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# Short-term associations between barbecue fumes and respiratory health in young adults

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ABSTRACT

*Background:* Epidemiological studies have associated biomass combustion with (respiratory) morbidity and mortality, primarily in indoor settings. Barbecuing results in high outdoor air pollution exposures, but the health effects are unknown.

*Objective:* The objective was to investigate short-term changes in respiratory health in healthy adults, associated with exposure to barbecue fumes.

*Methods:* 16 healthy, adult volunteers were exposed to barbecue smoke in outdoor air in rest during 1.5 h, using a repeated-measures design. Major air pollutants were monitored on-site, including particulate matter <2.5 μm ($PM_{2.5}$), particle number concentrations (PNC) and black- and brown carbon. At the same place and time-of-day, subjects participated in a control session, during which they were not exposed to barbecue smoke. Before and immediately after all sessions lung function was measured. Before, immediately after, 4- and 18 h post-sessions nasal expression levels of interleukin (IL)-8, IL6 and Tumor Necrosis Factor alpha (TNFα) were determined in nasal swabs, using quantitative polymerase chain reaction. Associations between major air pollutants, lung function and inflammatory markers were assessed using mixed linear regression models.

*Results:* High $PM_{2.5}$ levels and PNCs were observed during barbecue sessions, with averages ranging from 553 to 1062 μg/$m^3$ and 109,000–463,000 pt/$cm^3$, respectively. Average black- and brown carbon levels ranged between 4.1-13.0 and 5.0–16.2 μg/$m^3$. A 1000 μg/$m^3$ increase in $PM_{2.5}$ was associated with 2.37 (0.97, 4.67) and 2.21 (0.98, 5.00) times higher expression of IL8, immediately- and 18 h after exposure. No associations were found between air pollutants and lung function, or the expression of IL6 or TNFα.

*Discussion:* Short-term exposure to air pollutants emitted from barbecuing was associated with a mild respiratory response in healthy young adults, including prolonged increase in nasal IL8 without a change in lung function and other measured inflammatory markers. The results might indicate prolonged respiratory inflammation, due to short-term exposure to barbecue fumes.

## 1. Introduction

According to the World Health Organization, ambient air pollution caused an estimated 4.2 million premature deaths globally in 2016. Of these, 1.5 million deaths were caused by smoke emitted from biomass burning (Black, 2010; World Health Organization, 2016). Epidemiological studies have associated inhalation of biomass smoke from various sources (wood smoke, burning of animal dung or (char)coal) with respiratory and cardiovascular morbidity (*e.g.* aggravation of asthma, respiratory symptoms, increases in hospital admissions) and increased mortality due to cardiovascular, respiratory diseases and lung cancer (Kelly and Fussell, 2015; Sigsgