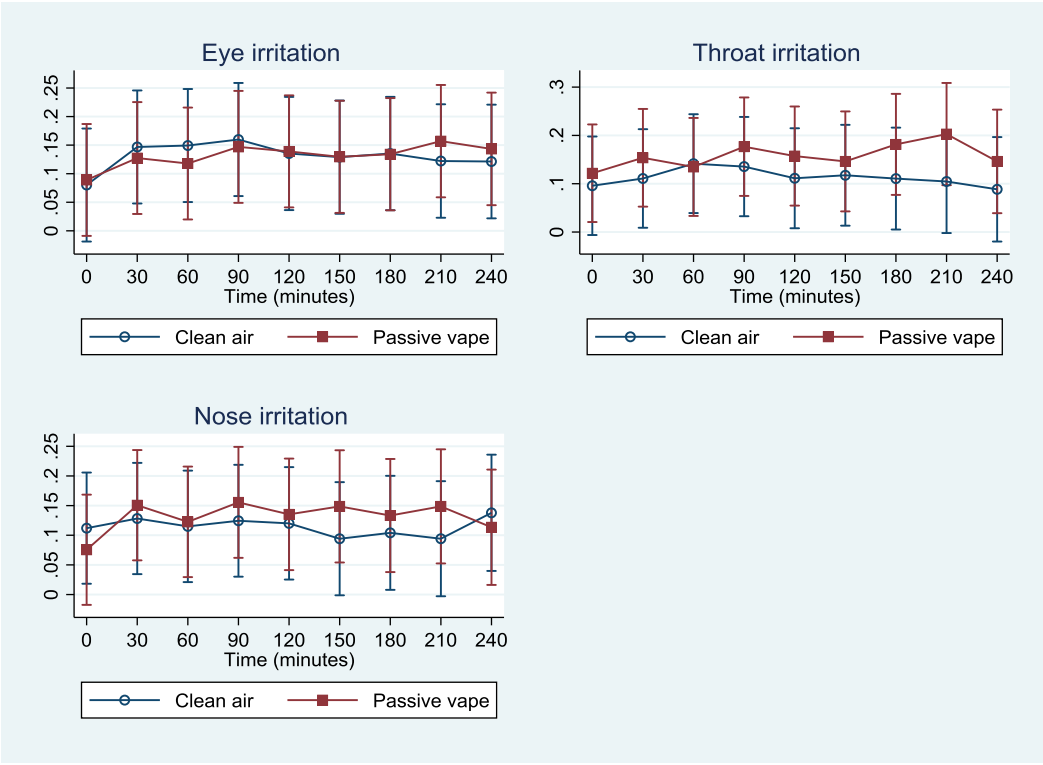

Figure 3. Mean symptoms (95% CI) as % of max[1] (y-axis) experienced over time (0 min to 240 min) during the two exposure scenarios: Eye irritation, throat irritation, and nose irritation.

[1] Symptoms were scored by placing a cross on a 130 mm open Visual Analogue Scale (VAS). The intensity of any discomfort was registered as the length in mm from the left of the scale to the marker. The scores were rated from 0 to 100% with highest number corresponding to highest discomfort. Discomfort was evaluated as changes over time (as percentage of max).

unclear, however, it has been shown that low levels increase the risk of infections, e.g. virus infections [22]. Therefore, in COPD-patients depleted levels of SP-A might increase the risk of exacerbations. We also observed a minor, however insignificant, increase after vape exposure alone (not taking time into account). This might be because SP-A initially increases to protect the lungs from, e.g. inhaled particles [23], followed by a depletion after having fought the invading micro-organisms.

While SP-A and albumin in exhaled air were the only primary outcomes in our study, several secondary outcomes were analyzed, however, they have to be viewed upon as hypothesis generating. Our findings of borderline significant negative changes in FEV_1 and FVC following passive vape exposure is differing from other experimental studies. In a chamber study, Flouris and colleagues exposed 15 never-smokers to machine-generated e-cigarette aerosol for a single hour after which they measured lung function. Never-smokers also underwent a control session with no emissions and a passive tobacco cigarette session. The assessment of lung function demonstrated that a one-hour passive e-cigarette vaping session did not significantly interfere with normal lung function, and the same result applied to active e-cigarette users [24]. A lack of change in lung function was also found in some of our previous exposure studies where we observed airway inflammation [25], indicating that lung function measurements may be less sensitive than other measurements. However, COPD-patients might be more sensitive with regard to changes in lung function than healthy volunteers. An important consideration in the present study was participant's discontinuation of corticosteroids seven days before exposure start possibly affecting the level of hyper-responsiveness in the lung. Increased responsiveness would render the participants more frail to passive vape, and we did see an indication of an effect. A possible explanation for the decline in lung function not reaching significance is low power, as we expected SD of 1.5 in our power calculation. Importantly, absence of significant short-term changes in spirometry does not mean that e-cigarettes are harmless [26].

In our study, we observed a minor, but insignificant, decrease in FeNO concentrations following exposure to passive vape. Tzortzi et al. examined the effects of passive exposure to e-cigarette emissions on respiratory mechanics in a crossover experimental study with 40 young healthy non-smokers [27]. All participants

underwent a 30-min control session with no emissions and two experimental sessions (0.5 and 1.5 ohm exposure). In their study, FeNO decreased significantly post exposure in the 0.5-ohm session indicating immediate alterations [27]. A decrease in nitric oxide (NO) is opposite to the findings in some air pollution studies, where NO production has been reported to increase with high levels of air pollution indicating airway inflammation [28,29], however, in accordance with studies on exposure to active and passive smoking of conventional cigarettes and active use of e-cigarettes [30–33]. A decrease in NO after exposure to passive vape could be explained by a negative feedback mechanism as the NO in vape downregulates NO synthases in the lungs through the nitric oxide it contains – similar to the mechanism observed by conventional cigarette smoking [27,34].

In the present study, we found increased levels of several plasma proteins, including albumin, acetoacetate, and citrate. Citrate is known for its activation potential for innate immune reactions and it plays a critical role in many normal physiological activities. Dysregulation can lead to several consequences such as impaired blood coagulability [35]. We found increased levels of free and esterified cholesterols, which are biomarkers associated with cardiovascular inflammation related to PM exposure [36].

We found no evident change in self-reported symptoms of eye and nose irritation, however, throat irritation was more pronounced on days with passive vape exposure, although to a limited extent. To our knowledge, no previous exposure study on passive vape has examined self-reported symptoms, however, our results complement studies with other designs and/or other subgroups of the population. Dicpinigaitis and colleagues found in their exposure study among 30 healthy volunteers a significant inhibition of cough reflex sensitivity after a single session of active e-cigarette use [37]. In a cross-sectional study among youths with asthma, Bayly and colleagues found that passive vape exposure was associated with higher odds of reporting an asthma attack, i.e. shortness of breath, chest tightness, cough, and/or wheezing in the past 12 months [38].

Strengths and limitations

The crossover design, the randomization, and double-blinding were the major strengths of the present study. In addition, we used an up-to-date exposure chamber in which all conditions other than the exposures were kept constant. Another strength was using human vapers, which gives a more realistic exposure than using a vaping machine, as the aerosol released to the surroundings are as in real-life with regard to composition of chemicals and particles exhaled from the user [39]. It is questionable whether smoking machines are able to replicate human vaping behavior, as the inhaled aerosol undergoes changes in the human lung that is assumed to be attributed to deposition and evaporation, and it is therefore uncertain whether results from studies relying on smoking machines are trustworthy [4,39]. However, using real vapers resulted in variability in the exposure, as the vapers did not use a standardized vape procedure, since they were only instructed to vape in shifts. From previous studies, we know that there is a large degree of variability in user exposure to these aerosol constituents across patterns of e-cigarette use among other things [40]. We feel that the blinding by using fruit gum as placebo worked well, however, we did not perform a systematic analyses on the effect of blinding.

Our study had limitations such as low power due to few participants, and furthermore, not all participants were able to complete all health examinations resulting in missing data. We found it difficult to recruit mildly to moderately affected COPD-patients, who were able to take the time to participate and at the same time cope with the discontinuation of corticosteroids even for a period of seven days. The activities of participants in the hours and days before the exposure sessions could not be standardized or completely controlled for and were likely to cause random effects. Low and varying levels of aerosol was another limitation in our study. The difference between median particle counts for exposure days with clean air and days with passive vape was not pronounced and neither were levels of formaldehyde, acetaldehyde or acetone. On two out of eight exposure days with passive vape, the level of particle counts were quite high as some very experienced vapers were present in the small chamber inhaling and exhaling forcefully. Previous studies show that composition of the aerosol that is generated depends on the ingredients of the e-liquid, the e-liquid levels left, the characteristics of the e-cigarette including the electrical characteristics of the heating element, and that production of harmful substances is influenced by both battery voltage output and temperature reached, which complicates research in this field [4,40].

Although nicotine-free liquids are available, the use of liquids containing nicotine is more common [41], why we chose e-liquids with 6 mg nicotine. This was the lowest amount possible, and one might expect worse health effects with higher nicotine levels [8]. The low amount of nicotine was chosen in order for the vapers not to become unwell during vaping for several hours.

In summary, we believe that a true, but very mild effect of passive vape occurred in this study, although chance is an alternative explanation, and therefore interpretation should be made with caution. The findings of this study do not necessarily pertain to the background population; however, they might be generalized to other people with chronic respiratory disease.

Conclusion

In conclusion, the results of this study indicate that passive vape is capable of exerting acute small inflammatory responses in lungs and blood as well as throat irritation. Despite the study being modest in its size with a narrow scope, it offers new findings on the potential harm of e-cigarettes. Although more research is required, it is clear that e-cigarette emissions are not merely harmless aerosol. In the future, we recommend more studies on passive vape exposure in sensitive subgroups, and studies of people chronically exposed to passive vaping, as such studies are virtually non-existent.

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Disclosure statement

Anna-Carin Olin is a board member and shareholder of PExA AB, and has a patent (wo2009045163) licensed to PExA AB. No potential competing interests was reported by the other authors.

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PExA AB Sweden lend us the PExA 1.0 instrument.

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Author's contributions to the study

KRL was responsible for conducting the study, she analyzed the health outcomes, and wrote the first draft of the article. JHB came up with the proposal to the study, designed the

study in collaboration with TS and he contributed to the manuscript. EB helped design the study and recruit participants, and she contributed to the manuscript. MB and MG planned and supervised the chemical and particle analysis and contributed to the manuscript. SM and PR measured particle exposure and contributed to the manuscript. PR also controlled the exposure chamber and analyzed the particle data. VHG and KØ helped conduct the study and contributed to the manuscript. ACO helped performing the PExA measurements and she contributed to the manuscript. TS was the principal investigator in designing and conducting the study, and he contributed to the manuscript. All authors read and approved the final manuscript before submission.

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Supplemental online material

An RCT of acute health effects in COPD-patients after passive vape exposure from e-cigarettes

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Methods

Design

A randomized double-blind, crossover design was applied to ensure that all possible exposure orders were represented. The experiment was carried out in groups of two or three participants. The groups were allocated to the possible exposure orders at random to counterbalance the influence of learning and other time-related changes. A statistician generated the random allocation sequence using Stata 16 software (StataCorp, College Station, Tex). All participants attended two exposure sessions for four hours: air mixed with vape from e-cigarettes (median mass of particles with diameter <2.5 µm, $PM_{2.5}$: 18 µg/m^3 (range: 8-333)) and clean filtered air ($PM_{2.5}$<6 µg/m^3) with at least two weeks between each exposure session to eliminate carry-over effects. The filtered clean air and e-cigarette vape sessions were identical except for the air quality. During the sessions, the investigators in contact with the participants were unaware of the exposure used and so were the participants. The investigator monitoring the exposure had no contact with the participants or clinical staff during measurement. The blinding was continued until basic statistical analyses had been conducted. The trial was conducted from April-June and August-November 2017.

Participants

Participants were recruited during winter-spring 2017 by means of advertising and through the outpatient clinic at the Department of Respiratory Diseases and Allergy at Aarhus University Hospital. Prior to the study, all participants underwent a standard medical assessment consisting of medical history and clinical examination. Participants had a known diagnosis of COPD determined by symptoms and spirometry using FEV_1/FVC below lower limit of normal and MRC (Medical Research Council) score≥2 and CAT (COPD Assessment Test) score≥10. Exclusion criteria were smoking and/or vaping and a medical history of

diseases, which could involve a risk for the participant or possibly influence the outcome measures. One week prior to exposure session patients were asked to change their long-acting bronchodilators (LABA-LAMA) to short-acting medication (SABA-SAMA) and discontinue corticosteroids. Participants had been free of infections or allergy for at least one week prior to the experiments and were not allowed to take any medication within the last 48 hours proceeding the exposure sessions. Eleven daily users of e-cigarettes with no serious disease participated in the study in order to establish the exposure in an adjacent chamber. They were recruited by means of advertising. All participants were enrolled on "first-come, first-serve" basis, and written consent was obtained from all included participants.

Exposure facilities

The study was conducted at the Climate Chamber facilities at Department of Public Health, Aarhus University, Denmark. Exposure sessions took place under controlled conditions in a 79m^3 climate chamber made of welded stainless steel optimized for experiments with gasses and particulate air pollutants, while exposure generation took place in a similar 29m^3 adjacent chamber. No run-in or priming period was used. During exposure in the chamber participants were seated around a desk at the centre of the chamber in a resting position. Participants were instructed not to discuss the environment in any form – verbally or by their attitude. This was controlled by surveillance of the climate chamber. The climate chamber was thoroughly cleaned before each exposure cleaning all surfaces with Extran® MA 01 solution in polished water following steaming all surfaces using polished water. Participants wore clean-suits (Microporous Cleanroom Disposable Coverall from Integrity®) over their clothes to avoid unintended contamination of the air by minimizing personal particle generation from clothes etc. For the safety of participants, maximum CO concentrations in the chamber was determined and specific alarms were included in the general precautionary procedures.

Exposure generation

The e-cigarette aerosol used for exposure was generated by 2-3 people vaping e-cigarettes by turn in an adjacent chamber. An established negative pressure of 10 Pascal in the exposure chamber relative to adjacent chamber (vapor chamber) transferred vapor through a pipe connection into the exposure chamber, where it was mixed into clean inlet air. The average air exchange in the exposure chamber was 2.1 ± 0.2. The most popular brand of e-cigarettes in Denmark was examined; Joyetech eGo AIO (with a 2 ml tank, standard battery capacity of 1500 mAh and a standard Cubis BF coil (0.6 ohm)). The included e-juices were pre-made "Tobacco" and "Strawberry" flavour (70% propylene glycol/30% vegetable glycerine) from "InSano" containing 6 mg of nicotine acquired in containers of 10 ml. All products were purchased from online stores or shopping malls. Batteries for the e-cigarettes were charged 12 hours prior to each exposure, and each e-cigarette mouthpiece was cleaned thoroughly after each exposure. The specific vape exposure levels used in this study were chosen to be comparable to real life levels. During clean air sessions the e-vapers did not use e-cigarettes. Instead, they were offered nicotine chewing gum (Nicotinell® Fruit) with 4 mg nicotine or normal chewing gum with fruit taste in order to mask the exposure.

Exposure characterization

Environmental conditions were routinely monitored and controlled by a HVAC (Heat Ventilation Air-Conditioning) system and kept as constant as possible throughout the experiment. Inlet air to the chambers was purified using HEPA filters and carbon filters. Data collection was done by using a logger system from Campbellsci with high quality sensors from different manufacturers and included temperature, humidity, CO_2, air flow rate, differential pressure, and ozone measurements. The airflow pattern in the exposure chamber was created using a slot inlet system in the ceiling to secure optimal mixing of vapor to get a

uniform vapor concentration in the chamber. A P-TRAK Ultrafine Particle Counter (TSI Inc.) model 8525 (particle size range 20-500 nm) and SMPS model 3936 (particle size range 7.4-500 nm) were placed in the control room to sample air through tubes one meter into the exposure chamber. The P-TRAK measured the total number of particles, while the SMPS measured the particle size distributions. The SMPS system consisted of an electrostatic classifier (EC-3080), X-ray neutralizer (model-3087), Differential Mobility Analyzer (DMA, model-3081) and a condensation particle counter (CPC, TSI-3776). The SMPS was operated with a total scan time of 210 seconds (180 s up scan, 30 s down scan, aerosol flow rate = 1.5 L min^{-1}, sheath flow rate = 10 L min^{-1}), which limited the size range measured between 7.7 and 300 nm on most exposure days, however using a flow rate = 5 L min^{-1}, measuring in the size range 10-500 on three exposure days. Density for particles was set to be water (density=1 g/ml). SKC PEM $PM_{2.5}$ sampling heads with PTFE filters were used in the exposure chamber at the center table in the height of participants' faces for gravimetric measurements of particle mass concentration. Nicotine was sampled during the exposure using SKC PTFE filters with PMP Support Ring 37mm 2.0 µm and saved at -20°C for later analyses. Nicotine in particle samples was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) after adsorption to Twisters. The carbonyl cartridges and nicotine filter units were placed close to the exhaust ventilator of the chamber. Carbonyl compounds were collected as 2,4-dinitrophenyl hydrazones using LpDNPH S10 cartridges (Sigma-Aldrich) with a sampling flow rate of 2 L min^{-1} for four hours. The cartridges were extracted with 5.00 mL acetonitrile and extracts were analyzed by high performance liquid chromatography (HPLC) (Dionex Ultimate 3000, Thermo Fisher Scientific) using an AcclaimTM 120 C18 column (3µm particles, 4.6 x 150 mm, Thermo Fisher Scientific) and UV detection (365 nm). The 2,4-dinitrophenyl hydrazones of formaldehyde, acetone and acetaldehyde were quantified using 8-point calibration curves with R^2>0.98 in the range 0.075-2 µg mL^{-1}. The air inside the

exposure chamber was characterized during each exposure session, however only measured with SMPS during six out of eight exposure sessions with passive vape.

Clinical measurements

Just before each exposure session, baseline clinical investigations were performed. The investigations were repeated immediately after exposure (4 hour post exposure start), and the next morning (24 hours post exposure start). The clinical investigations consisted of Particles in Exhaled Air (PExA), spirometry, exhaled NO measurements (FeNO), and venous blood sampling. All methods – except PExA and Proteomics (Nightingale) in blood – are standard methods used in the departments' previous exposure studies (E1–E3). For all outcomes participants served as their own controls.

PExA: Particles in Exhaled Air was measured using the novel PExA® instrument set-up (E4,E5), which is a non-invasive alternative to bronchoscopy in assessing the lining fluid from distal airways (E6). Participants performed repeated breath maneuvers allowing for airway closure and re-opening as previously described (E7). Exhaled particles were optically counted and collected on a membrane in the PExA® instrument. The subjects performed breathing maneuvers via a mouthpiece and a two-way, non-rebreathing valve into a thermostated box (36°C) containing a Grimm 1.108 optical particle counter and an impactor with a Teflon membrane impaction substrate. The instrument contain a vacuum pump that draw the exhaled air containing particles through the impactor where they are collected by impaction according to their size at hydrophilic Teflon membrane. Participants inhaled HEPA-filtered air for three breaths before the sampling in order to remove particles originating from ambient air. All participants wore a nose clip throughout the procedure. Participants were instructed to perform the following standardized breathing maneuvers to allow for airway closure and re-opening: i) exhale fully to residual volume and hold breath for three to five seconds, ii) inhale rapidly to vital capacity, iii) exhale normally, iiii) breathe

tidally in the instrument until particle concentration is < 150 particles/L. Only the exhalation in (iii) was sampled in the instrument. An ultrasonic flow sensor measured flow rates. The inhalation and exhalation flow-rate was displayed graphically in real-time on a computer screen, which helped participants to perform the required breathing maneuvers. Between breathing maneuvers, the test subject breathed particle-free air tidally. The procedure was repeated until a target sample of 120 ng was reached or a maximum sampling time of 30 minutes was reached. After collection, the Teflon membrane was immediately transferred to a low-binding Eppendorf polypropylene vial and stored at -80°C until analysis (E8). PEx-samples was analyzed for Surfactant Protein A (SP-A) and Albumin using mass spectrometry. Details on the analysis have been described previously (E7).

Spirometry: An EasyOne™ Spirometer (ndd Medical Technologies) was used to measure forced vital capacity (FVC) and forced expired volume in the first second (FEV_1). The ratio FEV_1/FVC was calculated from the maximal FEV_1 and FVC from all acceptable blows. Participants were tested at least three times and the best performance for FEV_1 and FVC was chosen according to the ATS/ERS guidelines (E9).

FeNO: Fractional exhaled nitric oxide (FENO) was measured using a chemiluminescence analyser (NIOX VERO® Airway Inflammation Monitor; Aerocrine AB, Sweden). The participant was instructed to inhale as deeply as possible to total lung capacity and consecutively exhale in the NIOX instrument at a fixed mouth flow rate of 50±5 mL/s for 10 s. The exhalation rate was held steady by applying a constant positive pressure while instructing the participant to exhale steadily using visual stimulation on the system screen in combination with audio signals. A continuous sound indicated correct pressure with a frequency proportional to the pressure. Exhaled nitric oxide levels are expressed in parts per billion (ppb). Measurements were performed in a standing position according to the 2005 ATS/ERS recommendations after at least 1 hour of fasting (E10).

Blood sample: We sampled 4 ml EDTA plasma and 5 ml serum. Blood plasma was frozen, and following thawing analyzed for proteomics using the Nightingale® platform examining more than 100 different proteins. Metabolic biomarkers were quantified from serum/plasma samples using high-throughput proton NMR metabolomics (Nightingale Health Ltd, Helsinki, Finland). This method provides simultaneous quantification of routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular metabolites including amino acids, ketone bodies and gluconeogenesis-related metabolites in molar concentration units. Details of the experimentation and applications of the NMR metabolomics platform have been described previously (E11).

Symptoms

During each exposure session, a questionnaire assessing subjective symptoms or perception was completed prior to exposure (baseline), during exposure every 30 min., and at the end of the exposure session. The questionnaire concerned subjective evaluations of 28 questions related to indoor air quality, symptoms and general well-being (E2). The participants were asked to score their evaluation (rate the strength) of symptoms and their environmental perception by placing a cross on a 130 mm open Visual Analogue Scale (VAS). The intensity of any discomfort was registered as the length in mm from the left of the scale to the marker. The scores were rated from 0 to 100% with highest number corresponding to highest discomfort. Discomfort was evaluated as changes over time (as percentage of max).

Statistics

Mixed models based on the univariate repeated measurements ANOVA were performed, taking into account the different design variables corresponding to the crossover design (E12). As fixed effects the models included the health outcome, time, exposure, exposure-

order, day, and time-exposure interaction (model 1). As a random effect we included participant ID. For analysis of plasma proteins gender was also included as there might be hormonal changes underlying gender differences in plasma lipids. Time was divided into baseline, four, and 24 hours, exposure was clean air or passive vape, order was corresponding to the order the participant received the exposure and coded either 12 or 21, while day indicated whether the exposure took place on participants' first or last day. The primary outcome of interest was the exposure and time-exposure interaction as an effect of any of these terms indicated a difference in the change from baseline associated with the exposure. Firstly, we fitted a mixed model with one-way interaction (Model 1). For those models where we did not find a statically significant interaction, the interaction term was left out (Model 2). The level of significance was assumed at $p<0.05$. All statistical analyses were performed using Stata 16 software (StataCorp, College Station, Tex).

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Figure E1 A-K.

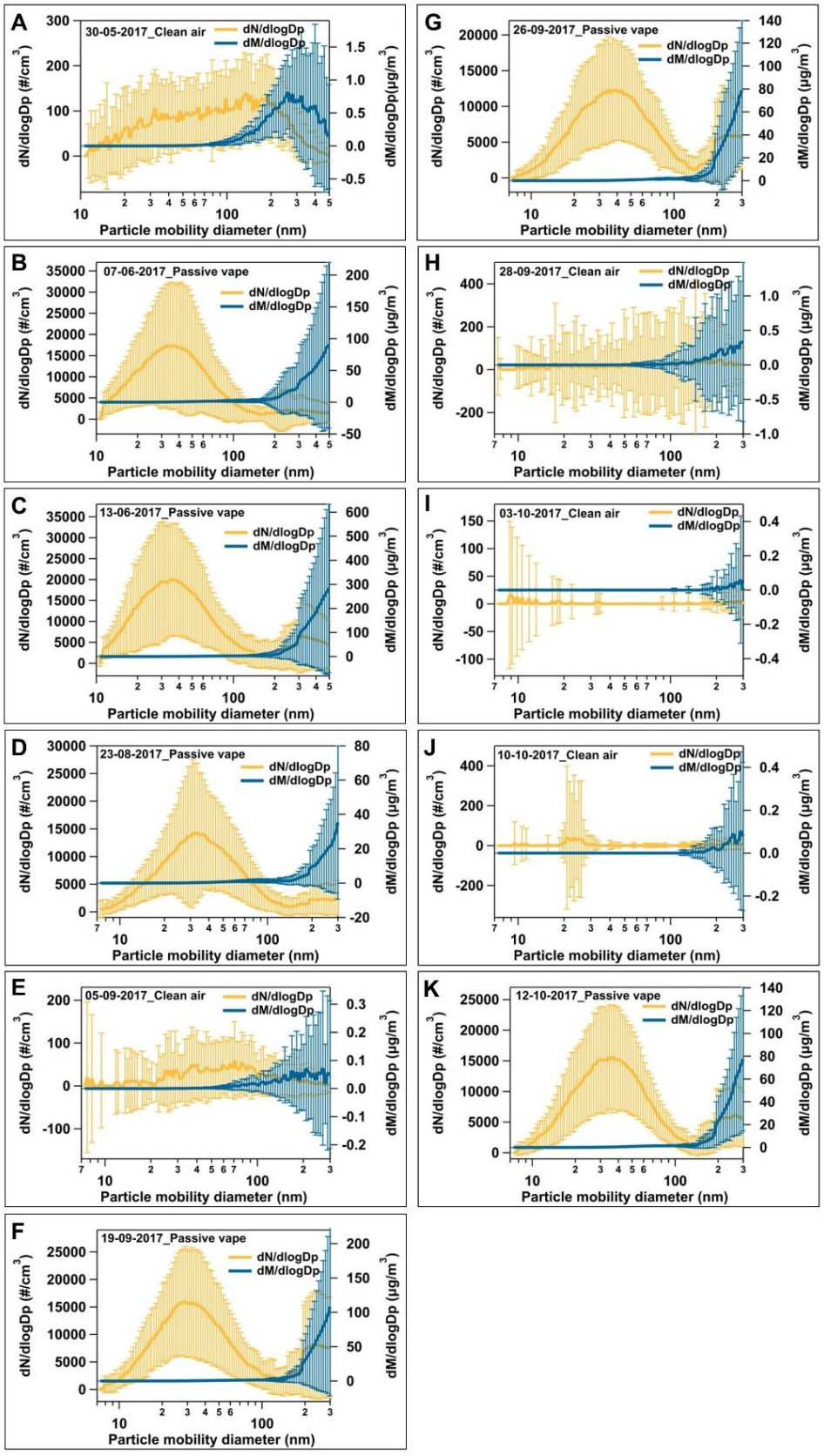
A
30-05-2017_Clean air
dN/dlogDp
dM/dlogDp
dN/dlogDp (#/cm3)
dM/dlogDp(µg/m3)
Particle mobility diameter (nm)
B
07-06-2017_Passive vape
C
13-06-2017_Passive vape
D
23-08-2017_Passive vape
E
05-09-2017_Clean air
F
19-09-2017_Passive vape
G
26-09-2017_Passive vape
H
28-09-2017_Clean air
I
03-10-2017_Clean air
J
10-10-2017_Clean air
K
12-10-2017_Passive vape

Figure E2.

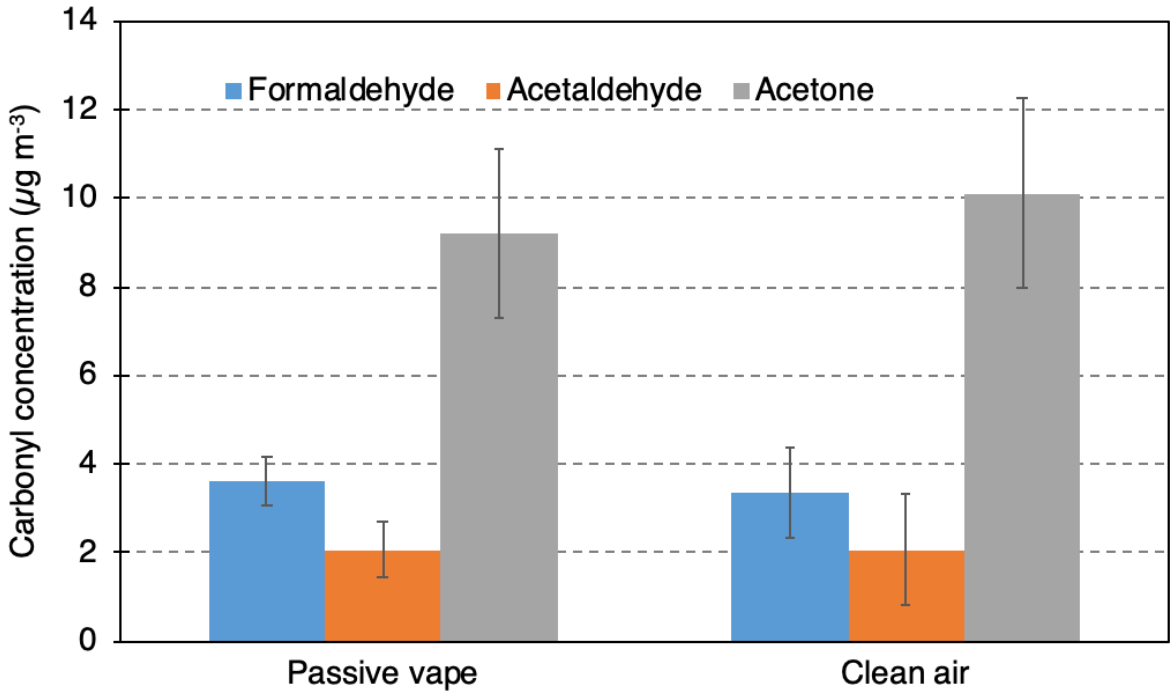
Formaldehyde
Acetaldehyde
Acetone
Carbonyl concentration (μg m-3)
14
12
10
8
6
4
2
0
Passive vape
Clean air

Figure captions

Figures E1 A-K. Individual curves for each exposure day with passive vape and clean air. Figures show the particulate number size distributions (dN/dlogDp) measured using the SMPS system and the corresponding mass size distributions (dM/dlogDp) obtained assuming spherical particles and a particle density of 1 g/ml. The lines represent averages over an entire experiment ± SD. Notice the different scales on both x- and y-axes.

Figure E2. Average concentrations ± SD (μg m^{-3}) of formaldehyde, acetaldehyde, and acetone in the exposure chamber on days with passive vape and clean air.

Paper II

Acute health effects from exposure to indoor ultrafine particles – A randomized controlled crossover study among young mild asthmatics.

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ORIGINAL ARTICLE

WILEY

Acute health effects from exposure to indoor ultrafine particles—A randomized controlled crossover study among young mild asthmatics

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Abstract

Particulate matter is linked to adverse health effects, however, little is known about health effects of particles emitted from typical indoor sources. We examined acute health effects of short-term exposure to emissions from cooking and candles among asthmatics. In a randomized controlled double-blinded crossover study, 36 young non-smoking asthmatics attended three exposure sessions lasting 5 h: (a) air mixed with emissions from cooking (fine particle mass concentration): ($PM_{2.5}$: 96.1 μg/m^3), (b) air mixed with emissions from candles ($PM_{2.5}$: 89.8 μg/m^3), and c) clean filtered air ($PM_{2.5}$: 5.8 μg/m^3). Health effects (spirometry, fractional exhaled Nitric Oxide [FeNO], nasal volume and self-reported symptoms) were evaluated before exposure start, then 5 and 24 h after. During exposures volatile organic compounds (VOCs), particle size distributions, number concentrations and optical properties were measured. Generally, no statistically significant changes were observed in spirometry, FeNO, or nasal volume comparing cooking and candle exposures to clean air. In males, nasal volume and FeNO decreased after exposure to cooking and candles, respectively. Participants reported additional and more pronounced symptoms during exposure to cooking and candles compared to clean air. The results indicate that emissions from cooking and candles exert mild inflammation in asthmatic males and decrease comfort among asthmatic males and females.

KEYWORDS
candles, cooking, human exposure, indoor air, inflammation, particles

1 | INTRODUCTION

Air pollution from both indoor and outdoor sources is a leading contributor to increased morbidity and mortality with more than seven million premature deaths worldwide every year.[1] As people spend up to 90% of their life indoor and approximately 16 h a day in their homes, indoor air pollution has equal or greater effects on health and well-being compared to ambient air pollution.[2,3] Chemicals, dust, dampness, and particulate matter (PM) cause indoor air pollution, and PM is a pollutant of special concern.[4] Many studies have

demonstrated that exposure to PM from traffic and smoking is associated with negative health impacts such as respiratory symptoms, allergic and inflammatory conditions of the lungs, cancer and cardiovascular diseases.[5-9] However, little is known about health effects of PM from common indoor sources, where especially cooking and burning candles has been shown to contribute to high levels of particles including fine ($PM_{2.5}$) and ultrafine ($PM_{0.1}$) particles.[10-12]

Fine and ultrafine particles are especially important for our health as they can penetrate deep into the respiratory system, depositing in the alveoli.[13] Due to their small size, ultrafine particles can enter the blood circulation, thereby reach target organs, for example, the heart and brain posing an even greater risk of systemic health impacts.[14-19] Regular and prolonged exposure to indoor PM may lead to adverse health effects, even at low concentrations. Furthermore, vulnerable individuals like children, the elderly, and people already suffering from respiratory disease, like asthma and COPD, are more susceptible.[6,20] Asthmatics are particularly vulnerable to particle exposure due to their chronic inflammation in the respiratory tract.[20]

Studies assessing potential acute health effects of exposure to PM from candles and cooking are not well represented in the literature. Existing exposure studies do not lead to definitive conclusions regarding the impact of PM from cooking and candles, however, most studies report adverse effects such as decreased lung function,[21] cardiovascular effects,[18,19,22] and negative impacts on human brain activity including declining cognitive performance among healthy individuals.[16,17] To date, health effects among individuals with asthma have not been examined in a controlled exposure study despite epidemiological studies showing that asthmatics are particularly susceptible to PM.[23,24] Thus, in a controlled chamber exposure study we examined acute health effects in the respiratory tract and self-reported symptoms of fine and ultrafine particles in indoor air generated by cooking and candles among young individuals with asthma. Our hypothesis was that exposure to cooking and burning candles would lead to respiratory inflammation and increased prevalence of symptoms compared to exposure to clean air.

Practical implications

- For cooking, the average mode diameter for particles was ~80 nm, while for candles it was ~7.5 nm.
- Consequently, particles from candles will be more difficult to filter out during ventilation.
- Our research shows, that there is a health potential by reducing particle emissions in our homes.
- Improving indoor air quality by reducing particulate air pollution from cooking and candles might reduce inflammation and increase comfort among people with respiratory disease.

2 | METHODS

2.1 | Design

A randomized double-blind crossover design was applied. Participants attended three exposure sessions each lasting 5 h; (a) air mixed with emissions from cooking (fine particle mass concentration ± SD): $PM_{2.5}$: 96.1 ± 13.1 μg/m^3, (b) air mixed with emissions from candles: $PM_{2.5}$: 89.8 ± 9.3 μg/m^3, and (c) clean filtered air $PM_{2.5}$: 5.8 ± 6.8 μg/m^3. The filtered clean air and particle sessions were identical except for the air quality. To eliminate impact of delayed effects, the three exposure sessions were separated by 14 days. The experiment included 36 participants, who were exposed in nine groups of four participants. In order to minimize the influence of learning and other time-related effects, the groups were randomized to the exposures. It was not possible to apply a fully balanced randomized design with an equal number of groups receiving the possible exposure orders; however, all six possible exposure orders were represented.

The study was double-blinded, as investigators in contact with the participants were unaware of the exposures as were the participants. The investigator conducting and monitoring the exposure had no contact with the participants or clinical staff during exposure days. The blinding was continued until basic statistical analyses had been conducted. The trial was conducted from May to June and from September to December 2019, with participants being exposed to all exposures within the same season.

2.2 | Ethics

The Ethical Committee in Central Denmark Region approved the study protocol (ref. no. 1-10-72-345-18) and written informed consent compiled by the Danish Ethical Committee was obtained from all participants prior to participation (see Supplementary Files for consent form).

2.3 | Participants

Non-smoking volunteers with mild asthma were recruited through social media, posters, and flyers at local high schools and university campuses in the municipality of Aarhus, Denmark. We aimed for 36 participants according to a power calculation made beforehand (power considerations can be found in Supplementary Files). Inclusion criteria were age between 18 and 25, a physician diagnosis of mild asthma, and >1 positive skin prick test-reactions towards common allergens. Exclusion criteria were use of any tobacco product, pregnancy, and a medical history of diseases, which could involve a risk for the participant or possibly influence the outcome measures. All participants were enrolled on "first-come, first-served" basis. Interested participants fulfilling the criteria were invited to a pre-examination including a medical doctor check-up and to have an introduction to the clinical measurements being conducted at the

exposure days. Atopy was confirmed by standard skin-prick test testing for 10 common aeroallergens, conducted according to standard procedures.[25] Most participants were treated only with short-acting β2-agonists when needed. In case participants were on long-acting asthma medication, it was converted to short-acting medication 2 weeks prior to participation and throughout the study. Before any exposure session, participants were required to be without signs of infections or airway symptoms and not to have taken steroids for at least one week, or any medicine (including antihistamines) during the previous 48 h. This was affirmed at a doctor check-up including an objective examination of mucous membranes in eyes, nose and throat the morning before each exposure session. If not confirmed, the exposure was rescheduled.

2.4 | Exposure facilities

The study was conducted at the Climate Chamber facilities at Department of Public Health, Aarhus University, Denmark. Exposure sessions took place under controlled conditions in a 72.9 m^3 climate chamber where walls, ceiling, and floor are made of welded stainless steel. Such material is optimized for experiments with gasses and particulate air pollutants as sink effects are minimized. Exposure generation took place in a similar 30 m^3 adjacent chamber. Participants were instructed to shower, carry clean clothes and not use perfume on days of exposure. During exposure, participants were seated around a desk in a resting position. Participants were instructed not to discuss the environment in any form – verbally or by attitude. This was controlled by surveillance of the climate chamber. Over their clothes, participants wore clean-suits (Cleanroom disposable coverall RS pro) to avoid unintended contamination of the air by minimizing personal particle generation from clothes, etc. For the safety of participants, CO-concentrations in the chamber were monitored and specific alarms were included in the general precautionary procedures. Both chambers and the pipes connecting the two chambers were thoroughly cleaned before each exposure session washing all surfaces with Extran® MA 01 solution in polished water followed by steaming using polished water.

2.5 | Exposure generation

Before the first participant in the group of four entered the exposure chamber, the exposure had been activated for approximately 2 h to ensure that the particle concentration had reached the required target concentration. Participants entered (and left) the exposure chamber in a sequence with 30 min between each participant. Each participant was exposed for 5 h. Because of an established negative pressure of 10 Pa in the large exposure chamber, particles and gases were directed from the adjacent chamber to the larger exposure chamber through a 10 m long pipe connecting the two chambers. On days with cooking exposure, four ovens (Anrätta, IKEA, Sweden) were placed in the adjacent chamber. One oven at a time was cooking breast of pork (28% fat) at 200°C as prescribed on the packaging. The meat was placed on a baking tray with approximately eight to 10 pieces (depending on size) corresponding to ~450 g distributed evenly on the tray. Before the first oven finished cooking the meat, the next oven started and so forth, until the first oven had to start over again with new meat. In total, the four ovens cooked meat five times in order for the exposure to last throughout the exposure day with participants being exposed to similar concentrations of particles during the whole session. Between exposure days, ovens were cleansed using the pyrolytic function. On exposure days with candles, four taper candles and six pillar candles, all made of 100% stearin, were lit and placed on a table with approximately 15–20 cm between each pillar candle. Taper candles were placed in a four-armed candlestick. A light circulation of air in the chamber made by a wide slow-rotating fan (95 rounds per minute) pointing towards the ceiling, made the candles flicker at a slow pace (average air velocity m/s 0.21 [±0.06] measured by Gill Windmaster HS ultrasonic Anemometer 32 Hz). A big funnel connected to the 10 m pipeline was placed above the table absorbing candle emissions. After transfer into the exposure chamber, the air was mixed with a constant supply of clean air. The air exchange rate was different between the three exposures, as we aimed for a specific mass concentration during particle exposure sessions (average air exchange during cooking: 4.4 h^{-1} [±0.2]; candles: 3.5 h^{-1} [±0.1]; clean air: 2.6 h^{-1} [±0.4]). When candles were almost burned down, they were extinguished in water in order to avoid an uneven exposure to soot and other large particles, and new candles were lit. On exposure days with clean air, the adjacent chamber was empty; the ovens and the candle set-up had been removed and all surfaces had been cleaned. Inside the large exposure chamber, target temperature was 23°C and relative humidity 45% throughout exposure sessions. All exposure sessions were conducted at the same time of the day to minimize the influence of diurnal variation in the outcome measurements.

2.6 | Exposure characterization

Environmental conditions were routinely monitored and controlled by a HVAC (Heat Ventilation Air-Conditioning) system and kept as constant as possible throughout the experiment. Inlet air to the chambers was purified using several HEPA- and carbon filters. Monitoring was done by using a logger system from Campbell Scientific with high-quality sensors for temperature, humidity, CO_2, airflow rate, differential pressure, and ozone measurements. The constant inflow of clean air in the large exposure chamber was created using a slot inlet system in the ceiling to secure optimal mixing of the exposure concentration added from the adjacent chamber. The particle exposure inside the exposure chamber was monitored and characterized during each exposure session from the first person entering the chamber until the last person leaving the chamber. For controlling the exposure level, online monitoring of particle mass was performed during each session by a DustTrak DRX 8533 equipped with a $PM_{2.5}$ inlet (TSI). Particles (PM_{10} and $PM_{2.5}$) were sampled during

exposure using SKC PTFE filters with PMP Support by means of PM-samplers (SKC PEM 2.5 μm, 2 L/min and ADI PM 2.5 μm & PM 10 μm, 10 L/min) and saved at −20°C for later mass analyses. NO_2 was measured during exposures (API Chemilumine-scent NO_2 analyzer model 200 A). Particle size distributions and number concentrations were measured at several exposure sessions (an overview of measurement dates is provided in Table S1). A Scanning Mobility Particle sizer (SMPS) with a soft x-ray neutralizer (TSI 3087) was used with either a nano Differential Mobility Analyzer (nano DMA, in the size range 2.4–79.1 nm) (DMA, TSI 3085, sheath/sample flow rate: 10/1.5 L/min) or a long DMA (long DMA, in the size range 14.6–661.2 nm) (TSI, 3081, sheath/sample flow rate: 3/0.3 L/min) connected to an Ultrafine Condensation Particle Counter (TSI UCPC, 3776). Scan and retrace time were 120 s and 30 s, respectively. The two size intervals were measured in sequence. The SMPS inlet was connected to the large chamber by a 1 m copper tubing (inner diameter: 4 mm). Data acquisition and processing were done using AIM 9.0 (TSI) with software diffusion correction and multiple charge correction applied to the data. Additionally, the particles' potential to scatter visible light was measured with a polar nephelometer (Ecotech Pty Ltd.; Aurora 4000) at wavelengths of 450, 525, and 635 nm. For this purpose, 1 m of conductive tubing was connected to the chamber and a flow rate of approximately 13 L/min was used.

Volatile organic compounds (VOCs) were collected onto two Tenax TA adsorbent tubes (Gerstel) in parallel for 6.6 (± 1.2) h (flow rate 11.8 [± 0.7] ml/min). Adsorbent tubes were thermally desorbed using a Gerstel thermal desorption unit and a Gerstel MPS autosampler followed by analysis with gas chromatography – mass spectrometry, GC-MS (Agilent 7890B GC and Agilent 5977A MSD). Thermal desorption of adsorbent tubes took place from 20 to 300°C followed by focusing at −100°C. The column was a Restek RTX-200 ms column (30 m × 0.25 mm × 0.25 μm) and carrier gas was Helium. The GC temperature program was 10°C min^{-1} from 35 to 300°C (initial hold time 2 min), and the MS scanned in the 30–500 m/z range. Calibration curves were prepared for hexanal, nonanal, 2-heptenal and 2,4-decadienal (selected based on preliminary investigations) with 1-bromotoluene as internal standard. Coefficient of variation (R^2) was better than 0.998 for all calibration curves.

2.7 | Outcome assessment of clinical measurements

Clinical investigations consisted of spirometry, fractional exhaled NO (FeNO), and measurement of nasal volume by acoustic rhinometry. Just before each exposure session, baseline clinical investigations were performed. The investigations were repeated immediately after exposure (5 h after exposure start), and the next morning (24 h after exposure start). All methods are standard used in the departments' previous exposure studies.[26,27] For all outcomes, the participants acted as their own controls. All outcomes reported in this study are secondary outcomes of interests, why they have to be viewed upon as hypothesis generating. Other outcomes from this study (systemic inflammatory biomarkers), including the primary outcome, will be reported in a later publication.

2.7.1 | Spirometry

An EasyOne™ Spirometer (ndd Medical Technologies) was used to measure Forced Vital Capacity (FVC) and Forced Expired Volume in the first second (FEV_1). Subsequently, the ratio FEV_1/FVC was calculated. Participants were tested at least three times and the best performance for FEV_1 and FVC was chosen. Testing was performed in accordance with the ATS/ERS guidelines.[28] Data are presented in liters.

2.7.2 | FeNO

Fractional exhaled nitric oxide (FeNO) is an objective biomarker of airway inflammation and was measured using a chemiluminescence analyzer (NIOX VERO® Airway Inflammation Monitor; Aerocrine AB). The participant was instructed to inhale as deeply as possible to total lung capacity and consecutively exhale in the NIOX instrument at a fixed mouth flow rate of 50 ± 5 ml/s for 10 s. The exhalation rate was held steady by applying a constant positive pressure while instructing the participant to exhale steadily using visual stimulation on the system screen in combination with audio signals. A continuous sound indicated correct pressure with a frequency proportional to the pressure. Measurements were performed in a standing position according to the 2005 ATS/ERS recommendations.[29] Exhaled NO-concentrations are expressed in parts per billion (ppb).

2.7.3 | Nasal volume

Acoustic rhinometry precisely locates the nasal cross-sectional area and volume of each nasal cavity by analysis of sound reflection. Acoustic rhinometry was performed only before and immediately after exposure (5 h after exposure start) using an A1 Acoustic Rhinometer, GM INSTRUMENTS.[30] The details of the procedure has been described elsewhere.[30] The volume of each nasal cavity was determined by integration of the area–distance curve from 0 to 5 cm into the nose. The left and right nasal cavity were examined alternatively until four reproducible measurements were obtained. Presented data are the summed average of the volumes recorded for the right and left side of the nose in cm^3.

2.8 | Self-reported symptoms and discomfort

During each exposure session, a questionnaire assessing symptoms or perception was completed at the start of exposure (0 min), during

TABLE 1 Participant characteristics for the study population by means and standard deviations (SD) in all participants and stratified by sex

Characteristic	All	Females	Males
Participants, *N (%)*	36 (100.0)	20 (55.6)	16 (44.4)
Age in years	22.3 (1.5)	22.0 (1.6)	22.6 (1.4)
Height (cm)	174.1 (7.2)	169.9 (4.9)	179.4 (6.0)
Weight (kg)	70.2 (10.0)	67.5 (9.9)	73.6 (9.3)
BMI (weight/(height^2))	23.2 (3.2)	23.4 (3.7)	22.8 (2.6)
FEV_1 (liter)[a]	3.76 (0.74)	3.39 (0.41)	4.30 (0.79)
FEV_1 (% predicted)	3.90 (0.54)	3.49 (0.19)	4.48 (0.3)
FVC (liter)[a]	4.64 (0.99)	4.11 (0.43)	5.41 (1.09)
FVC (% predicted)	4.53 (0.72)	3.99 (0.22)	5.32 (0.36)
FEV_1/FVC[a]	0.81 (0.08)	0.83 (0.07)	0.80 (0.08)
FeNO (ppb)[a]	33.07 (27.3)	30.70 (22.44)	37.08 (34.67)

Abbreviations: FeNO, Fractional exhaled Nitric Oxide; FEV_1, Forced Expiratory Volume in the first second; FVC, forced vital capacity.

[a]The reported FEV_1, FVC and FeNO values were measured at participant's pre-examination, which was held before final inclusion in the trial. FEV_1 and FVC are depicted for 34/36 participants. FeNO is for 32/36 participants.

TABLE 2 Characterization of the environmental exposures in the large exposure chamber for clean air, cooking, and candles exposure (climate and air quality factors) described by means and standard deviations (SD)

Measurement	Unit	Clean air exposure	Cooking exposure	Candle exposure
Number of sessions, *N*		10	11	11
Temperature	°C	22.9 ± (0.2)	22.9 ± (0.2)	23.1 ± (0.2)
Humidity	RH%	43.8 ± (1.2)	43.1 ± (1.0)	43.2 ± (0.7)
CO_2	ppm	629 ± (74)	542 ± (43)	915 ± (66)
NO_2^*	ppb	2.1 ± (0.5)	6.47 ± (1.8)	52.94 ± (1.8)
$PM_{2.5}$	µg/m^3	5.8 ± (6.8)	96.1 ± (13.1)	89.8 ± (9.3)
PM_{10}	µg/m^3	3.0 ± (1.0)	97.2 ± (11.7)	91.4 ± (7.6)
Total particle number conc. (2.4–79.1 nm)**	#/cm^3	1.1×10^3 (1.2×10^3)[a]	5.9×10^3 (6.5×10^3)[b]	1.7×10^6 (1.8×10^5)[c]
Total particle number conc. (14.6–661.2 nm)**	#/cm^3	8.8×10^2 (3.4×10^2)[a]	7.2×10^4 (2.5×10^4)[b]	3.7×10^5 (1.3×10^5)[c]

Note: Clean air exposure: Mean PM_{10} has a smaller mass than $PM_{2.5}$ due to instability in collection of particles.

Abbreviations: CO_2, carbon dioxide; Conc., concentration; NO_2, nitrogen dioxide; PM, particulate matter; SMPS, Scanning Mobility Particle sizer.

[a]Average of two sessions.

[b]Average of three sessions.

[c]Average of four sessions.

*Mean NO_2 might be underestimated for all exposures as the instrument (API Chemiluminescent NO_2 analyzer model 200 A) had an off-set about 22% at the end of the study, which happened gradually during the trial.; **Total particle number concentrations are SMPS average values for the total of the measured time intervals. The explanation for some SDs being higher than the mean is fluctuations in particle number concentration over time and between sessions.

exposure every 30 min, and at the end of the exposure session (5 h) – in total 11 times. The questionnaire consisted of 28 questions related to indoor air quality, symptoms, and general comfort. Details on the questions have been published elsewhere.[31] Participants were asked to rate the strength of symptoms and their environmental perception by placing their finger on a Surface Pro touch screen on a rating scale from 0 to 10, with the highest number corresponding to highest discomfort.

2.9 | Exit poll

The morning after the third exposure, participants completed an exit poll on which exposures they thought they had been exposed to during the three exposure days. Comparing their actual exposure to their appraised exposure made it possible to evaluate the participant blinding effectiveness of the study. Participants were asked to think about this only once to avoid speculation about the exposures during the study.

2.10 | Statistics

We used linear mixed models based on the univariate repeated measurement analysis of variance (ANOVA) to evaluate the change in health outcomes (post-exposure vs. baseline measures) between clean air and candles and cooking, respectively. The models included the outcome of interest, and as fixed effects, it included the exposure, time, exposure-order, day, and time-exposure interaction. As a random effect, we included participant ID. Time was divided into baseline, 5 h, and 24 h, exposure was clean air, candles or cooking, order was corresponding to the order the participant received the exposure at, while day indicated whether the exposure took place on participants' first, second, or third day. The statistical measures of interest were the exposure and time-exposure interaction as an effect of any of these terms indicated a difference in the change from baseline associated with the exposure. We initially fitted a model with interaction (Model 1). In the case of no significant interactions, the next step was an analysis without interactions (Model 2). Finally, the analyses were stratified by sex. When examining symptoms and discomfort reported during exposures, we fitted linear mixed models followed by contrast tests to identify significant differences between particle exposures and clean air at each time point. Margins plots were plotted to illustrate mean symptom development during the three exposures. A Chi^2-test was performed to examine whether there was a significant difference on the actual versus appraised exposure of candles and clean air. The level of significance was assumed at $p < 0.05$. All statistical analyses were performed using Stata 16 software (StataCorp).

3 | RESULTS

Thirty-six non-smoking individuals (20 females; 16 males) with mild asthma participated in the exposure study comprising all three exposures (mean age 22.3 years). Table 1 summarizes the characteristics of the study participants.

3.1 | Exposure characteristics

In Table 2, characterization of the environmental exposures (temperature, humidity, particle mass and number concentrations, etc.) are listed. Due to air conditioning, temperature and relative humidity remained nearly constant throughout all exposures. Levels of CO_2 and NO_2 increased during candle exposures (915 ppm CO_2 and 52.9 ppb NO_2). The total particle number concentrations reached the highest mean level during candle exposure experiments compared to cooking experiments, with the mean values for candle exposures being 1.7×10^6 particles/cm^3 using nano DMA and 3.7×10^5 using long DMA, respectively. For cooking, mean total particle number concentrations were 5.9×10^3 (nano DMA) and 7.2×10^4 (long DMA).

3.1.1 | Particles

A representative example of particle characteristics from cooking exposure is shown in Figure 1. The average particle diameters were in the range 32 to 104 nm with the mode ~80 nm. Both particle number concentration and mode diameter varied depending on the timing of the four ovens (Figure 1D). Figure 2 shows an example of particle characteristics during a candle exposure. The highest particle number concentration was found for particle diameters ~7.5 nm (Figure 2A). The average particle mode diameters derived for the candle exposure sessions were in the range 6.2 to 9.2 nm. Figure 2C shows the particle volume distribution for particles when using long DMA. The particle number and size distributions were fairly stable during the candle exposure (Figure 2D). Similar particle characteristics to those shown in Figure 2 were observed during other exposure sessions with candles. The temporal evolution of the scattering coefficients shows that the emissions by candles were relatively constant throughout the experiment (Figure 2E) while emissions by cooking exhibited more variation (Figure 1E). During exposure to clean air, particle concentrations were very low; see Figures S1 and S2.

3.1.2 | Volatile organic compounds

As seen from Figure 3, cooking resulted in high levels of VOCs, especially aldehydes including pentanal, hexanal, heptanal, and nonanal, as well as unsaturated aldehydes such as 2-heptenal and 2,4-decadienal. Concentrations of VOCs were low during sessions with clean air, and only few VOCs, including nonanal, were observed above the limit of detection. During exposure to candle emissions, concentrations of VOCs were also low, and the VOCs mainly detected included benzoic acid, isopropyl alcohol, 1-butanal, toluene, and benzene (data not shown).

3.2 | Health outcomes

No significant changes were observed in FEV_1 and FVC, when comparing cooking and candles to clean air (Table 3). Similarly, FeNO and nasal volume were not affected by exposure from either cooking or candles, when compared to clean air exposure. However, analysis stratified by sex revealed that nasal volume decreased significantly in males after exposure to cooking compared to clean air (mean: −0.49 cm^3 [95% CI −0.97; −0.01] [$p = 0.048$]). Also, FeNO declined in males after being exposed to candles, though, estimates were borderline significant (mean: −3.03 ppb [95% CI −6.30; 0.24] [$p = .069$]).

3.3 | Self-reported symptoms and discomfort

More participants reported additional and significantly more serious symptoms such as watering eyes and blocked nose when exposed to

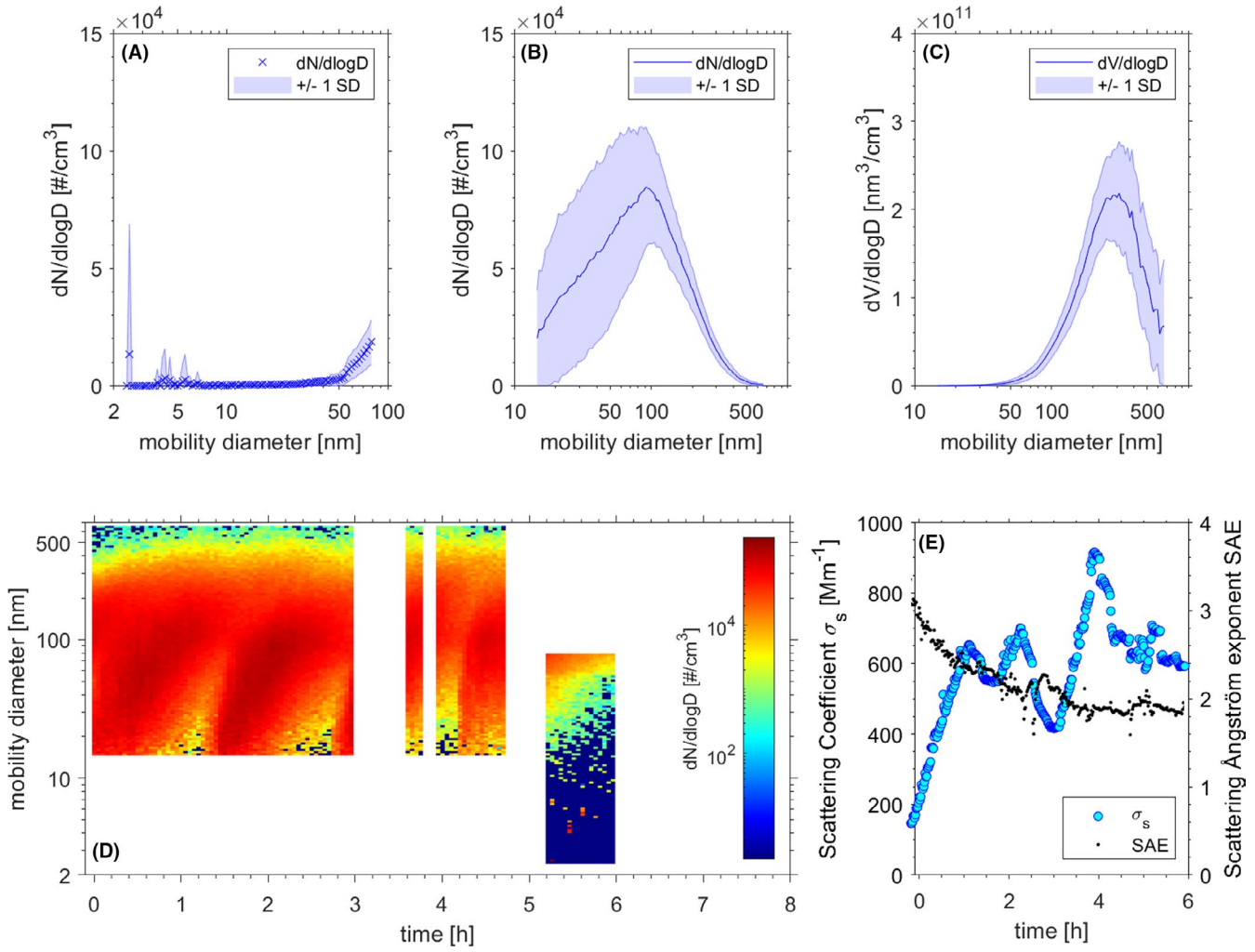

FIGURE 1 Example of particle characteristics during cooking exposure (30/10/2019). (A) Average particle number size distribution measured in the size range 2.4–79 nm (nano DMA). (B) Average particle number size distribution measured in the size range 14–661 nm (long DMA). (C) Average particle volume size distribution for the size range 14–661 nm (long DMA). (D) Temporal evolution of particle number and size. (E) Temporal evolution of scattering coefficients and scattering Ångström exponents (at a wavelength of 525 nm). Notice that the two different SMPS size intervals were measured in sequence. Hence, the empty areas in (D) are due to stopping measurements when changing between long DMA and nano DMA. DMA, Differential Mobility Analyzer; SMPS, Scanning Mobility Particle sizer

candles compared to clean air as seen from Figure 4. Participants felt significantly more serious symptoms including eye irritation, dry eyes, watering eyes, running nose, blocked nose, head ache, nausea, and general discomfort during exposure to cooking compared to during clean air exposure. Of the included symptoms of asthma (wheezing, coughing, shortness of breath, and chest tightness) only chest tightness was significantly more severe during cooking exposure compared to during clean air exposure. In general, levels of reported asthma symptoms were between 0 and 1 (on a scale from 0 to 10) during the three exposure sessions (data not shown). As seen from Figure 4, participants did not become habituated to their surroundings when exposed to cooking or candles. Overall, females reported more severe symptoms throughout the questions, however, differences between males and females were not significant (data not shown).

3.4 | Exit poll

On exposure days with cooking, 35/36 (97.2%) participants were able to identify the exposure (Figure 5). Participants were not able to identify whether they had been exposed to clean air or candles in a systematic way; when exposed to candles 20/35 (57.1%) participants guessed the exposure correctly. One participant marked it with a "?" indicating he had no idea whether he had been exposed to clean air or candles, however, he was excluded from the analysis as he did not provide a qualified guess as all other participants. Chi^2-test showed no significant difference (p = 0.250) whether participants thought they had been exposed to candles or clean air on days with candle exposure and vice versa.

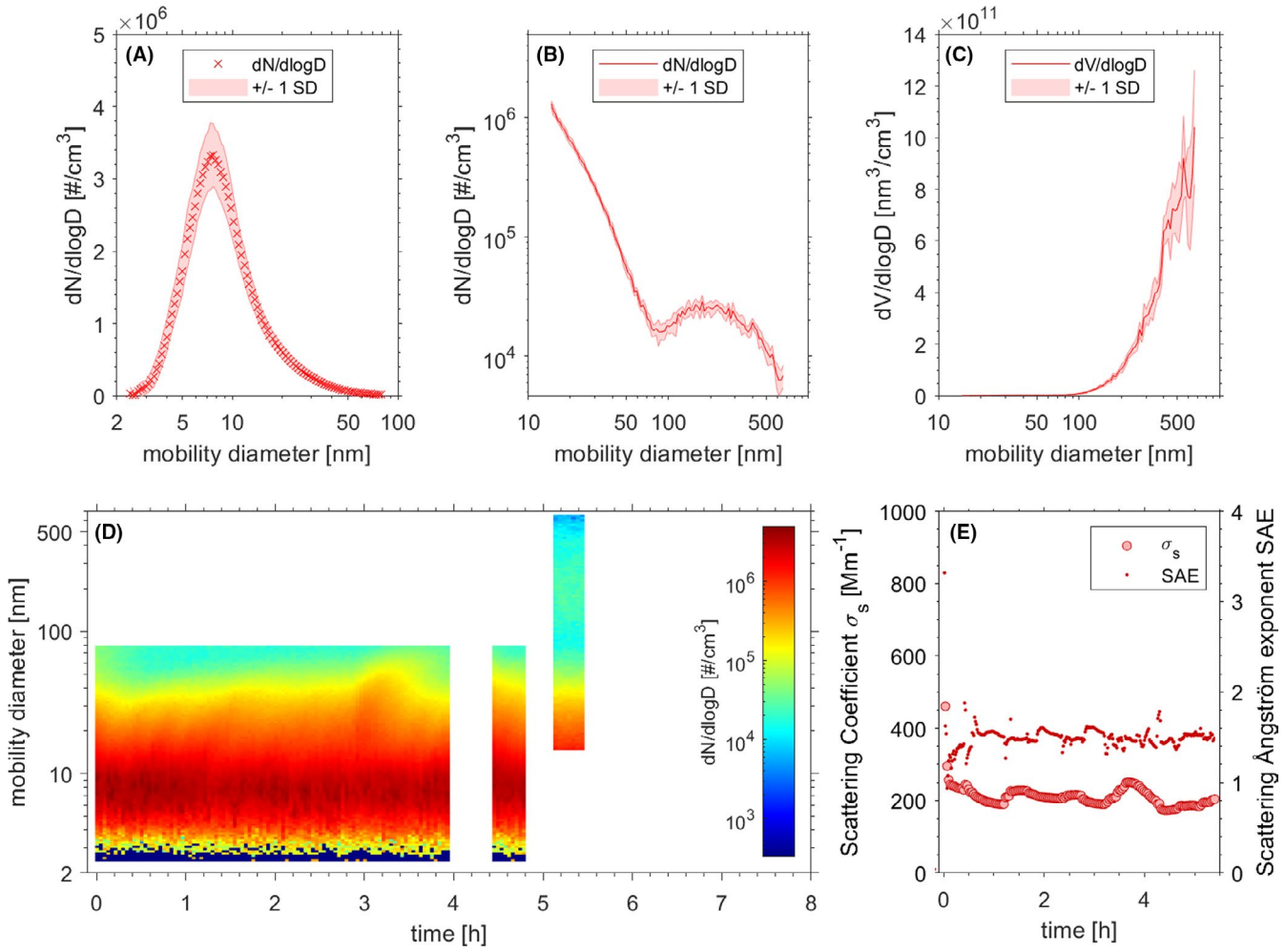

FIGURE 2 Example of particle characteristics during candle exposure (21/11/2019). (A) Average particle number size distribution measured in the size range 2.4–79 nm (nano DMA). (B) Average particle number size distribution measured in the size range 14–661 nm (long DMA). (C) Average particle volume size distribution for the size range 14–661 nm (long DMA). (D) Temporal evolution of particle number and size. (E) Temporal evolution of scattering coefficients and scattering Ångström exponents (at a wavelength of 525 nm). Notice that the two different SMPS size intervals were measured in sequence. Hence, the empty areas in (D) are due to stopping measurements when changing between long DMA and nano DMA. DMA, Differential Mobility Analyzer; SMPS, Scanning Mobility Particle sizer

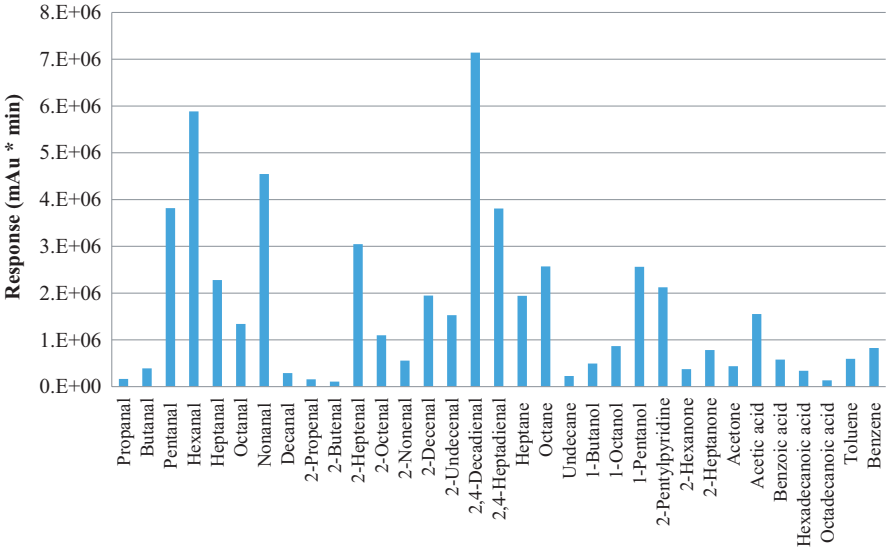

FIGURE 3 Relative GC-MS response of Volatile organic Compounds (VOCs) in air samples collected during exposure to emissions from cooking.

TABLE 3 Mean change in objective health outcomes caused by cooking and candle exposure in all participants and stratified by sex (Clean air = reference). Results are reported from Model 2 without interaction[a]

	All (n = 36)				Females (n = 20)				Males (n = 16)			
	Coefficient	**95% CI**		***p*-Value**	**Coefficient**	**95% CI**		***p*-Value**	**Coefficient**	**95% CI**		***p*-Value**
Spirometry												
FEV_1												
Cooking	0.02	−0.03	0.06	.451	0.02	−0.04	0.08	.573	0.01	−0.04	0.07	.675
Candles	0.03	−0.01	0.07	.203	0.05	−0.01	0.11	.117	0.02	−0.04	0.07	.607
FVC												
Cooking	0.03	−0.02	0.07	.202	0.03	−0.04	0.09	.437	0.03	−0.03	0.09	.284
Candles	0.04	−0.01	0.08	.084	0.05	−0.01	0.11	.103	0.04	−0.02	0.10	.193
FEV_1/FVC												
Cooking	−0.001	−0.006	0.004	.656	−0.001	−0.01	0.01	.880	−0.002	−0.01	0.01	.569
Candles	−0.001	−0.006	0.004	.732	−0.01	−0.01	0.01	.906	−0.003	−0.01	0.01	.503
FeNO												
Cooking	−0.31	−3.05	2.44	.827	1.58	−2.63	5.79	.460	−2.44	−5.71	0.83	.142
Candles	0.01	−2.74	2.76	.995	1.95	−2.26	6.16	.361	−3.03	−6.30	0.24	.069
Nasal volume												
Cooking	−0.24	−0.61	0.13	.204	−0.08	−0.62	0.46	.769	−0.49	−0.97	−0.01	.048*
Candles	0.12	−0.25	0.49	.51	0.3	−0.23	0.84	.265	−0.10	−0.58	0.39	.690

Note: Nasal volume is specified as cm^3.

Abbreviations: FeNO, Fractional exhaled Nitric Oxide (ppb); FEV_1, Forced Expiratory Volume in the first second (liter); FVC, Forced Vital Capacity (liter).

[a] Results are from linear mixed models.

*The level of significance was assumed at $p < .05$.

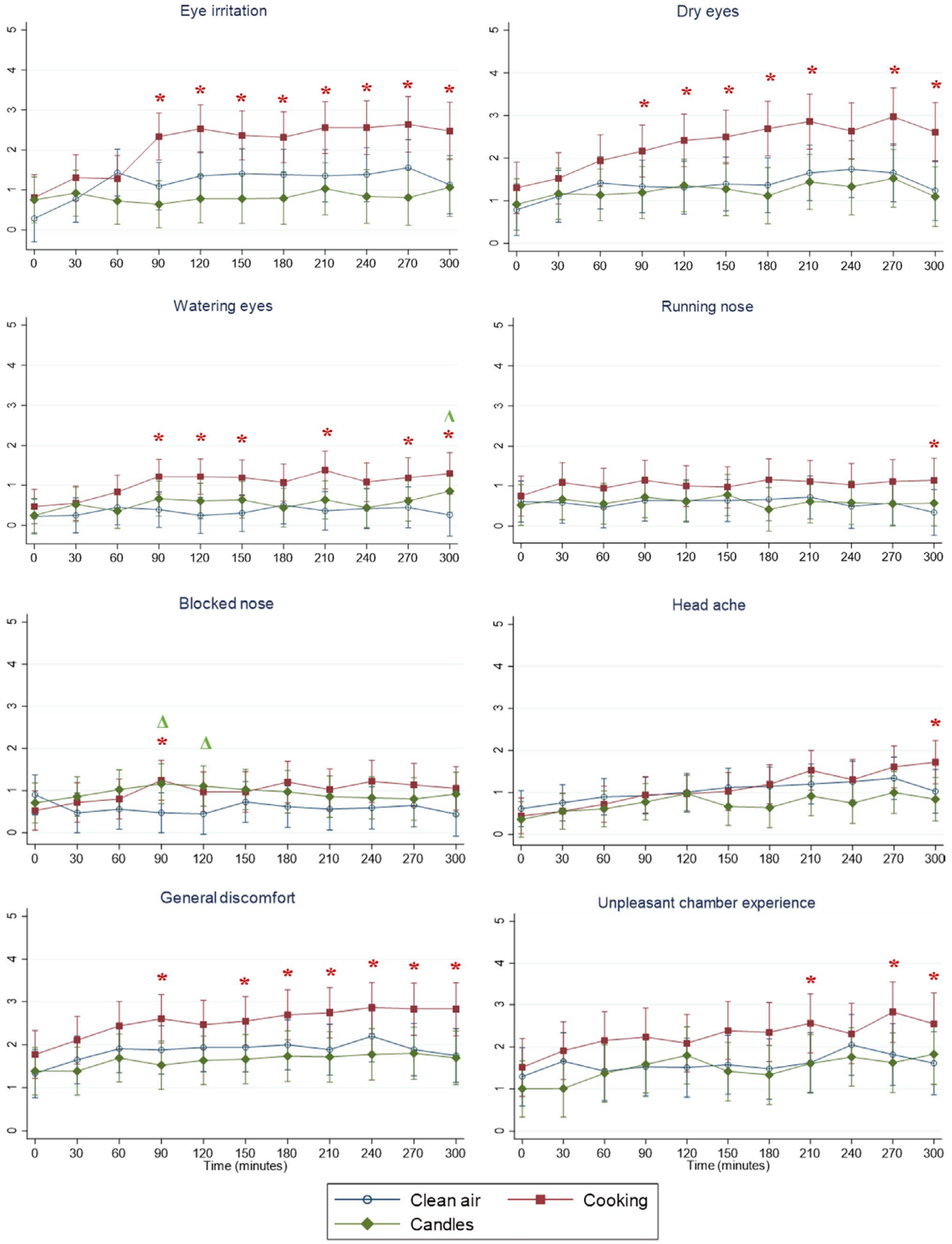

FIGURE 4 Symptoms and discomfort reported by all participants during the three exposures. *Y*-axis: Symptoms and discomfort reported on scale from 0 to 10, with 10 being worst. *X*-axis: Time in minutes – in total 5-h exposure duration. Symptoms with significant differences between cooking and clean air, and candles and clean air are shown ($p < 0.01$ indicated by a star for cooking and a triangle for candles). Despite significant differences from clean air, nausea, and chest tightness are not shown, as symptom levels were low

4 | DISCUSSION

A main finding in this study was decreasing nasal volume in males after exposure to cooking compared to when exposed to clean air. A decline in FeNO, but not significant, was observed among males after exposure to emissions from candles. We observed no change in FEV_1 and FVC when participants had been exposed to cooking or candles compared to clean air. More participants reported additional and significantly more pronounced symptoms of irritation, when exposed to cooking and candles. Females tended to report more serious symptoms than males; however, differences between males and females were not statistically significant. Participants were able to smell exposure to cooking, meaning that blinding of cooking was not achieved.

Particles emitted from cooking were larger than particles emitted from candles with the average mode diameter of particles being ~80 nm and ~7.5 nm, respectively. We, however, also observed a peak around 200–300 nm for candle emissions (see Figure 2B). Hence, when exposed to almost the same mass concentration level ($PM_{2.5}$), participants were exposed to fewer particles during cooking exposure compared to when exposed to candles. Emissions from cooking exhibited more variation in particle size range than emissions from candles, due to bursts of fat when broiling. Differences in size and composition in particles might be a reason for the difference observed in health effects between the two exposures. Candle exposure was found to reduce FeNO concentrations in the lungs among males while cooking emissions led to decreasing nasal volume, thereby only affecting the upper respiratory tract, supporting the existing knowledge that the smallest particles affect the deeper airways.[15] We observed a borderline significantly larger drop in nasal volume when comparing changes after cooking exposure to changes after candle exposure the difference was (−0.36 [95% CI −0.73; 0.00], p = 0.052). There was no significant difference when comparing change in FeNO for candle versus cooking (p = 0.882).

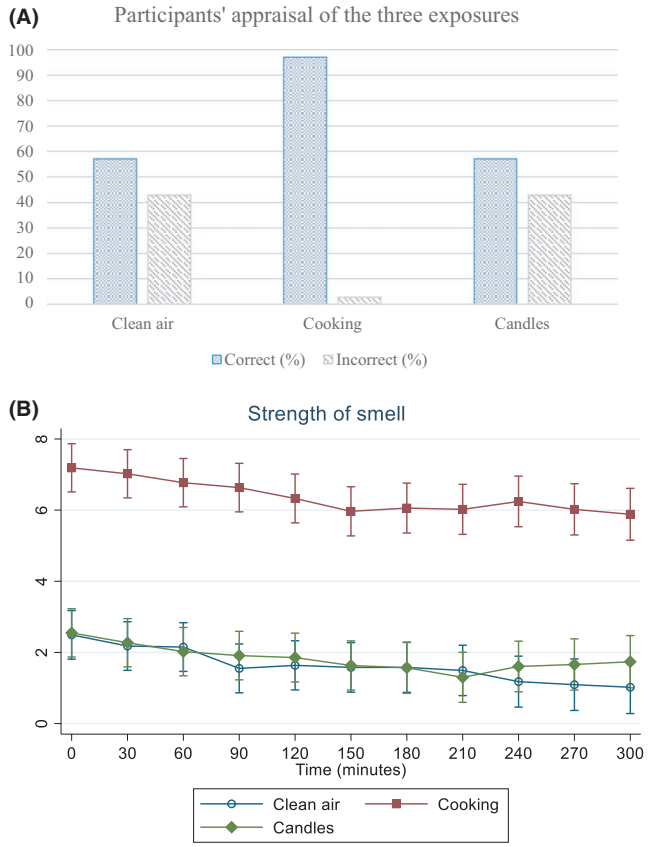

FIGURE 5 Results from exit poll and self-reported strength of smell during the exposures. (A) Participants' appraisal of the three exposures (correct/incorrect) reported in percent. (B) Participants' experience of "strength of smell" during the three exposures. *Y*-axis: Symptoms are reported on a scale from 0 to 10 with 10 being worst. *X*-axis: time in minutes

The scattering coefficients from cooking were on average 500 Mm^{-1}, in the range of data for biomass burning.[32] We found lower values of 200 Mm^{-1} for candles like for cleaner environments.[33] The Scattering Ångström Exponent (SAE) expresses the scattering dependence on wavelength and can be used to infer the size of the particles responsible for the light scattered. For large particles, SAE will typically be close to zero, while it increases for predominately small particles. SAEs for cooking start with high values around 3 and level at values of 2, while they are quite constant for candles at a value of 1.5, both indicating a major fraction of small particles. Such SAE values are in line with data from organic and elemental carbon measurements.[34]

When generating the two exposures, we aimed for the same mass concentration level for both exposures each time. In the present study, average particle mass concentrations ($PM_{2.5}$) were 96.1 $\mu g/m^3$ and 89.8 $\mu g/m^3$, for cooking and candles, respectively. These levels are comparable to other exposure studies with indoor particle exposures, for example frying sausages and burning candles.[16,21,35] We assume the particle concentrations observed in our study to be comparable with indoor levels during daily activities in private homes, although we note that indoor PM concentrations are the product of not only particles emitted, but also ventilation conditions and building design. Sufficient ventilation can lead to lower exposure levels while insufficient ventilation might lead to even higher personal exposure levels, when cooking using several cooktops or burning several candles at once as shown in several observation studies.[35-38] During candle exposure in the present study comprising 10 candles, NO_2 levels (~100 $\mu g/m^3$) exceeded the yearly average threshold level for ambient NO_2 (40 $\mu g/m^3$) (EU).[39] CO_2 levels were affected by the persons in the chamber as well as the air exchange rate. On days with particle exposures, we had to increase ventilation rates in order to maintain a constant exposure. Consequently, CO_2 levels were higher on days with clean air than on days with cooking. During days with clean air as exposure, sources of particles were the participants and the lunch served in the chamber. On days with clean air and cooking exposure, we detected peaks of particles below 3 nm. Only few particle counts close to the lower limit of the nano DMA (2.4 nm) gave a high concentration due to loss corrections explaining the peak below 3 nm in figures and the high SDs in Table 2. We consider these high counts to be noise. For candle

exposure, particle number concentration was high in this area, and so the spikes are most likely vanishing in the high concentration.

In the present study, measurements of identified VOCs showed low levels during exposure to clean air and candle emissions. Nonanal, detected during exposure sessions with clean air, has previously been detected from human subjects.[40] Cooking resulted in high levels of VOCs, including aldehydes, which has also been shown in previous studies of cooking activities.[41,42] Aldehydes can be auto-oxidation products of unsaturated fatty acids. Of these, 2,4-heptadienal and 2-heptenal are from degradation of linolenic acid, while pentanal is from linoleic acid, and 2-decenal is degraded from oleic acid; all three are fatty acids found in pork meat.[43] Furthermore, compounds such as 2-pentyl-pyridine was observed, which stems from the Maillard reaction that occurs during grilling of meat including breast of pork.[42] This reaction creates molecules with a distinct smell.

We observed a decrease in nasal volume after exposure to cooking – in males this decrease was statistically significant. Nasal volume indicates the degree of patency of the nose. Inhalation of pollutants such as particles can cause an inflammatory response, leading to a swelling of the nasal mucosa thereby lowering nasal volume. Our results are in concordance with two observational studies conducted among Swedish school children and school personal, where exposure to elevated levels of indoor air pollutants (PM_{10}, dust, formaldehyde, NO_2, and molds) in class rooms lead to decreasing nasal patency.[44,45] Depending on the size of inhaled particles, they deposit in the nasopharynx, while ultrafine particles can enter the lower airways.[30] As the nasal mucosa is the first part of the airways in contact with the environment, it seems reasonable that the larger particles emitted from cooking, can affect nasal patency by being trapped in the nasal cavity, thereby preventing them from reaching the lower airways.

In the present study, exposure to candles seemed to reduce FeNO concentrations among males. This decline was not observed among females. To our knowledge, no previous exposure study has examined changes in FeNO after exposure to candles, the reason why this is a novel finding. FeNO is a simple objective method measuring biomarkers of eosinophilic airway inflammation,[29] and several studies examined other particles sources and their effect on FeNO.[46-51] A decrease in nitric oxide (NO) from the lungs is in accordance with studies on exposure to smoking of conventional cigarettes and electronic cigarettes.[27,46-49] However, it is opposed to the findings in some air pollution studies, where NO production in the lungs has been reported to increase with high levels of air pollution.[50,51] NO, which is present in the exhaled breath of all humans, is produced by cells involved in the inflammatory response and elevated levels may be indicative of airway inflammation and injury.[29] A decrease in NO after exposure to candle emissions could be explained by a negative feedback mechanism as the NO emitted from candles downregulates NO synthases in the lungs.[52,53] The physiological consequences of such a downregulation is unknown.

We observed that males were more susceptible to the above respiratory effects (nasal volume and FeNO) of emissions from cooking and candles than females. Similarly, observational studies evaluating associations between ambient air pollution and asthma-related symptoms in children and adolescents, found that long-term exposure to PM may increase asthma-related symptoms, especially among boys.[54,55] They explain it by sex-related differences in the deposition of PM, with boys developing larger lungs than girls, yet with smaller airway diameters relative to volume.[55] Additionally, hormonal status and body size influence the biological transport of environmentally derived chemicals.[56] An exposure study of cooking fumes found significant effects on the human brain with the brain abnormality mainly being driven by males rather than females.[16] However, the general literature is far from consistent regarding evidence of different associations between air pollution and health effects for males and females.[56]

We found no change in lung function (FEV_1 and FVC) after exposure to cooking and candles, respectively. Other studies examining non-asthmatics and asthmatics, have found strong evidence for short-term effects of fine and ultrafine particles on lung function, especially in children.[15,21,57] Yet, in some of our previous controlled exposure studies with fine and ultrafine particles from wood smoke and electronic cigarettes, we found airway inflammation, but no significant effect on lung function indices,[27,58] indicating that the lung function measurements may be less sensitive to short exposures as seen in the current study – especially in young, relatively healthy individuals.

In general, we observed an increase in self-reported symptoms and discomfort when participants were exposed to emissions from candles and cooking, with cooking emissions resulting in several significantly increased symptoms such as eye irritation, nose irritation, headache, nausea, etc. Previous exposure studies on cooking and candles seem to focus on objective measures, thereby not including self-reported items. Yet, the prevalence of symptoms in the present study corresponds well with findings from studies of the indoor environment based on population samples.[59] Also, previous chamber studies have shown that exposure to airborne dust causes increased prevalence of complaints about air quality, discomfort, and symptoms related to irritation of eyes, nose, or throat.[60,61] In our study, symptoms of asthma were not significantly worse during PM exposure compared to clean air exposure (except for chest tightness being significantly worse at one time point during cooking), which may be explained by the included participants having only mild asthma. In the present study, females tended to report more severe symptoms than males throughout most questions, which is consistent with previous studies on the indoor environment.[59,62] This might be explained by hormonal differences and the fact that females are more aware of their surrounding environment as discussed by Stenberg et al.[62,63]

4.1 | Strengths and limitations

Strengths of the present study were the crossover design, the randomization of exposures, the multiple effect measurements including baseline, and double-blinding for candles and blinding of investigators for cooking. In addition, we used a state-of-the-art exposure chamber in which all conditions other than the exposures were kept

constant. The study design eliminates confounding from personal characteristics, while the chamber set-up eliminates pollutants such as dust, chemicals, and particles from others sources affecting the exposures and thereby outcomes. The successful blinding to candle exposure strengthens both the subjective and objective results. The exposure levels were very constant throughout and across exposure days with regard to the same exposure, meaning that all participants have been exposed to the same concentration of particles and gases. In the present study, particle emission levels from candles and cooking are comparable to real-life scenarios.

Our study also had limitations. Not being able to blind cooking emissions may have affected symptom awareness causing over-reporting of the experienced symptoms during cooking. Nevertheless, it is reasonable that participants had more dry eyes, more head ache, etc., as a consequence of the emitted particles and gases themselves.[63-67] It is implausible that participants' knowledge about the exposure affected objective results. As participants were left unattended in their homes between exposure days, another limitation is that exposure to indoor particles as well as traffic emissions on the day of the experiments might impact the results. However, due to randomization of the exposures, activities of participants in the hours and days before the exposure sessions are most likely to cause random effects. Thirdly, only one type of candle emission and one kind of cooking emission were examined. Health effects might have changed differently when examining other types of candles under other conditions and other ways of cooking. We chose the two stearin candles as they are among the most frequently sold candles in Denmark, and roasting pork in the oven is very common in Denmark and other Nordic countries as well. In order to generate controlled and standardized exposure scenarios, which were as similar as possible across study exposure days, there were some differences to real-life scenarios in a common household. In this study, candles were replaced before burning down and pork was kept in the turned off oven when finished, in order to reduce emissions from soot and burning fat. A fourth limitation is that we had no biomarker of neutrophilic inflammation. FeNO, as the only included marker of inflammation, measures eosinophilic inflammation.[29] Having included neutrophilic markers of inflammation in our study might have revealed important immune responses to PM exposures.[68] Furthermore, since we only measured health effects up to 24 h after exposure start, there might have been delayed effects not detected in the present study. However, as the exposures are not receptor-mediated as, for example, endotoxin showing systemic effects persisting for weeks,[69] we did not expect a cascade of inflammation, but instead general mild inflammation. Soppa et al. examined the effect of cooking and candles on lung function and found negative changes immediately after exposure, but not persistent 24 h after exposure.[21] Similarly, we expected changes in lung function, FeNO, and nasal volume in our study to be reversible within hours. Nevertheless, in case of delayed effects, the health effects of cooking and candles may have been underestimated in the present study. The findings of mild inflammatory responses do not necessarily pertain to the general population as individuals with asthma are particularly vulnerable to particle exposure due to their chronic inflammation in the respiratory tract. However, the results might be generalized to vulnerable subgroups such as children, the elderly, and other individuals with chronic respiratory disease – known to be susceptible to PM exposure due to some of the same reasons (increased minute ventilation and impaired host defense).[70,71]

Finally, we believe that a true but very mild effect of the exposure to cooking and candles occurred in this study, chance is an alternative explanation as outcomes were classified as secondary, and therefore interpretation should be made with caution.

5 | CONCLUSIONS

In the present study on young asthmatics, results indicated that short-term exposure to emissions from cooking and candles led to mild inflammatory responses in males and decreasing comfort among both males and females. Differences in size and chemical composition of particles by different sources may cause the differential health effects. Knowledge on the impact of exposure to indoor fine and ultrafine particles on health is restricted, and this study adds to the field of health consequences due to indoor particle exposures including detailed and novel exposure assessments.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests.

AUTHOR CONTRIBUTIONS

Karin Rosenkilde Laursen: Funding acquisition (supporting), Project Administration (lead), Methodology (equal), Conducting the study (equal), Formal analysis of health data (lead), Writing – Original Draft Preparation (lead), Writing – review and editing (lead). **Berit Brøndum Rasmussen**: Conducting the study (equal), Formal analysis of exposure data (equal), Writing – review and editing (equal). **Bernadette Rosati**: Conducting the study (equal), Formal analysis of exposure data (equal), Writing – review and editing (equal). **Vibeke Heitmann Gutzke**: Conducting the study (equal), Writing – review and editing (equal). **Kirsten Østergaard**: Conducting the study (equal), Writing – review and editing (equal). **Peter Ravn**: Methodology (equal), Conducting the study (equal), Writing – review and editing (equal). **Søren K. Kjærgaard**: Methodology (equal), Writing – review and editing (equal). **Merete Bilde**: Formal analysis of exposure data (equal), Writing – review and editing (equal), Supervision of exposure data (equal). **Marianne Glasius**: Formal analysis of exposure data (equal),

Writing – review and editing (equal), Supervision of exposure data (equal). **Torben Sigsgaard**: Conceptualization (lead), Funding acquisition (lead), Methodology (equal), Conducting the study (equal), Supervision (lead), Writing – review and editing (equal).

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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SUPPLEMENTARY FILES

Acute health effects from exposure to indoor ultrafine particles – a randomized controlled crossover study among young mild asthmatics

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Power considerations

From a study on the effect of "frying-pan" particles performed by our colleagues in Gothenburg, we received the following information on the change in Surfactant-Protein A (SP-A) during the experiment. The difference and SD are 1 and 1.1, respectively. This translates to a power of approximately 100 using standard power calculation with the inclusion of 36 persons.

Informed consent to participate in a health science research project

Title of the research project:

THE ULTRAFINE PROJECT

- A study of the acute health effects following exposure to ultrafine particles from cooking and candles

Statement from the test subject:

I have received written and oral information and I know enough about the aim, methods, advantages and disadvantages to agree to participate.

I know that it is voluntary to participate and I can withdraw my consent anytime without losing my current or future right to treatment.

I give my consent to participate in the research project, for collection of my biological material and for storage of this in a research biobank. I have a copy of this consent form and a copy of the written information about the project for my own use.

Name of test subject: _____

Date: _____ Signature: _____

In case new essential health information emerge about you from the research project, you will be informed. If you do **not** wish to receive any new essential health information emerging from this research project, please mark it here: _____ (write x)

Do you wish to be informed about the results of the research project and potential consequences for you?

Yes _____ (write x) No _____ (write x)

Statement from the person, providing the project information:

I declare, that the test subject has received oral and written information about the research project. To the best of my knowledge and ability, sufficient information has been given in order for the participant to make an informed decision about participating in this research project.

Name of the person providing the project information:

Date: _____ Signature: _____

Table S1. SMPS measurements: Details on flow rate and impactor size

	Date of experiment	DMA	Size interval (nm)	Aerosol flow (L/min)	Sheath flow (L/min)	Impactor size (cm)
Cooking	07-05-2019	Nano	2.41 to 79.1	1.5	10	0.071
		Long	14.6 to 661.2	0.3	3	0.0457
	30-10-2019	Nano	2.41 to 79.1	1.5	10	0.071
		Long	14.6 to 661.2	0.3	3	0.0457
	07-11-2019	Nano	4.45 to 156.8	0.3	3	0.071
		Long	14.6 to 661.2	0.3	3	0.071
Candle	09-05-2019	Nano	2.41 to 79.1	1.5	10	0.071
		Long	14.6 to 661.2	0.3	3	0.0457
	13-05-2019	Nano	2.41 to 79.1	1.5	10	0.071
		Long	14.6 to 661.2	0.3	3	0.0457
	05-11-2019	Nano	2.41 to 79.1	1.5	10	0.071
		Long	14.6 to 661.2	0.3	3	0.071
		Long	7.37 to 289	1.5	10	0.071
	21-11-2019	Nano	2.41 to 79.1	1.5	10	0.071
		Long	14.6 to 661.2	0.3	3	0.0457
Clean air	15-05-2019	Nano	2.41 to 79.1	1.5	10	0.071
		Long	14.6 to 661.2	0.3	3	0.0457
	19-11-2019	Nano	2.41 to 79.1	1.5	10	0.071
		Long	14.6 to 661.2	0.3	3	0.071

Definition of abbreviations: DMA = Differential Mobility Analyzer. Measurements were done with two different DMA's; a nano DMA and a long DMA. nm = nanometer. L/min = Litre per minute. cm = centimetre.

Figure S1

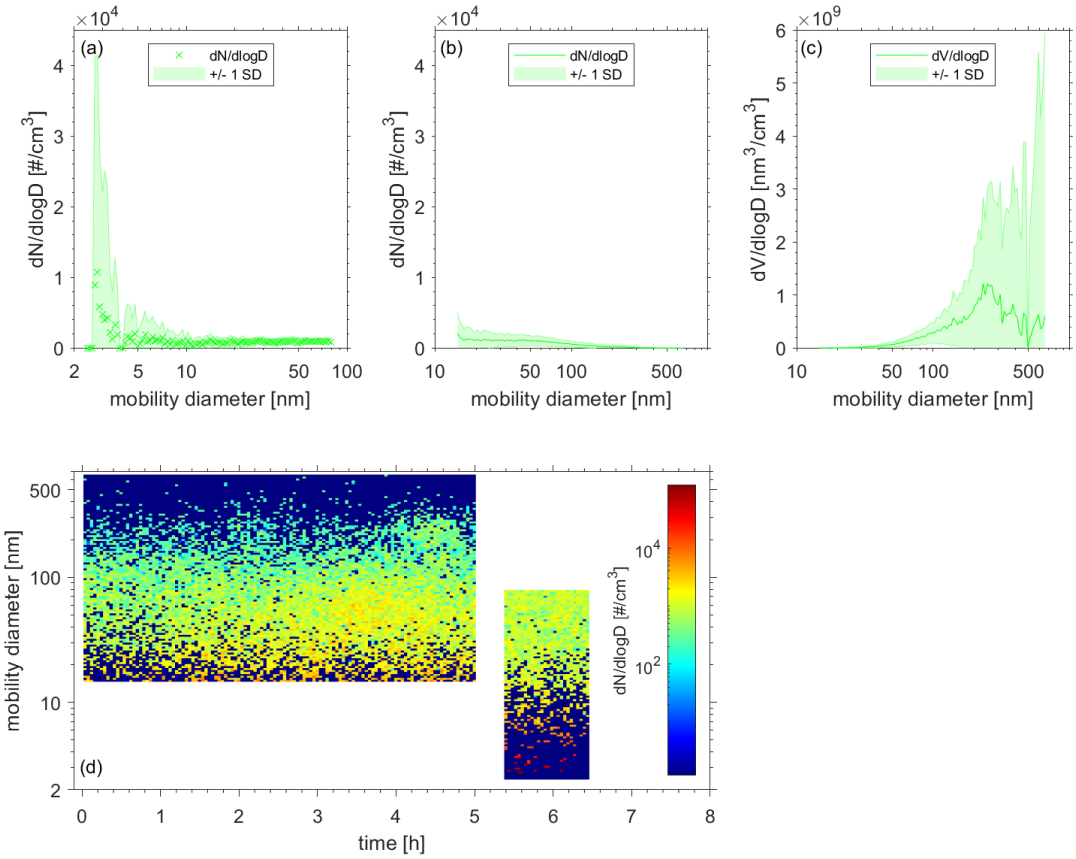

Figure S1. Particle characteristics during a clean air session (15/05/2019)[1]. a) Average particle number size distribution measured in the size range 2.4 to 79 nm (nano DMA) b) Average particle number size distribution measured in the size range 14 to 661 nm (long DMA). c) Average particle volume size distribution for the size range 14 to 661 nm (long DMA). d) Temporal evolution of particle number and size. No nephelometer data from this day. Notice that the two different SMPS size intervals were measured in sequence. Hence, the empty areas in d) are due to stopping measurements when changing between long DMA and nano DMA.

[1] This clean air session was the first clean air session in the trial. It is deviating from other clean air sessions as we had a small negative pressure (10 Pa) in the large exposure chamber thereby transferring air from the smaller chamber to the larger chamber, where participants were staying.

Figure S2

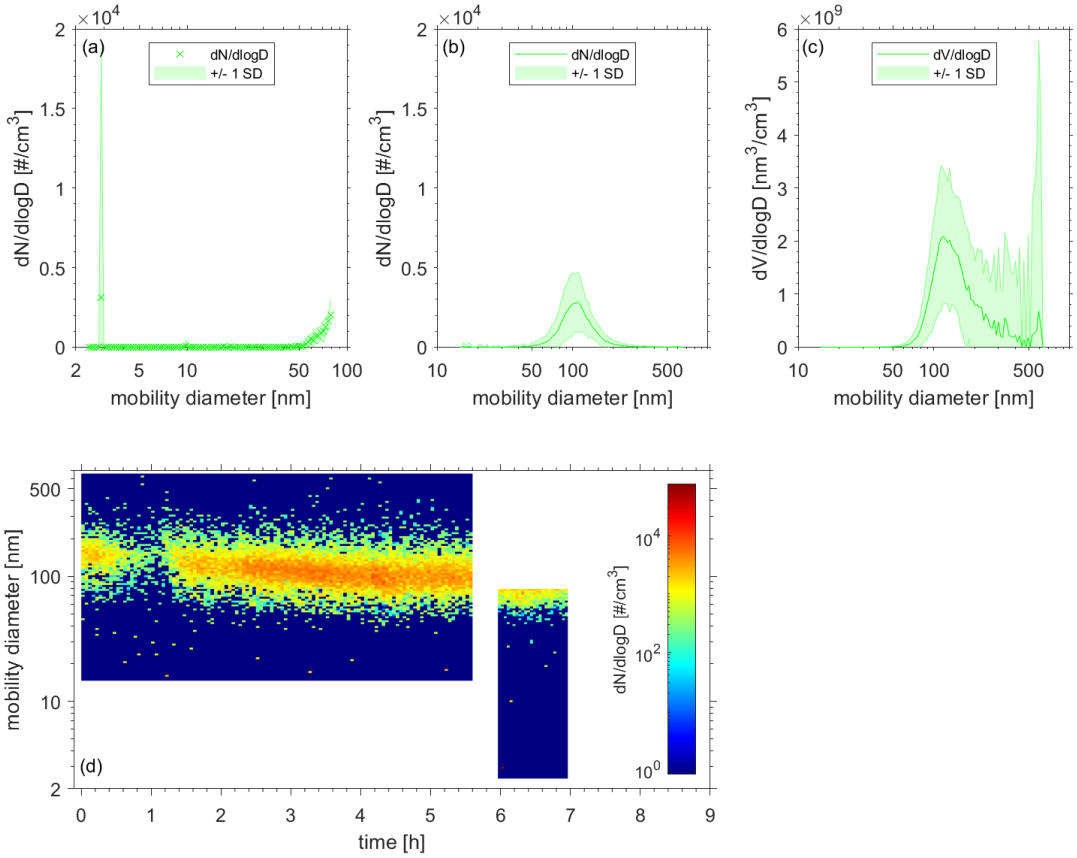

Figure S2. Particle characteristics during a clean air session (19/11/2019)[1]. a) Average particle number size distribution measured in the size range 2.4 to 79 nm (nano DMA) b) Average particle number size distribution measured in the size range 14 to 661 nm (long DMA). c) Average particle volume size distribution for the size range 14 to 661 nm (long DMA). d) Temporal evolution of particle number and size. No nephelometer data is shown as it was below the detection limit of the instrument. Notice that the two different SMPS size intervals were measured in sequence. Hence, the empty areas in d) are due to stopping measurements when changing between long DMA and nano DMA.

[1] During this and the eight other clean air sessions, no negative pressure was established in the large exposure chamber.

Paper III

Airway and systemic inflammation biomarkers after short-term exposure to indoor ultrafine particles – A randomized controlled double-blind crossover study among mild asthmatic subjects

(Manuscript in preparation)

Airway and systemic inflammation biomarkers after short-term exposure to indoor ultrafine particles – A randomized controlled double-blind crossover study among mild asthmatic subjects

(MANUSCRIPT IN PREPARATION)

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KEY WORDS: Indoor air; Ultrafine particles; Human exposure; Cooking; Candles; Inflammation; SP-A; Albumin; Metabolomics; Biomarkers

ABSTRACT

Background: There is insufficient knowledge about systemic health effects of exposure to particles emitted from typical indoor sources. In high-income countries, candlelight burning and cooking are major indoor contributors to particle pollution. We examined whether short-term exposure to emissions from cooking and burning candles causes inflammatory changes in young individuals with mild asthma. Thirty-six non-smoking asthmatics participated in a randomized controlled double-blind crossover study attending three exposure sessions (mean $PM_{2.5}$): a) air mixed with emissions from cooking (96.1 $\mu g/m^3$), b) air mixed with emissions from candles (89.8 $\mu g/m^3$), and c) clean filtered air (5.8 $\mu g/m^3$). Particles were generated in an adjacent chamber and let into a full-scale exposure chamber where participants were exposed for five hours. Biomarkers were assessed in relation to airway and systemic inflammatory changes; the primary outcomes of interest were surfactant Protein-A (SP-A) and albumin in droplets in exhaled air – novel biomarkers for changes in the surfactant composition of small airways. Secondary outcomes were cytokines in nasal lavage, and cytokines, C-reactive protein (CRP), epithelial progenitor cells (EPCs), gene expression related to DNA-repair, oxidative stress, and inflammation, as well as metabolomics in blood. Samples were collected before exposure start, right after exposure, and the next morning. *Results*: SP-A in droplets in exhaled air was differently affected by the three exposures, showing almost stable concentrations following candle exposure, while concentrations

decreased following clean air and cooking exposure. Albumin in droplets in exhaled air increased following exposure to cooking and candles compared to clean air exposure, although not significant. We found only weak associations between cooking and candle exposure and systemic inflammation biomarkers, while concentrations of some lipids and lipoproteins in blood increased significantly following exposure to cooking.
Conclusion: Emissions from cooking and candles may affect the small airways and increase concentrations of lipids and lipoproteins in blood among young individuals with asthma. We found only weak associations between the exposures and systemic inflammatory biomarkers. Together, the results suggest existence of mild inflammation following cooking and candle exposure. Candles and cooking induced different effects on health, however, further studies are needed to confirm the findings.

BACKGROUND

Indoor air quality is not well-regulated nor well understood with respect to health effects. This knowledge gap is critical, as people spend up to 90% of their time indoors, and most of that time is spend in their home (1,2). Pollutants of indoor origin such as dust, chemicals, and particulate matter (PM) are of great importance to personal exposure and presumably health (3). Numerous epidemiological studies have found high levels of PM in residences (4–10), with activities contributing to high levels of indoor particulate air pollution including cooking and burning candles (4–7,9). PM is a key pollutant from a health and environmental perspective both indoors and outdoors. Fine ($PM_{2.5}$) and ultrafine ($PM_{0.1}$) particles (UFP) are especially important for our health as they can penetrate into the deepest regions of the lungs and may deposit there (11–13). Due to their small size, high number concentration and large surface area to volume ratio, UFP have unique characteristics, including enhanced ability to enter blood circulation, thereby reaching target organs e.g. the heart and brain posing an even greater risk of systemic health impacts (11–14).
Regular and prolonged exposure to indoor PM may lead to adverse health effects, even at low concentrations, with vulnerable individuals such as children, the elderly and people suffering from respiratory disease, like asthma, being particularly susceptible (15–17). Asthmatics are susceptible to particle exposure due to their chronic inflammation in the respiratory tract (17). To date, little is known about adverse health effects related to exposure to emissions from cooking and candle burning as only a handful of studies assessing short-term health effects

have been conducted. In the published studies on healthy subjects in exposure chambers with candle or cooking emissions, negative effects on lung function (18), cardiovascular outcomes including blood pressure, arterial stiffness and heart rate variability (19–21), and brain activity (22,23) have been demonstrated, though no single effect has been observed in all studies. In a previous publication from the present study, we have reported on changes in nasal mucosa and FeNO-concentrations, and decreasing self-reported well-being following exposure to cooking and candle emissions among subjects with mild asthma (24). In observational and intervention studies, indoor exposure to particles in the fine and ultrafine size range have been associated with systemic inflammatory biomarkers such as declining levels of endothelial progenitor cells, oxidative stress, and release of several cellular mediators, such as cytokines (25–28), all of above mechanisms relevant in the causal pathway to cardiovascular and pulmonary disease (28–30).

Lower airway responses to PM can be assessed by evaluating early biomarkers including Surfactant Protein-A (SP-A) and albumin found in the lining fluid of small airways (31,32). SP-A poses several functions that make it an interesting potential biomarker for inflammation in the small airways (33). Besides contributing to reduced surface tension in the alveoli during respiration, SP-A is a critical component of the respiratory innate immune defence; it is able to opsonize or bind pathogens and other invading micro-organisms to enhance phagocytic removal from the airways (34,35). It may also act as modulator of the immune response (34). Albumin, the most prominent blood protein, is the primary determinant for colloid osmotic pressure in the vascular space and possibly also in lining fluid of the small airways, but it is also suggested as a marker of membrane permeability (36). Changes in concentrations of SP-A and albumin may indicate an inflammatory reaction (35). Levels of SP-A and albumin are typically assessed by bronchoalveolar lavage (BAL) or similar invasive methods, but new technology makes it possible to measure these proteins in droplets in exhaled air (31,32). To date, the measurement of SP-A and albumin in exhaled air has not been studied in relation to exposure to air pollution, but effects on SP-A have been found in an exposure study of second-hand emissions from electronic cigarettes and in a cross-sectional study of smokers (37,38).

Altered levels of serum metabolites e.g. GlycA and cholesterols may be associated with inflammation related to PM exposure (30,39,40). Metabolomics offers valuable insight into the metabolic changes in response to low-dose PM exposure (30,41), and it allows suggestion of hypotheses on mechanisms of toxicity in order to better understand causes of diseases (41).

A recent intervention study showed associations of several serum metabolites with indoor $PM_{2.5}$ exposure (30).

In the present study, the aim was to examine whether short-term respiratory and systemic effects of indoor particle exposure could be observed in a population of young asthmatic volunteers. Information on effects were collected in terms of SP-A and albumin in droplets in exhaled air, cytokines in nasal lavage, and cytokines, C-reactive protein (CRP), Epithelial Progenitor Cells (EPCs), gene expression related to DNA-repair, oxidative stress, and inflammation, as well as metabolomics in blood. The hypothesis tested was that short-term exposure to cooking and candle emissions could induce acute responses in airways and blood.

RESULTS

Results are presented as mean (± SD) unless specified otherwise.

Particle exposure

The detailed characterization of exposure levels has been reported previously (24) and can be found in supplementary files (Table S1). Due to air conditioning, temperature and relative humidity remained nearly constant throughout all exposures (~23°C and ~43%). During candle exposure, levels of CO_2 and NO_2 increased to 915 (± 66) ppm CO_2 and 52.9 (± 1.8) ppb NO_2 compared to CO_2: 629 (± 74) and NO_2: 2.1 (± 0.5) during clean air exposure. During cooking, levels of CO_2 and NO_2 were 542 (± 43) ppm and 6.5 (± 1.8) ppb, respectively. Representative examples of particle characteristics (size and number distribution, volume distribution and temporal evolution of the scattering coefficients) during a cooking, a candle, and a clean air exposure session are shown in our previous publication (24). For the convenience of the reader, mean particle number size distributions from cooking and candle exposures, are shown in Figure 1. During cooking exposures, the average particle mode diameters were in the range 71 (± 39) nm to 87 (± 17) nm. Both particle number concentration and mode diameter varied depending on the timing of the ovens. During candle exposure, the highest number concentration was found for particles with diameters below 10 nm, with the average particle mode diameters in the range 7.0 (± 0.8) nm to 8.0 (± 1.2) nm. Particle emissions were fairly stable during the candle exposures, whereas particle emissions by cooking exhibited more variation over the course of an exposure session.

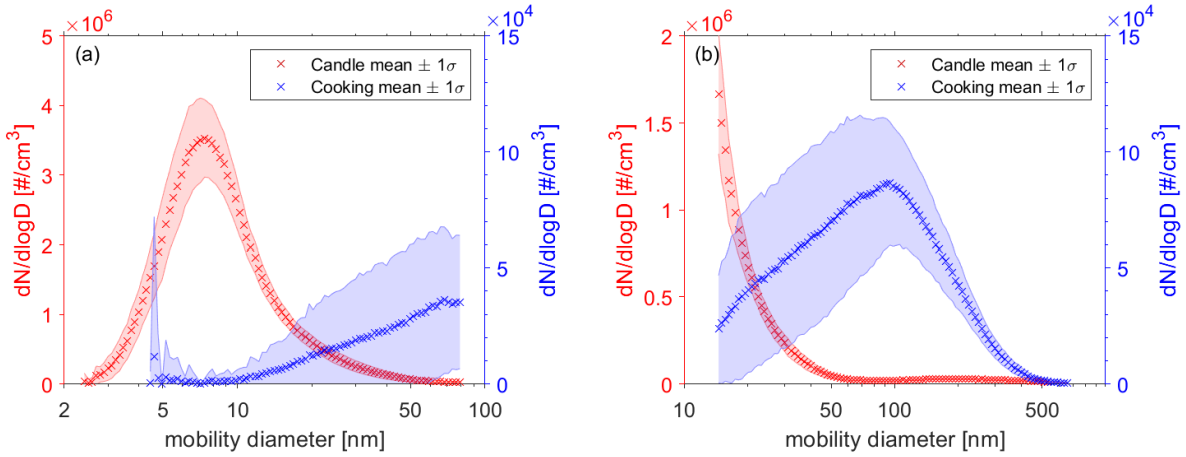

Figure 1. Mean particle number size distributions recorded during cooking (in blue; right y-axis) and candle exposures (in red, left y-axis) as calculated from two cooking experiments carried out on 07.11.19 and 30.10.19 and two candle experiments carried out on 21.11.19 and 05.11.19 using SMPS. (a) Mean particle number size distributions in the size range 2.4 to 79.1 nm (nano DMA). (b) Mean particle number size distributions in the size range 14.6 to 661.2 nm (long DMA). The two different SMPS size intervals were measured in sequence. Notice the different y-axis for the two exposures.

The hygroscopicity of cooking emissions was inconclusive, primarily due to the fact that the particle distributions varied strongly with time. Thus, subsequent measurements of dry and humid distributions were difficult to interpret. As seen from Figure 2, candle emissions in the size range 2.4 to 79.1 nm (nano DMA) showed some growth when exposed to high humidity. The geometric mean of the dry distribution, as calculated from the ten scans before and ten scans after humidification, shifted from 8.6 (± 0.2) nm to 11.1 (± 1.1) nm at humid conditions (mode diameter changed from 7.4 to 9.5 nm). The RH in the humidifier was set to 90% but as the particles had to subsequently travel through the SMPS system it was expected that the RH was slightly lower in the SMPS itself. Figure 2 illustrates candle emission size distributions before, during and after humidification on two exposure days. In order to interpret the data we had to consider that a larger fraction of the small particles would be lost inside the setup including the humidifier. Therefore, we performed calculations with the Particle Loss Calculator (42) that suggest that less than 10% of particles with a diameter of 5 nm would be additionally lost in the expanded setup with humidifier. Thus, we expect the shift to larger sizes, illustrated in Figure 2, to be mainly due to growth of the particles by water uptake rather than loss of small particles in the system. Candle emissions in the larger size ranges did not seem to exert the same hygroscopic growth as observed in the smaller size ranges (Figure 2 b and c).

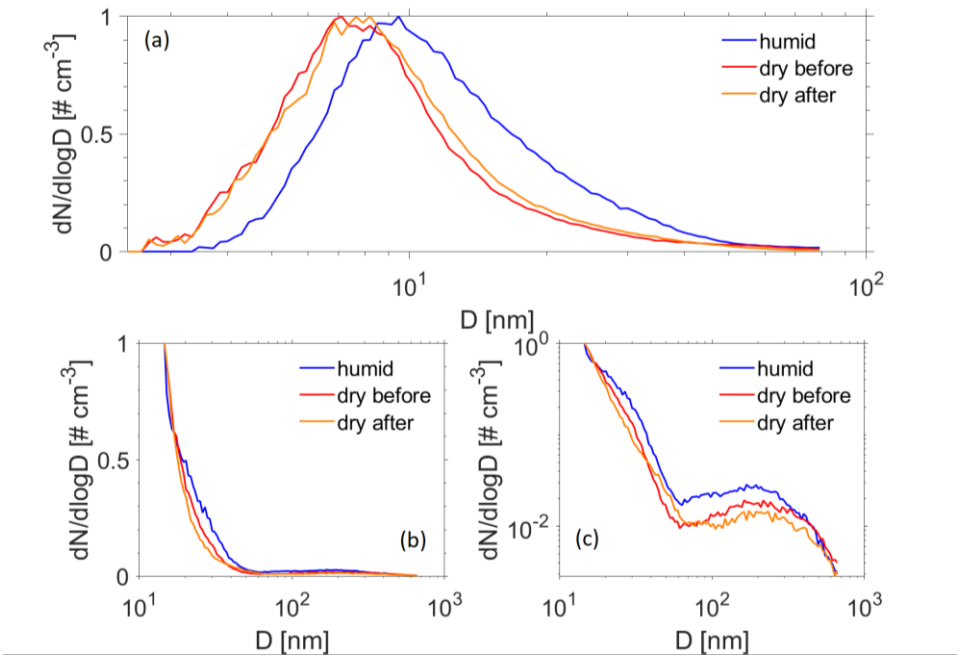

Figure 2. Normalized particle size distributions from candle experiments on 21.11.19 (upper panel) and 05.11.19 (lower panels). Each curve was calculated as the median from 10 scans. The blue lines depicts the humidified distribution (RH ~90%), whereas the red and orange lines show the dry distributions recorded before and after humidification (RH ~43%; conditions in the exposure chamber).

Scanning Electron Microscope images

Figure 3 shows SEM images of filters from a) a cooking exposure, b) a candle exposure and c) an unused reference filter. From b) candle particles and agglomerates down to 20 nm can be observed. We were not able to distinguish the cooking filter sample from the reference filter, thereby not able to see particles from the cooking exposure session. The fibers from the filter itself can be seen clearly in both a) and c).

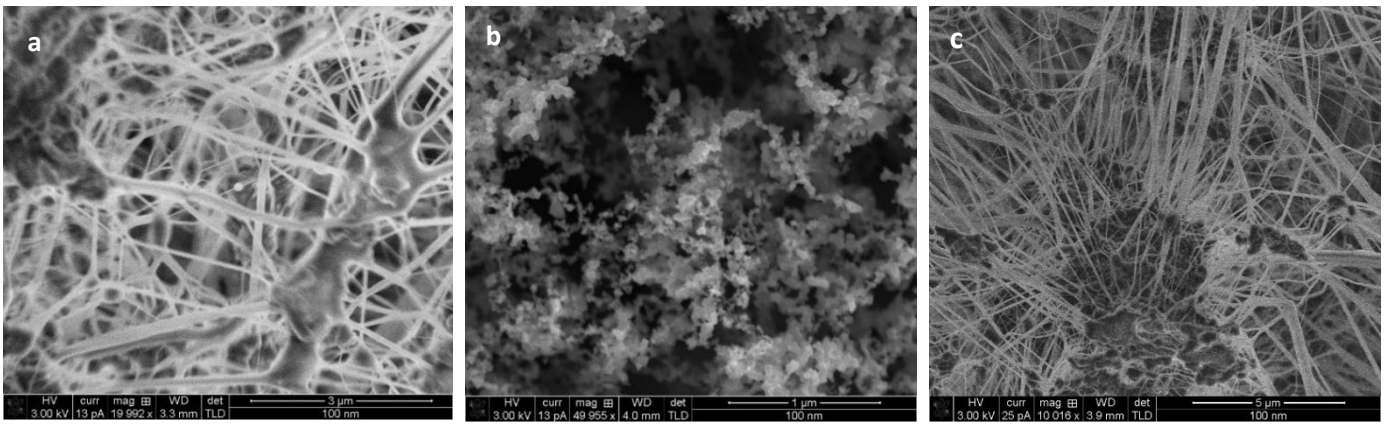

Figure 3. Scanning Electron Microscopy images of filter from a) cooking exposure, b) candle exposure c) and reference filter. Image b) is in 3D. Note that images are shown at different magnifications; a) 20 x 10^3 b) 50 x 10^3 c) 10 x 10^3.

Biomarkers

Table 1 and 2 present the estimated changes following cooking and candle exposures for the included respiratory and systemic biomarkers, respectively. Table 3 presents changes in metabolites and macromolecules. In Tables S2 and S3 unadjusted means and standard deviations for the included biomarkers can be found.

SP-A and albumin in exhaled air: Figure 4 illustrates the adjusted mean change in concentration of SP-A and albumin for the three exposures over time. The level of SP-A was nearly stable over time, when participants were exposure to candles, however, decreasing SP-A concentrations were observed for clean air and cooking five hours after exposure start. Compared to days with clean air exposure, mixed models showed that concentrations of SP-A in the samples was increased following candle exposure (0.31% (95% CI -0.02; 0.63)) (Table 1). The difference between candle and clean air exposure on SP-A was persistent across analyses, but with varying significance (Table 1, S4 and S5). There was no difference between cooking and clean air exposure on SP-A when observing changes following the exposures adjusted for baseline values (Table 1). Exposure to cooking and candles numerically increased the concentration of albumin in samples compared to clean air exposure; cooking: 0.24% (95% CI -0.26; 0.74) and candles: 0.25% (95% CI -0.25; 0.75). The numerical increase in albumin was persistent across analyses (Tables S4 and S5). Albumin/SP-A was 0.08 (95% CI -0.10; 0.25) for cooking and -0.05 (95% CI -0.22; 0.13) for candles.

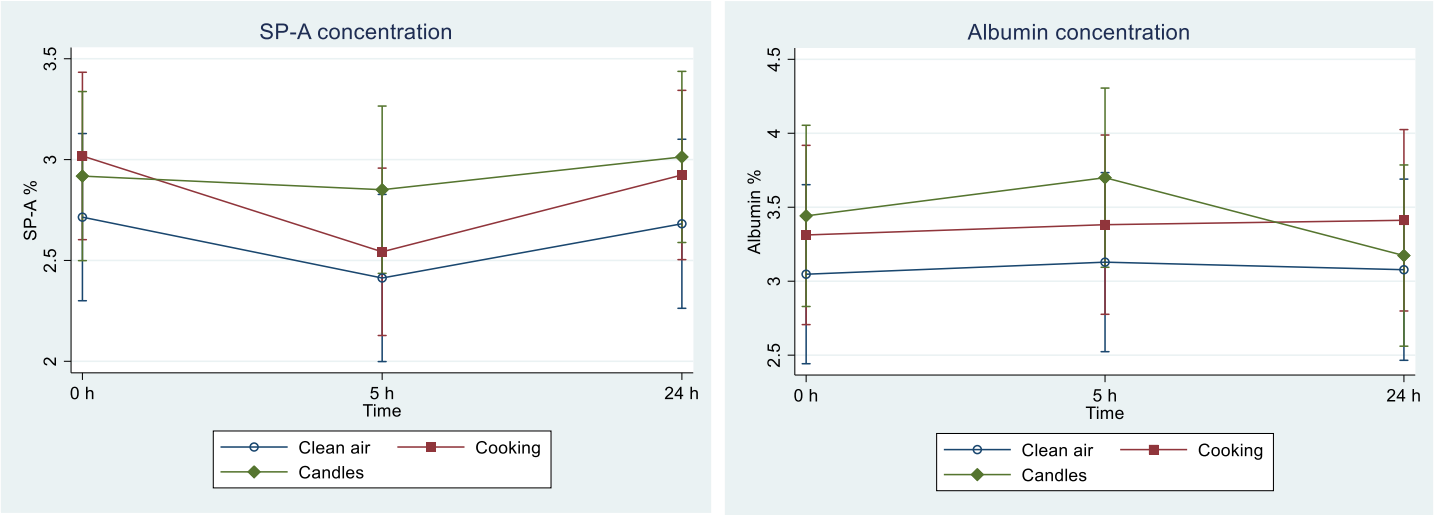

Figure 4. Margins plot of the adjusted mean change in biomarkers in exhaled air (Surfactant Protein-A and albumin) for each of the three exposures (clean air, cooking and candles). Biomarkers were measured before exposure (0 hours), and following exposure corresponding to 5 hours after and 24 hours after exposure start as depicted on the x-axis. SP-A and albumin are reported in % of the sampled material.

Table 1. Mean change (5h-24h) in respiratory outcomes following cooking and candle exposure compared to clean air exposure [†]

	Cooking exposure			Candle exposure		
	Coefficient	95% CI	*p*-value	Coefficient	95% CI	*p*-value
Biomarkers in exhaled air						
SP-A %	0.02	(-0.30; 0.35)	0.888	0.31	(-0.02; 0.63)	0.065
Albumin %	0.24	(-0.26; 0.74)	0.343	0.25	(-0.25; 0.75)	0.325
Albumin/SP-A	0.08	(-0.10; 0.25)	0.243	-0.05	(-0.22; 0.13)	0.591
Nasal lavage biomarkers						
IL-1β	-0.20	(-0.40; -0.01)	0.044*	-0.09	(-0.29; 0.11)	0.370
IL-8	-0.05	(-0.23; 0.14)	0.634	-0.03	(-0.21;0.16)	0.777

Mean changes for nasal lavage biomarkers correspond to differences on logarithmic scale.

[†] Results are from linear mixed models with no interaction. Changes in biomarkers in exhaled air are reported from 5 h to 24 h post exposure adjusted for baseline. For nasal lavage biomarkers no baseline values exist. SP-A and albumin are expressed as weight percent. IL-1β and IL-8 are reported in pg/ml. *Definition of abbreviations*: SP-A = Surfactant Protein-A, IL = interleukin. * The level of significance was assumed at $p < 0.05$.

Nasal lavage biomarkers: We observed a significant decrease in IL-1β from 5h to 24 h following cooking exposure (-0.20 (95% -0.40; -0.01)), but no clear change in IL-1β following candle exposure (-0.09 (95% CI -0.29; 0.11)) compared to clean air exposure (Table 1). No significant differences between the exposures were observed for IL-8.

Cytokines in serum: Several of the measurements were below the lower detection limit. This was true for IL-1β, IL-8, and TNF-α and missing data were excluded from the analyses. The results of the remaining cytokines are presented in Table 2. IL-1β and TNF-α showed a significant or near-significant decline from 5 h to 24 h following cooking and candle exposure compared to clean air exposure (Table 2). No significant association between the exposures and IL-8 was observed. CCL2 increased significantly from 5h to 24 h following candle exposure compared to when exposed to clean air: 18.3 pg/ml (95% CI 3.97; 32.7). There was a significant difference in CCL2 changes following candle vs. cooking exposure, with candles increasing levels of CCL2 significantly more than cooking: 15.2 pg/ml (95% CI 1.12; 29.2) (data not shown).

Table 2. Mean change in systemic inflammation biomarkers following cooking and candle exposure (clean air = reference)[†]

	Cooking exposure			Candle exposure		
	coefficient	95% CI	*p*-value	coefficient	95% CI	*p*-value
Cytokines in serum						
IL-1β	-0.14	(-0.31; 0.02)	0.086	-0.17	(-0.32; -0.01)	0.036*
IL-8	0.14	(-0.70; 0.97)	0.743	0.18	(-0.63;0.99)	0.660
CCL2	3.19	(-11.1; 17.5)	0.660	18.3	(3.97; 32.7)	0.013*
TNF-α	-0.42	(-0.78; -0.06)	0.023*	-0.54	(-0.91; -0.17)	0.004*
C-Reactive Protein						
CRP	0.14	(0.03; 0.25)	0.010*	0.10	-0.01; 0.20	0.075
EPCs						
Early	0.74	(-55.7; 57.2)	0.979	-4.55	(-61.7; 52.6)	0.875
Late	-2.55	(-33.7; 28.6)	0.871	-9.08	(-41.8; 23.7)	0.585
Gene-expression						
IL-8	0.22	(-0.20; 0.63)	0.313	0.39	(-0.03; 0.80)	0.068
CCL2	-0.13	(-0.54; 0.29)	0.554	0.01	(-0.41; 0.42)	0.983
TNF-α	-0.10	(-0.53; 0.33)	0.637	-0.04	(-0.46; 0.39)	0.855
HMOX1	-0.06	(-0.37; 0.25)	0.700	0.01	(-0.30; 0.32)	0.966
OGG1	-0.20	(-0.51; 0.09)	0.175	-0.07	(-0.36; 0.22)	0.645

Mean changes for CRP and Gene-expression correspond to differences on logarithmic scale.

[†] Results are from linear mixed models with no interaction term. Changes are reported from 5 h to 24 h post exposure adjusted for baseline. For cytokines, only CCL2 had complete data; for IL-1β: 167/324, IL-8: 207/324, and TNF-α: 204/324 observations were included in the analyses. *Definition of abbreviations*: CCL2 = C-C motif chemokine ligand 2, EPCs = Endothelial Progenitor Cells. HMOX1 = heme oxygenase (decycling) 1, IL = interleukin, log = natural logarithm of x. TNF-α = tumor necrosis factor α, OGG1 = oxoguanine DNA glycosylase 1. Cytokines in serum are reported in pg/ml. CRP is reported in ng/ml. EPCs are reported in number of endothelial cells per standard unit. * The level of significance was assumed at $p < 0.05$.

C-reactive protein (CRP) in serum: As shown in Table 2, significant differences between cooking and clean air and borderline significant differences between candles and clean air were found for CRP in serum (cooking: 0.14 ng/ml (95% CI: 0.03; 0.25) and candles: 0.10 ng/ml (95% CI: -0.01; 0.20)). Estimates indicated increasing CRP following particle exposure, however, margins plot showed an actual decline in CRP following clean air exposure and almost stable levels for particle exposures.

Endothelial Progenitor Cells (EPCs): Table 2 shows EPC levels stratified by phenotypes; early and late. No significant effect of cooking and candle exposure was observed for neither early nor late EPCs. Linear mixed models supplemented with Student's t-test for changes over time for each exposure showed significant and borderline significant increases for early and late EPCs between 0h and 5h for all exposures suggesting diurnal effects (data not

shown). Sensitivity analyses of samples stratified by dilution, showed no significant associations between the exposures and EPCs (data not shown)

Gene expression: The measured gene expression related to DNA-repair and pro-inflammatory responses did not show any significant variations following cooking or candles exposure (Table 2), except from borderline significant increases in IL-8 following candle exposure (0.39 (95% CI: -0.03; 0.80)). Analyses showed significant variations in time for HMOX1, OGG1 and TNF-α following all exposures with increasing values from 0h to 5h (data not shown).

Metabolomics: From the analysis of NMR data (see Figure S1 for example spectra) several significant peaks in metabolites and macromolecules were observed. In particular, we observed increasing concentrations of lipids and lipoproteins following cooking exposure compared to when participants were exposed to clean air (Table 3). Peaks around ~2 ppm correspond to glycoprotein acetylatoin (GlycA) (40), however, due to the untargeted metabolomics approach, it was not possible to further specify macromolecules. No significant associations were found for candles and metabolites.

Table 3. Mean change in metabolites and macromolecules on days with cooking exposure compared to clean air exposure. Metabolites and macromolecules are shown if ($p \leq 0.03$) †

	Cooking exposure			
Metabolite / macromolecule	**Chemical shift (ppm)**	**Cooking#24 hours**	**95% CI**	***p*-value**
Unsaturated fatty acid =C<u>H</u>	~5.25	-43.50	(-82.37; -4.63)	0.028
Unsaturated fatty acid =CH-C<u>H</u>$_2$	~2.04	94.31	(20.83; 167.80)	0.012
Unsaturated fatty acid =CH-C<u>H</u>$_2$	~2.04	106.02	(30.20; 181.85)	0.006
Unsaturated fatty acid =CH-C<u>H</u>$_2$	~2.04	135.28	(15.57; 255.00)	0.027
Unsaturated fatty acid =CH-C<u>H</u>$_2$	~1.97	99.31	(12.31; 186.30)	0.025
Alanine	~1.45	120.47	(32.22; 208.71)	0.008
Unidentified	~1.45	99.21	(12.72; 185.70)	0.025
Unidentified	~1.45	95.51	(9.81; 181.20)	0.029
Unidentified	~1.45	94.54	(9.27; 179.81)	0.030
Lipid -C<u>H</u>3 (+Valine)	~1.00	107.89	(15.07; 200.71)	0.023
Lipid -C<u>H</u>3 (+Valine)	~0.94	144.14	(14.50; 272.78)	0.028

† Results are derived from linear mixed models of NMR data using Model 1 with interaction between exposure and time.
Definition of abbreviations: ppm = parts per million.

DISCUSSION

To our knowledge, this study represents the first controlled human exposure study of the impact of cooking and candle exposure in subjects with mild asthma. The study performed a detailed characterization of cooking and candle emissions, with physical and hygroscopic growth profiling of the particles within the emissions. We found suggestive evidence that five hour exposure to emissions from cooking and candles, respectively (at $PM_{2.5}$ mass concentrations ~90 $\mu g/m^3$), slightly changed the primary outcome measures, SP-A and albumin in droplets in exhaled air, with SP-A affected differently by the three exposures, and albumin increasing numerically, although not significant. Furthermore, cooking exposure was followed by increased concentrations of some lipids and lipoproteins in the blood. Only weak, no or reducing effects were observed for other secondary outcomes in terms of upper airway and systemic inflammatory biomarkers, EPC levels and gene expression. Serum CRP decreased following clean air exposure.

During cooking exposure, participants were exposed to lower numbers of particles compared to when exposed to candles. The average mode diameter of particles emitted from cooking was ~80 nm, while for candles it was ~7.5 nm. However, many particles were also observed around 80 nm for candles. The modes observed in the present study are similar to those in the literature with candles emitting high number concentrations of ultrafine particles with diameter <10 nm (43–45), and soot mode of particles having a mean diameter of ~270 nm (21,46) corresponding to the second peak mode in the present study. In a comprehensive review of PM from cooking, Abdullahi *et al*. report, that most of the measured particles in the included studies was in the ultrafine size range, with modes reported primarily in the range of 20-100 nm (47). In a similar exposure study with frying sausages, the majority of particles were in the size range of 50-100 nm (20). For candle particles, we observed different behavior of the size ranges with regard to water uptake with smaller particles showing more growth than larger particles when exposed to high humidity. Previous candle emission studies showed that especially the small particle sizes contain considerable amounts of salts, while soot particles govern the larger size ranges (46,48), which might be an explanation of the different particle behavior. Differences between the two exposures regarding particle size and chemical composition of the emissions might explain the difference observed in health effects (13).

When generating the two particle exposures we aimed for the same mass concentration level for the two exposures and across sessions. In the present study, average particle mass

concentrations ($PM_{2.5}$) were 96.1 (± 13.1) µg/m^3 and 89.8 (± 9.3) µg/m^3, for cooking and candles, respectively, comparable to other exposure studies with similar exposures (4,18,22). Likewise, the particle concentrations in our study are comparable with indoor concentrations during daily activities in private homes. When cooking using several cooktops or burning multiple candles, maximum mass concentrations ~300 µg/m^3 (up to 351 µg/m^3) have been found in several observational studies (4,6,8,49).

Lower airway effects were assessed by evaluating novel and early biomarkers from the distal part of the lungs (31,32). SP-A and albumin are abundant proteins in the lung lining fluid that forms an interface between lung epithelial cells and the external environment (50). In the present study we observed different effects on SP-A concentrations following the three exposures, with differences between candle and clean air exposure on SP-A concentrations being significant or borderline significant across statistical analyses. We were not able to establish whether the difference in effects are caused by a decreasing effect of clean air or an increasing effect of candles on SP-A, however, stable levels during candle exposure and recent research on diurnal variation in healthy non-exposed individuals, showing minor increases in SP-A during the day, point to a decreasing effect of clean air exposure on SP-A (51). This may be explained by an increase in respiration rates as a consequence of the particle-free clean air, hence, a greater use of surfactant with the small airways opening and closing more frequently. Decreasing levels of NO in exhaled air has been shown in healthy individuals following particle-free clean air exposure (52), indicating that the airways may be subject to small inflammatory effects during everyday life as a consequence of continuous, minor exposure to pollutants. This may be especially true for individuals with asthma (53). The cause of the observed decrease in SP-A following cooking exposure is unknown. It may be explained by changes in the lung milieu (35), however, further studies are needed. Damage to the small airways may increase the permeability of the blood-air space barrier, leading to the passage of plasma proteins into the airway space and possible leakage of lung proteins out from the airways. This in turn may change the protein content (54). Inflammation in general is associated with a leakage of albumin from the vasculature into the airways (55,56) – a possible explanation for the observed tendencies towards increasing albumin concentrations in the small airways following cooking and candle exposure. When albumin increase in the small airways, interstitial osmotic pressure may be increased (36).

In an exploratory approach in the present study, we found increased levels of several lipoproteins following cooking exposure – a metabolic change, which is commonly observed

following inflammation due to increased apolipoprotein synthesis (57). For all reported metabolites, parallel increases were observed following candle exposure, however, not as pronounced as for cooking, and not significant at $p \leq 0.03$. Had we chosen a significance level < 0.05, more than 100 significant peaks in metabolites were found for cooking exposure when compared to clean air possibly indicating further metabolic changes. However, with ~1000 bins tested, a number of these were likely to be false positives. In a randomized blinded intervention study using air purifiers in dormitories among healthy young adults, high $PM_{2.5}$ exposure was likewise associated with alterations in serum lipid metabolites, indicating an enhancement of lipid metabolism and oxidation (30). The changes in lipids and lipoproteins that occur during inflammation are part of the innate immune response and therefore likely to play an important role in protecting the host (57,58). Evidence shows, that acute inflammation and infection induce various alterations in lipid metabolism, but if the inflammatory response persists it may contribute to increased risk of atherosclerosis (57). We found that significant changes in peaks of unsaturated fatty acids following cooking corresponded to GlycA (40,58), which may be consistent with inflammation. Results from recent observational and interventional studies have demonstrated that GlycA is elevated in acute and chronic inflammation, suggesting GlycA being a marker that tracks systemic inflammation and subclinical vascular inflammation (40,58). Previous results have suggested that GlycA captures systemic inflammation at least as good as CRP (40,58,59). GlycA is a composite biomarker integrating protein levels and glycosylation states of the most abundant acute phase proteins in serum, allowing for a stable measure of inflammation (58).

In general, low concentrations of the different cytokines in nasal lavage fluid were observed. We found slightly lower levels of interleukins after cooking exposure significant for IL-1β. Previous studies show a clear downward shift in concentrations of all cytokines and cells from the first nasal lavage to the subsequent ones (60). In order to avoid this, we refrained from sampling at baseline, since this might have induced artificially lower levels after exposure.

In several studies, enhanced levels of serum cytokines have been used to determine the systemic inflammation level in humans exposed to air pollution (12). Overall, no evident effects in systemic biomarkers (EPCs, gene expression, CRP, and cytokines) were found in the present study. Though, following candle exposure we observed a significant increase in circulating CCL2 (from 5 to 24 hours) indicating continuous and increasing inflammation from baseline. CCL2 recruit cells of the immune system (monocytes, lymphocytes etc.) to the

sites of inflammation produced by tissue injury or infection (61). Inhaled particles can provoke an inflammatory response in the lungs, with consequent release of inflammatory cytokines into circulation – typically including interleukins and TNF-α (12,62). We found borderline significant increases in gene expression related to IL-8 following candle exposure. In contrast, we observed very small, however, decreasing levels of IL-1β and TNF-α following cooking and candle exposure compared to clean air exposure. This might indicate recruitment from the blood of these cytokines into the cell lining as a first response (60). There was a slight increase in some serum cytokines following clean air exposure, which is probably an effect caused by the stay in the exposure chamber. Despite the fact that the exposure order was randomized, baseline values between exposures clearly deviated from each other for some of the outcomes. In the statistical analyses, we therefore had to adjust for baseline values. We have no reasonable explanation for this variation other than a low number of participants. By using a randomized cross-over design, and by preparing participants before taking part in the study, we did everything possible to prevent this variation.

Systemic inflammation may be observed by elevated CRP as found in several cross-sectional studies among children and healthy adults (63), however, in the present study, cooking and candle exposure did not alter CRP levels in serum. CRP decreased following clean air exposure, particularly at 24 hour, which might be explained as an effect of very clean air in the chambers during the clean air sessions compared to standard indoor and ambient air. The air delivered to the chambers were filtered through a series of filters including a final stage with HEPA- and carbon filters. Similarly, in a recent intervention study, air filtration was associated with decreased concentrations of inflammatory markers including CRP (64).

Strengths and limitations

Strengths of the present study were the design including randomization, and double-blinding. Combining this design with a state-of-the-art exposure chamber, in which all conditions other than the exposures were kept constant, eliminates confounding from personal characteristics. In general, controlled human exposure studies, make it possible to separate effects of the specific PM component and size fraction of different combustion sources from effects associated with the complex mixtures of air pollution examined in epidemiological studies (65,66). Confirmed by an "exit poll" among the participants on their final visit, as described previously (24), blinding of candle exposure proved successful, strengthening the results. Contrary, we were only able to blind cooking to investigators, not participants, because of the

smell of roasted pork. Nevertheless, it is implausible that participants' knowledge about the exposure affected the objective measures reported here. All participants have been exposed to the same concentration of particles and gases, as exposure levels were constant throughout and across exposure days. In the present study, particle levels from candles and cooking are comparable to real-life scenarios (4,6,8,49). A particular strength of our study is the thorough exposure characterization performed with several instruments including SMPS giving particle size and number concentrations down to 2.4 nm. For candles in particular this is important, as evidence, including findings from the present study, indicates high number concentrations of particles below 10 nm (43,44). We examined a comprehensive array of biomarkers previously associated with air pollution and from several places in the human body, providing a thorough understanding of how individuals may be affected by indoor particles.

The present study also has limitations. First, exposure to indoor and ambient pollution between days of the experiments might impact the results, as participants were left unattended in their homes with no instructions regarding behaviour except for not using tobacco products and not taking medicine. However, due to the crossover design and randomization of the exposures, activities of participants in the hours and days before the exposure sessions are expected to cause random effects, thereby attenuating the exposure-outcome association. Secondly, in case of delayed effects, the health effects of cooking and candles may have been underestimated in the present study. However, as the exposures are not assumed to be receptor-mediated as e.g. endotoxin showing systemic effects persisting for weeks (67), we do not expect a cascade of inflammation, but instead, general mild inflammation to occur – which might, however, not decrease – within a short amount of time (68). Thirdly, the clinical outcomes might have changed differently, if we had examined candles composed of other materials and/or under other burning conditions. Multiple cooking or other cooking styles most likely would have emitted different profiles of compounds and different levels of PM (47) affecting deposition in the respiratory tract and consequently health reactions (69). Yet, the examined exposures were chosen as being representative for Denmark and other Nordic countries. Fourth, in order to generate similar exposure scenarios across study exposure days, there were some differences to real-life exposure patterns in a common household. In order to reduce uneven emissions from soot and burning fat, we replaced candles before burning down and pork was kept in the turned off oven when finished. Fifth, as individuals with asthma are particularly vulnerable to particle exposure due to chronic inflammation in the respiratory tract, the findings indicating mild inflammatory

responses do not necessarily pertain to the general population. Nevertheless, the results may apply to susceptible individuals such as children, the elderly and other individuals with chronic respiratory disease – also known to be susceptible to PM exposure (70,71). However, as several of our key biomarkers showing possible effects of the exposures (biomarkers in exhaled air as well as GlycA and other lipid metabolites) are new, but promising in relation to air pollution, the interpretation towards actual health effects is difficult.

CONCLUSIONS

In conclusion, the results of this study suggest that emissions from cooking and candles can affect the respiratory system thereby causing a shift in some local and systemic biomarkers in young individuals with asthma, thus, possible pointing to the existence of mild inflammation following cooking and candle exposure. Candles and cooking induced different effects on health, which may be explained by differences in particle size and chemical composition of the emissions. As key findings in the present study are related to novel biomarkers, the findings warrant confirmation in future studies, nevertheless, strategies to reduce indoor particle pollution should be considered to minimize potential disease progression.

METHODS

Details on study design, participants, exposure facilities, exposure generation, and exposure characterization have been described elsewhere (24) and are only briefly described below.

Study design

In short, the study was designed as a randomized, double-blind, controlled crossover exposure experiment. Participants took part in three exposure sessions, each lasting five hours; a) air mixed with emissions from cooking (mean fine particle mass concentration (± SD)) ($PM_{2.5}$: 96.1 (± 13.1) $\mu g/m^3$), b) air mixed with emissions from burning candles ($PM_{2.5}$: 89.8 (± 9.3) $\mu g/m^3$), and c) clean filtered air ($PM_{2.5}$: 5.8 (± 6.8) $\mu g/m^3$). The filtered clean air and particle sessions were identical except for the air quality. Participants were exposed in groups of four with each participant attending all three exposure sessions, with a gap of two weeks between each exposure.

Study population

Non-smoking volunteers with mild asthma were recruited through spreads at local campuses and social media. According to power considerations, we aimed for 36 participants (24). To

be included in the study, participants had to be between 18 and 25 years of age, have a physician diagnosis of mild asthma, and >1 positive skin prick test-reactions towards common allergens. Participants were excluded if they were using tobacco products, were pregnant, or had a medical history of diseases, which could involve a risk for the participant or possibly influence the outcome measures. Thirty-six non-smoking individuals (20 female; 16 male) with mild asthma participated in the study (mean age (± SD): 22.3 (± 1.5) years) (24). For those participants using long-acting asthma medication when included in the study, medication was converted to short-acting medication two weeks prior to participation and throughout the study. Participants had to be without signs of infections or airway symptoms and not have taken steroids for at least one week, or any medicine during the least 48 hours before participating in an exposure session. This was affirmed at a doctor check-up in the morning before each exposure session.

Ethics

The study protocol was approved by the Ethical Committee in Central Denmark Region (ref. no. 1-10-72-345-18) and reported to the Danish Data Protection Agency (journal no. 2016-051-000001/780). The project was conducted in accordance with The Declaration of Helsinki and written informed consent was obtained from all participants.

Exposure facilities and exposure description

Exposure sessions took place in a 72.9 m^3 exposure chamber made of stainless steel, while exposure generation took place in a similar, but smaller adjacent chamber. Because of an established negative pressure of 10 Pa in the large exposure chamber, particles and gases were directed from the adjacent chamber to the large exposure chamber through a 10 meter pipe connection. On days with cooking as exposure, four ovens were placed in the adjacent chamber. One oven at a time was cooking breast of pork (28% fat) at 200°C as prescribed on the packaging. Before the first oven finished cooking the meat, the next oven started and so forth, until the first oven had to start over again with new meat. In total, the four ovens cooked meat five times in order for the exposure to last throughout the exposure session. On exposure days with burning candles, four taper candles and six pillar candles made of 100% stearin were lit and placed on a table. In the chamber, a light circulation of air was made by a wide slow-rotating fan, which made the candles flicker at a slow pace. A big funnel was placed above the table, absorbing emissions from the candles, thereby transferring them into the exposure chamber, where it was mixed with a constant inflow of clean air. During clean air sessions, the adjacent chamber was not in use. In order to maintain a stable exposure level,

different average air exchange rates were applied for the three exposures; (average air exchange (± SD) during cooking: 4.4 h^{-1} (± 0.2); candles: 3.5 h^{-1} (± 0.1); clean air: 2.6 h^{-1} (± 0.4)). Throughout exposure sessions, target temperature was 23°C and relative humidity 45% inside the large exposure chamber. Before the first participant in the group of four entered the exposure chamber, the exposure had been activated for approximately two hours to ensure that the particle concentration had reached the required target concentration. Participants entered the exposure chamber with 30 minutes in between starting their five hour exposure session. During exposure, participants were seated around a desk in a resting position wearing clean-suits to avoid unintended contamination of the air from clothes etc.

Data collection

Exposure characterization

The particle exposure inside the exposure chamber was monitored and characterized during each exposure session from the first person entering the chamber until the last person leaving the chamber. For controlling the exposure level, online monitoring of particle mass was performed by a Dusttrak Aerosol Monitor 8520 equipped with a $PM_{2.5}$ inlet (TSI, St Paul, Minnesota). Particles (PM_{10} and $PM_{2.5}$) were sampled using SKC PTFE filters with PMP Support by means of PM-samplers (SKC PEM 2.5 µm, 2 L/min and ADI PM 2.5 µm & PM 10µm, 10 L/min). Particle size distributions were measured at several exposure sessions using a Scanning Mobility Particle sizer (SMPS) equipped with a nano Differential Mobility Analyzer (DMA) (TSI, 3085) (nano DMA, size range 2.4-79.1 nm) or a long DMA (TSI, 3081) (long DMA, size range 14.6-661.2 nm). The two size intervals were measured in sequence during an exposure session. During short periods of the experiments, a humidifier was placed in front of the SMPS to measure the particle size distribution after exposure to a relative humidity of 90 (± 2) % at the inlet of the SMPS. By comparing the size distributions with and without humidifier, the hygroscopic growth of the poly-disperse particle distribution could be addressed.

Images of SKC PTFE filters ($PM_{2.5}$) from cooking and candle sessions and a reference filter were taken using scanning electron microscopy (JEOL Magellan XHR 400 FE-SEM 3kV nominal current 13pA spot size ~1-1.5nm). Filter samples were imaged without any added conducting coating to prevent changes to the sample materials. It was not possible to apply higher magnification or longer exposure times of filters in the microscopy as this could lead to beam induced damage of the filter material.

Clinical measurements and biomarkers

Prior to (0h), right after (5h) and the morning after (24h) exposure, each participant underwent several health examinations including sampling of exhaled air, nasal lavage and blood. For all outcomes participants served as their own controls. All clinical investigations were timed, so that they were performed at approximately the same time of the day before and after each exposure session. Proteins in droplets in exhaled air, comprising surfactant protein-A and albumin, were the primary outcome of interest in the study. Other outcomes reported in this study are secondary outcomes of interests, why they have to be viewed upon as hypothesis-generating. The effect of cooking and candle exposure on respiratory markers of inflammation and self-reported well-being has been reported elsewhere (24).

SP-A and albumin in exhaled air: Droplets in exhaled air, also termed particles in exhaled air, were collected using the PExA® instrument set-up (31,32), which is a non-invasive method to assess the lining fluid from the distal airways (72). Endogenous particles, formed in the airways, are exhaled and reflect chemical composition of the respiratory tract lining fluid (31). Participants performed repeated breath maneuvers allowing for airway closure and re-opening as described previously (54). The subjects exhaled through a mouthpiece and a two-way, non-rebreathing valve into the thermostated PExA instrument (36°C), containing a Grimm 1.108 optical particle counter and an impactor with a Teflon membrane impaction substrate. Participants inhaled HEPA-filtered air for three breaths before the sampling in order to remove particles originating from ambient air. Participants wore a nose clip throughout the procedure. They were instructed to perform the following standardized breathing maneuvers to allow for airway closure and re-opening: i) exhale fully to residual volume and hold breath for five seconds, ii) inhale rapidly to total lung capacity, iii) exhale to residual volume capacity at a flow of 1000-1500 mL/s. The exhalation flow was shown to the participant on a computer screen. Only the exhalation in (iii) was sampled in the instrument. The maneuver was repeated until 120 ng was collected or a maximum sampling time of 30 minutes was reached, with normal tidal breathing in-between. After collection, the Teflon membrane was immediately transferred to a low-binding Eppendorf polypropylene vial and stored at -80°C until analysis (73). Samples were analyzed for SP-A and albumin using mass spectrometry. Details on the instrument and analysis have been described elsewhere (54). Four of 324 samples were excluded from the statistical analyses, as they were contaminated with saliva, detected by extremely high levels of albumin. Results are reported as weight percent, herein % of the sampled material.

Cytokines in nasal lavage: Participants were sitting with a fully flexed neck when sampling nasal lavage. Through a nasal cork plug attached to a syringe, 5 mL of 0.9% sterile saline water (~37°C) was injected into one nostril. The saline water was kept in the nasopharyngeal region for 30 seconds followed by collection of the fluid in a cup. The lavage was then repeated in the other nostril. The first nasal lavage sample (flush from the right and left nostril) was collected after exposure (5h) and then again at follow up (24h). No baseline sample was performed to avoid "cleaning" the nasal cavity prior to exposure. Each nasal lavage sample was transferred to a vial, 30mM DDT was added with the amount of fluid determined by differential weighing, and the sample was separated into a pellet and the supernatant. The supernatant samples were kept on ice during processing (approximately 15 minutes), following centrifuge (10 minutes at 755 g and 4°C). Supernatant samples (2x1 mL per sample) were stored in cryo-tubes at -80°C until analysis. The supernatant samples were analyzed for interleukin-1β (IL-1β) and interleukin-8 (IL-8) using Magnetic Luminex Performance assay (R&D Systems, Minneapolis, MN). 100 µL undiluted sample and 25 µL of a suspension of capture-antibody-conjugated beads were mixed in plate wells. After three hours of incubation, the beads were washed three times and subsequently reacted for 1.5 hours with a 50 µL mixture of biotin antibody cocktail detection antibodies. 50 µL of streptavidin-phycoerythrin was added to the wells and the incubation was continued for an additional 30 minutes. Finally, the beads were washed three times and re-suspended in 100 µL buffer and analyzed on the Luminex® MAGPIX platform using xMAP technology. All samples were measured in duplicate. Results are reported in pg/ml.

Blood samples: Four mL of peripheral venous blood was sampled in K_2-tubes (BD Vacutainer®, Denmark) containing EDTA as anticoagulant for endothelial progenitor cells (EPC). Next, 8 mL blood used for measurement of gene expression was collected in CPT vials (BD Vacutainer®, Denmark). Finally, for analyses of cytokines, CRP and metabolomics, 10 mL blood was sampled in SST advance tubes (BD Vacutainer®, Denmark). A Safety-Lok™ blood collection set (BD Vacutainer®, Denmark) was applied. Following gradient centrifugation, the peripheral blood mononuclear cells (PBMCs) for measurement of gene expression were stored at -80°C in freezing medium containing 50% fetal bovine serum (GibcoRBL), 40% RPMI-1640 medium, and 10% dimethyl sulfoxide. Samples for cytokines, CRP and metabolomics were stored at room temperature for 20 minutes before centrifuged (15 minutes at 755 g and 4°C). Serum blood was transferred to

three 1.8 mL micro tubes (Sarstedt, Nümbrecht) before being stored at -80°C until analysis. The samples underwent different procedures as described below.

Cytokines in serum: After thawing, serum samples (2 x 1.8 mL) were analyzed using Magnetic Luminex® Performance assay (R&D Systems, Minneapolis, MN). A portion of 50 µL standard undiluted sample and 50 µL diluted microparticle cocktail were mixed in plate wells. After three hours of incubation on a microplate shaker (800 rpm, room temperature), samples were washed three times using a magnetic device for microplates. 50 µL Biotin-Antibody Cocktail was added to each well following incubation for one hour in a microplate shaker (800 rpm). Subsequently, samples were washed three times. Streptavidin-phycoerythrin (50 µL) was added to each well and the incubation was continued for an additional 30 minutes. Finally, the beads were washed and resuspended in 100 µL wash buffer, following incubation for two minutes at room temperature on a microplate shaker (800 rpm). Within 90 minutes the samples were analyzed on the Luminex® MAGPIX platform using xMAP technology. All samples were measured in duplicate. The concentration was measured for Tumor Necrosis Factor-α (TNF-α), C-C motif chemokine ligand 2 (CCL2), IL-1β, and IL-8. Results are reported in pg/ml.

C-reactive protein (CRP): Serum samples were analysed using Quantikine® ELIZA kit, Human C-Reactive protein (R&D Systems, Minneapolis, MN). 50 µL undiluted sample was diluted 1:50-1:400 dependent on CRP levels in the sample. 50 µL standard and diluted samples and 100 µL of Assay dilutent were mixed in plate wells, following incubation for two hours at room temperature. Subsequently, samples were washed four times. 200 µL of Human CRP Conjugate was added to each plate well, then incubated for two hours and washed once. 200 µL substrate solution was added to plate wells and incubated 30 minutes while protected from light. 50 µL stop solution was added to each well. The optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm (Walvelenght correction was set to 570 nm) using GENS software. All samples were measured in duplicate. Results are reported in ng/ml.

Endothelial Progenitor Cells (EPCs): Fresh EDTA blood from the participants (4 mL) was analyzed 24 hours after exposure start. Thus, blood from before, 5 h after and 24 h after exposure start was analyzed at the same time for one exposure session. The blood had been stored at 5ºC until analyses. The collected blood samples were analyzed for EPCs using polychromatic flow-cytometry, defining EPCs as events within the leukocyte gate with a

$CD34^{+}KDR^{+}$ antigenic profile expressed as per cent EPCs per leukocyte, as described by Jantzen *et al*. (74). We further used the presence or absence of the differential progenitor marker $CD133^{+/-}$ to separate the EPCs into early or late subpopulations, respectively, as the surface marker CD133 expressed in EPCs upon release into circulation is lost upon maturation allowing discrimination between early or late EPCs (75). Blood samples (1 mL) from the study participants were hemolysed with Ammonium Chloride buffer at RT in the dark for 20 minutes and centrifuged (10 minutes at 400 g). The supernatant was discarded and the remaining 100 µL cells were stained with CD133 BV480 (1 µL, BD Catalog NR 747562), CD34 PerCP Cy5.5 (20 µL, BD Catalog No. 347222) and CD309 PE (20 µL, BD Catalog no. 560494) and 30 µL Brilliant Violet binding buffer (BD Catalog no. 563794) in a master mix (15 minutes, 25ºC, dark). The samples were diluted to 2 mL, and aliquoted in 500 µL onto a 98-well deep-well plate. The samples were acquired at 500 µL/minute with an Attune Flow Cytometer from Thermo Fisher with a threshold set on violet Forward Scatter. Leukocytes were gated on a SS:FS scatter plot, and CD34+ cells were gated on a SS:CD34 plot. $CD309^{+}$ cells were divided into $CD133^{+}$ (early) and $CD133^{-}$ cells (late), avoiding neutrophil background. With an Attune Flow Cytometer, all cells in 1 mL blood were processed and equivalent fractions of the samples was compared. For the first 36 of 324 samples, the dilution factor was double (4 mL). Accordingly, these were analyzed separately in sensitivity analysis. Results are reported in number of endothelial cells per standard unit (1 mL).

Gene expression: The expression of the genes related to DNA repair (oxoguanine DNA glycosylase 1 (OGG1), GenBank sequence accession ID: 4968)) and oxidative stress (heme oxygenase (decycling) 1 (HMOX1), Gene ID: 3262), as well as genes related to inflammation interleukin 8 (IL-8, Gene ID: 3576), TNF-α (Gene ID: 7124), and chemokine (C-C motif) ligand 2 (CCL2, Gene ID: 6347) were analyzed in PBMCs. Total RNA was isolated using Direct-zolTM RNA MiniPrep kit (Zymo Research, Irvine, CA, USA), which included a DNase I treatment. The PBMCs diluted in freezing-medium was centrifuged (10 minutes at 400 g and 4°C), and the TRI Reagent was added to the precipitate, as stated in the protocol for biological liquids. The quantitative PCR reactions were carried out in ABI PRISM 7900HT (Applied Biosystems), using probes and primers from Applied Biosystems. The assay IDs for the genes were as follows: CCL2, Hs00234140_m1; IL6, Hs00985641_m1; IL8, s00174103_m1; TNF, Hs00174128_m1; HMOX1, Hs00157965_m1; OGG1, Hs01114116_gl. The 18S rRNA was used as a reference gene (Eukaryotic 18S rRNA

Endogenous Control, 4352930E, Applied Biosystems). The PCR reactions were performed as described by Jensen *et al*. (76). The level of gene expression is reported as the ratio between the level of the target gene and the 18S rRNA reference gene using the comparative $2^{-\Delta Ct}$ method.

Metabolomics: Nuclear Magnetic Resonance (NMR) spectroscopy was used for metabolomics. Frozen samples of serum blood were thawed and 1 mL was transferred to SampleJet NMR tubes (Bruker®, Karlsruhe, Germany). A small amount of paramagnetic gadoteridol 'contrast solution' was added in order to guarantee the quantitative response of the NMR spectrometer (77), to a final concentration of 0.3 mM. NMR samples received at the NMR facility were kept at 6°C. Time to experimentation varied from 4 to 24 hours as samples were automatically taken from 96-tube racks in succession. NMR analyses were done on a Bruker 500MHz spectrometer, equipped with a SampleJet automatic sample changer, using 5mm sample tubes. All measurements were done at 310K (37°C) and automation was run from the Bruker IconNMR module. In order to ease comparison of intensities of all spectra the autogain option (rga) was disabled and all experiments were recorded with oversampling and a receiver gain of 90.5. All samples placed in the SampleJet were kept cooled at 6°C. Drying and heating was done for 60 seconds prior to loading samples into the magnet core to prevent condensed air on the tubes. Once the sample was positioned the temperature was equilibrated for 120 seconds until the temperature stability was better than 0.2 K, followed by automatic shimming and tune/match. The time spent on each sample change totaled 5 minutes. Each 1D proton spectrum measurement (experiment NOESYGPPR1D) consisted of 4 dummy scans, 96 scans, with 1 s relaxation delay between scans and 1 s for signal acquisition. Total acquisition time per sample was three and a half minutes. 1D-NOESY NMR spectra were pre-processed in parallel in TopSpin 4.0.9, with a small line broadening of 0.3 Hz, a phase correction, water peak removal, and a spline-corrected baseline correction. Following the pre-processing, all data was gathered in a matrix using *nmrglue* (78) prior to spectral alignment with *Icoshift* (79). The alignment was performed with an initial co-shift of 0.004 ppm following a squared average alignment of manually defined bins surrounding the critical areas in the spectra. Furthermore, all spectra were referenced to the glucose peak at 5.22 ppm. Following the alignment, all spectra were binned and integrated in two regions, namely from 9.60 ppm to 5.16 ppm and 4.30 ppm to -0.500 ppm, in bin sizes of 25 points (≈0.009 ppm), giving rise to a total of 1007 bins, which

were Pareto scaled for each sample. With that, the dataset of 1007 variables for each sample were used in further statistical analysis as described below.

Statistics

We used linear mixed models based on the univariate repeated measurement analysis of variance (ANOVA) to evaluate the change in health outcomes between clean air and candles and cooking, respectively. The models included the outcome of interest, and as fixed effects exposure, time, exposure-order, day, and time-exposure interaction. As a random effect we included participant ID. Time was divided into baseline (0 hours), five hours, and 24 hours, exposure was clean air, candles or cooking, order was corresponding to the order the participant received the exposure at, while day indicated whether the exposure took place on participants' first, second or third day. The statistical measures of interest were the exposure and time-exposure interaction as an effect of any of these terms would indicate a difference associated with the exposure. We initially fitted a model with interaction (Model 1). For models where the interaction term was not statistically significant, the interaction term was left out and instead we examined mean change in the outcomes following the three exposures (5 h to 24 h) adjusted for baseline values (0 h) (Model 2). In case of non-normal distributions, analyses were performed on log-transformed outcome variables. This was true for cytokines in nasal lavage, serum CRP, and gene expression. Before conducting the statistical analyses on the 1007 metabolomics bins, we decided to use a false-discovery rate of $p \leq 0.03$ in order to keep spurious findings low, but still enabling explorative analyses. For other outcomes, the level of significance was assumed at $p < 0.05$. All statistical analyses were performed using Stata 17 software (StataCorp, College Station, Tex).

Abbreviations

CCL2 = C-C motif chemokine ligand 2, CRP = C-reactive protein, CO_2 = carbon dioxide, EPC = endothelial progenitor cells, GlycA = glycoprotein acetylation, HMOX1 = heme oxygenase (decycling) 1 gene, IL-8 = interleukin-8, IL-1β = interleukin-1β, ng = nanogram, nm = nanometer, NMR = Nuclear Magnetic Resonance, NO_2 = nitrogen dioxide, OGG1 = oxoguanine DNA glycosylase 1, PExA = Particles in Exhaled Air, pg = picogram, PM = particulate matter, ppb = parts per billion, ppm = parts per million, SP-A = Surfactant Protein A, TNF-α = Tumor necrosis factor-α, UFP = ultrafine particles, µg = microgram, µL = microliters.

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Conflicts of Interest

Anna-Carin Olin was one of the inventors of the PExA-method and is a board member and shareholder in PExA AB. No potential competing interests was reported by the other authors.

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SUPPLEMENTARY FILES

Airway and systemic inflammation biomarkers after short-term exposure to indoor ultrafine particles – A randomized controlled double-blind crossover study among mild asthmatic subjects

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Table S1. Characterization of the environmental exposures in the large exposure chamber for clean air, cooking and candles exposure (climate and air quality factors) described by means and standard deviations (SD)

Measurement	**Unit**	**Clean air**	**Cooking**	**Candles**
Number of sessions, *N*		10	11	11
Temperature	°C	22.9 ± (0.2)	22.9 ± (0.2)	23.1 ± (0.2)
Humidity	RH%	43.8 ± (1.2)	43.1 ± (1.0)	43.2 ± (0.7)
CO_2	ppm	629 ± (74)	542 ± (43)	915 ± (66)
NO_2[†]	ppb	2.1 ± (0.5)	6.5 ± (1.8)	52.9 ± (1.8)
$PM_{2.5}$	$\mu g/m^3$	5.8 ± (6.8)	96.1 ± (13.1)	89.8 ± (9.3)
PM_{10}	$\mu g/m^3$	3.0 ± (1.0)	97.2 ± (11.7)	91.4 ± (7.6)
Total particle number conc. (2.4-79.1 nm)[‡]	$\#/cm^3$	1.1×10^3 (1.2×10^3)[a]	5.9×10^3 (6.5×10^3)[b]	1.7×10^6 (1.8×10^5)[c]
Total particle number conc. (14.6-661.2 nm)[‡]	$\#/cm^3$	8.8×10^2 (3.4×10^2)[a]	7.2×10^4 (2.5×10^4)[b]	3.7×10^5 (1.3×10^5)[c]

Definition of abbreviations: CO_2 = Carbon Dioxide, NO_2 = Nitrogen dioxide, PM = Particulate Matter. Conc. = Concentration. Clean air exposure: Mean PM_{10} has a smaller mass than $PM_{2.5}$ due to instability in collection of particles. [†] Mean NO_2 might be underestimated for all exposures as the instrument (API Chemiluminescent NO_2 analyser model 200 A) had an off-set about 22% at the end of the study, which happened gradually during the trial. [‡] Total particle number concentrations are SMPS average values for the total of the measured time intervals. The explanation for some SDs being higher than the mean is fluctuations in particle number concentration over time and between sessions. [a] Average of two sessions [b] Average of three sessions. [c] Average of four sessions.

Table S2. Unadjusted means and standard deviations (SD) for biomarkers at baseline (0h) and after exposure (5h and 24h).

	Clean air			Cooking			Candles		
Outcome	0h	5h	24h	0h	5h	24h	0h	5h	24h
Biomarkers in exhaled air									
SP-A %	2.73 (1.4)	2.43 (1.1)	2.71 (1.2)	2.98 (1.4)	2.51 (1.1)	2.86 (1.4)	2.96 (1.2)	2.87 (1.1)	3.04 (1.4)
Albumin %	3.04 (2.0)	3.12 (1.6)	3.07 (1.7)	3.29 (1.4)	3.36 (1.5)	3.36 (2.0)	3.47 (2.4)	3.72 (2.0)	3.21 (2.1)
Albumin/SP-A	1.11 (0.5)	1.31 (0.5)	1.16 (0.5)	1.26 (0.6)	1.40 (0.6)	1.31 (0.9)	1.24 (0.8)	1.37 (0.6)	1.08 (0.4)
Nasal lavage biomarkers									
IL-1β	-	30.1 (38.7)	27.3 (31.2)	-	27.6 (36.9)	23.5 (32.0)	-	25.0 (27.2)	27.3 (37.6)
IL-8	-	173 (127)	157 (107)	-	180 (156)	162 (128)	-	177 (125)	170 (170)
Cytokines in serum									
IL-1β	0.50 (0.4)	0.60 (0.7)	0.65 (0.5)	0.59 (0.5)	0.53 (0.3)	0.45 (0.4)	0.47 (0.4)	0.41 (0.3)	0.42 (0.3)
IL-8	3.04 (2.4)	2.83 (2.0)	3.12 (2.1)	4.23 (3.3)	4.08 (3.7)	3.11 (2.4)	3.43 (2.3)	4.22 (3.5)	4.06 (2.2)
CCL2	84.3 (67.8)	71.4 (58.8)	82.6 (66.5)	106 (69.2)	92.8 (74.5)	92.0 (69.5)	118 (81.9)	107 (67.6)	120 (67.3)
TNF-α	2.71 (1.4)	2.80 (1.4)	3.31 (1.6)	3.21 (1.5)	2.62 (1.5)	2.66 (1.3)	3.12 (1.8)	2.82 (1.6)	3.03 (1.6)
C-Reactive Protein									
CRP	1907 (2730)	1690 (2482)	1509 (2167)	1943 (2619)	1937 (2583)	1896 (2713)	2173 (2748)	2187 (2973)	2053 (2936)
EPCs									
Early	632 (265)	742 (330)	693 (334)	448 (194)	545 (254)	520 (224)	409 (218)	513 (279)	463 (265)
Late	360 (132)	425 (155)	395 (159)	309 (101)	367 (121)	348 (100)	257 (112)	317 (151)	287 (160)
Gene-expression									
IL-8	1.01×10^{-6}	4.43×10^{-7}	6.59×10^{-7}	4.08×10^{-7}	5.95×10^{-7}	4.45×10^{-7}	1.11×10^{-6}	5.76×10^{-7}	2.05×10^{-6}
	(2.0×10^{-6})	(7.0×10^{-7})	(2.2×10^{-6})	(5.4×10^{-7})	(1.1×10^{-6})	(7.7×10^{-7})	(3.5×10^{-6})	(1.5×10^{-6})	(7.1×10^{-6})
CCL2	4.21×10^{-7}	4.88×10^{-7}	2.20×10^{-7}	2.82×10^{-7}	3.33×10^{-7}	4.42×10^{-7}	1.71×10^{-7}	2.27×10^{-7}	9.91×10^{-7}
	(9.7×10^{-7})	(1.2×10^{-6})	(2.5×10^{-7})	(3.4×10^{-7})	(3.9×10^{-7})	(1.4×10^{-6})	(2.2×10^{-7})	(3.8×10^{-7})	(3.4×10^{-6})
TNF-α	2.39×10^{-6}	6.85×10^{-6}	3.40×10^{-6}	2.70×10^{-6}	6.70×10^{-6}	3.21×10^{-6}	3.14×10^{-6}	2.43×10^{-3}	3.21×10^{-4}
	(2.8×10^{-6})	(1.4×10^{-5})	(4.4×10^{-6})	(2.9×10^{-6})	(1.8×10^{-5})	(2.3×10^{-6})	(5.6×10^{-6})	(1.5×10^{-2})	(1.9×10^{-3})
HMOX1	1.84×10^{-5}	3.05×10^{-5}	1.81×10^{-5}	1.47×10^{-5}	1.72×10^{-5}	1.58×10^{-5}	1.68×10^{-5}	7.66×10^{-5}	4.66×10^{-5}
	(2.0×10^{-5})	(6.8×10^{-5})	(2.4×10^{-5})	(1.4×10^{-5})	(1.5×10^{-5})	(1.3×10^{-5})	(1.5×10^{-5})	(2.7×10^{-4})	(1.9×10^{-4})
OGG1	6.35×10^{-6}	1.66×10^{-5}	9.24×10^{-6}	6.33×10^{-6}	1.15×10^{-5}	6.85×10^{-6}	6.43×10^{-6}	2.28×10^{-5}	3.66×10^{-5}
	(6.4×10^{-6})	(4.3×10^{-5})	(1.2×10^{-5})	(4.2×10^{-6})	(1.5×10^{-5})	(5.2×10^{-6})	(5.4×10^{-6})	(7.5×10^{-5})	(1.7×10^{-5})

Definition of abbreviations: CCL2 = C-C motif chemokine ligand 2, EPCs = Endothelial Progenitor Cells. HMOX1 = heme oxygenase (decycling) 1, IL = interleukin, TNF-α = tumor necrosis factor α, OGG1 = oxoguanine DNA glycosylase 1. Cytokines nasal lavage and serum are reported in pg/ml. CRP is reported in ng/ml. EPCs are reported in number of endothelial cells per standard unit.

Table S3. Unadjusted means and standard deviations (SD) for metabolites and macromolecules at baseline (0h) and after exposure (5h and 24h).

	Chemical shift	**Clean air**			**Cooking**			**Candles**		
Metabolites and macromolecules	ppm	0h	5h	24h	0h	5h	24h	0h	5h	24h
Unsaturated fatty acid =C<u>H</u>	~5.25	-1210 (69.9)	-1174 (78.6)	-1173 (75.1)	-1192 (80.7)	-1178 (66.8)	-1198 (77.6)	-1190 (76.2)	-1154 (102)	-1190 (67.3)
Unsaturated fatty acid =CH-C<u>H</u>$_2$	~2.04	3330 (167)	3321 (206)	3260 (176)	3311 (185)	3320 (157)	3335 (169)	3308 (170)	3286 (158)	3299 (152)
Unsaturated fatty acid =CH-C<u>H</u>$_2$	~2.04	3962 (169)	4019 (226)	3883 (156)	3939 (169)	4008 (157)	3965 (166)	3940 (181)	3979 (168)	3921 (159)
Unsaturated fatty acid =CH-C<u>H</u>$_2$	~2.04	5660 (273)	5711 (355)	5574 (267)	5634 (292)	5715 (286)	5683 (281)	5652 (312)	5669 (303)	5644 (281)
Unsaturated fatty acid =CH-C<u>H</u>$_2$	~1.97	4676 (167)	4690 (222)	4583 (168)	4632 (199)	4674 (183)	4637 (157)	4666 (222)	4691 (263)	4658 (189)
Alanine	~1.45	3692 (187)	3668 (214)	3622 (207)	3686 (193)	3666 (182)	3737 (203)	3679 (198)	3630 (202)	3705 (206)
Unidentified	~1.45	3263 (152)	3179 (200)	3187 (195)	3243 (184)	3188 (154)	3267 (168)	3255 (159)	3164 (199)	3255 (149)
Unidentified	~1.45	3145 (151)	3056 (197)	3068 (193)	3126 (184)	3068 (150)	3144 (164)	3132 (156)	3040 (195)	3131 (148)
Unidentified	~1.45	3096 (154)	3013 (198)	3014 (190)	3077 (184)	3025 (151)	3089 (162)	3078 (157)	2992 (193)	3075 (149)
Lipid -C<u>H</u>3 (+Valine)	~1.00	3778 (152)	3649 (224)	3692 (179)	3743 (188)	3661 (162)	3765 (185)	3772 (171)	3660 (229)	3763 (156)
Lipid -C<u>H</u>3 (+Valine)	~0.94	5626 (202)	5480 (324)	5518 (243)	5588 (233)	5486 (236)	5624 (222)	5630 (236)	5496 (305)	5611 (214)

Definition of abbreviations: ppm = parts per million.

Table S4. Mean change (0h, 5h, and 24 h) in biomarkers in exhaled air on days with cooking and candle exposure compared to clean air exposure [†]

	Cooking exposure			**Candle exposure**		
Biomarker	Coefficient	95% CI	*p*-value	Coefficient	95% CI	*p*-value
SP-A %	0.23	(-0.06; 0.51)	0.125	0.33	(0.04; 0.61)	0.027*
Albumin %	0.28	(-0.15; 0.72)	0.202	0.36	(-0.08; 0.79)	0.109
Albumin/SP-A	0.11	(-0.03; 0.25)	0.124	0.04	(-0.11; 0.18)	0.627

[†] Results are from linear mixed models with no interaction term. SP-A and albumin are expressed as weight percent. *Definition of abbreviations*: SP-A = Surfactant Protein-A. * The level of significance was assumed at $p < 0.05$.

Table S5. Mean change (5h-24 h) in biomarkers in exhaled air following cooking and candle exposure compared to clean air exposure [†]

	Cooking exposure			**Candle exposure**		
Biomarker	Coefficient	95% CI	*p*-value	Coefficient	95% CI	*p*-value
SP-A %	0.18	(-0.18; 0.54)	0.322	0.40	(0.04; 0.76)	0.032*
Albumin %	0.32	(-0.20; 0.84)	0.228	0.35	(-0.17; 0.87)	0.184
Albumin/SP-A	0.10	(-0.07; 0.27)	0.243	-0.02	(-0.19; 0.15)	0.821

[†] Results are from linear mixed models with no interaction term and no adjustment for baseline values. SP-A and albumin are expressed as weight percent. *Definition of abbreviations*: SP-A = Surfactant Protein-A. * The level of significance was assumed at $p < 0.05$.

Figure S1

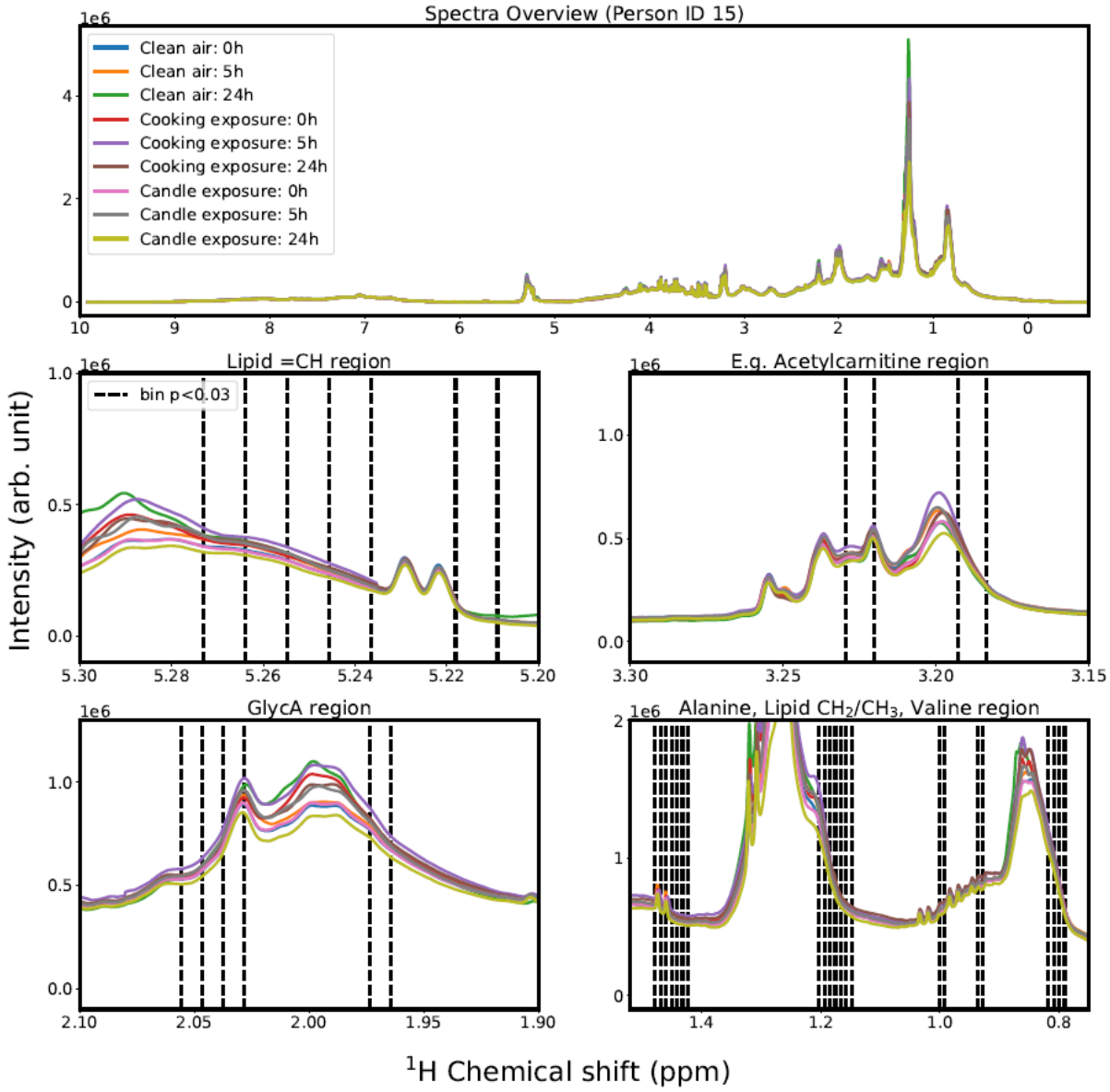

Figure S1. Spectra overview of example NMR dataset of person with ID 15. Interesting regions are shown in subfigures where significant changes of metabolites and macromolecules were observed between exposure to clean air and exposure to candle / cooking (exact bins lie between dotted black lines).

Co-authorship declarations

AARHUS UNIVERSITY

Declaration of co-authorship concerning article for PhD dissertations

Full name of the PhD student: Karin Rosenkilde Laursen

This declaration concerns the following article/manuscript:

Title:	An RCT of acute health effects in COPD-patients after passive vape exposure from e-cigarettes
Authors:	Karin R. Laursen, Jakob H. Bønløkke, Elisabeth Bendstrup, Merete Bilde, Marianne Glasius, Vibeke H. Gutzke, Shamjad P. Moosakutty, Anna-Carin Olin, Peter Ravn, Kirsten Østergaard, Torben Sigsgaard

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If accepted or submitted, state journal:

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E. No or little contribution (<10%)
F. N/A

Category of contribution	Extent (A-F)
The conception or design of the work:	C
Free text description of PhD student's contribution ***(mandatory)*** The study was designed in overall terms, when KRL began writing her PhD-protocol, however, she helped in designing the details.	
The acquisition, analysis, or interpretation of data:	A
Free text description of PhD student's contribution ***(mandatory)*** KRL was responsible for conducting the study, she analyzed the health outcomes	
Drafting the manuscript:	A

Free text description of PhD student's contribution ***(mandatory)*** KRL wrote the first draft of the article. Co-authors commented and KRL revised the manuscript.	
Submission process including revisions:	A
Free text description of PhD student's contribution ***(mandatory)*** KRL submitted and revised the manuscript.	

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Title:	Acute health effects from exposure to indoor ultrafine particles – A randomized controlled crossover study among young mild asthmatics
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D. Has contributed (10-33 %)
E. No or little contribution (<10%)
F. N/A

Category of contribution	Extent (A-F)
The conception or design of the work:	B
Free text description of PhD student's contribution ***(mandatory)*** KRL helped design the study.	
The acquisition, analysis, or interpretation of data:	A
Free text description of PhD student's contribution ***(mandatory)*** KRL was responsible for conducting the study (acquisition of data), and she analyzed and interpreted the health outcomes	
Drafting the manuscript:	A

Free text description of PhD student's contribution ***(mandatory)*** KRL wrote the first draft of the article. Co-authors commented and KRL revised the manuscript.	
Submission process including revisions:	A
Free text description of PhD student's contribution ***(mandatory)*** KRL submitted and revised the manuscript.	

Signatures of first- and last author, and main supervisor

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Declaration of co-authorship concerning article for PhD dissertations

Full name of the PhD student: Karin Rosenkilde Laursen

This declaration concerns the following article/manuscript:

Title:	Airway and systemic inflammation biomarkers after short-term exposure to indoor ultrafine particles – A randomized controlled double-blind crossover study among mild asthmatic subjects
Authors:	Karin Rosenkilde Laursen, The Climate Chamber Group, Nichlas Vous Christensen, Frans AA Mulder, Jörg Schullehner, Hans Jürgen Hoffmann, Annie Jensen, Peter Møller, Steffen Loft, Anna-Carin Olin, Bernadette Rosati, Merete Bilde, Marianne Glasius, Torben Sigsgaard

The article/manuscript is: Published ☐ Accepted ☐ Submitted ☐ In preperation ☒

If published, state full reference:

If accepted or submitted, state journal:

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No ☒ Yes ☐ If yes, give details:

Your contribution

Please rate (A-F) your contribution to the elements of this article/manuscript, **and** elaborate on your rating in the free text section below.

A. Has essentially done all the work (>90%)
B. Has done most of the work (67-90 %)
C. Has contributed considerably (34-66 %)
D. Has contributed (10-33 %)
E. No or little contribution (<10%)
F. N/A

Category of contribution	Extent (A-F)
The conception or design of the work:	B
Free text description of PhD student's contribution ***(mandatory)*** KRL helped design the study.	
The acquisition, analysis, or interpretation of data:	A
Free text description of PhD student's contribution ***(mandatory)*** KRL was responsible for conducting the study (acquisition of data), and she analyzed and interpreted the health outcomes	
Drafting the manuscript:	A
Free text description of PhD student's contribution ***(mandatory)*** KRL wrote the first draft of the article. Co-authors commented and KRL revised the manuscript.	

Submission process including revisions:	A
Free text description of PhD student's contribution ***(mandatory)*** KRL will be responsible for submitting and revising the manuscript.	

Signatures of first- and last author, and main supervisor

Date	Name	Signature
26/7 2021	Karin Rosenkilde Laursen	Karin R. Laursen
26/7 2021	Torben Sigsgaard	TSigsgaard
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Date: 26/7 2021

Karin R. Laursen

Signature of the PhD student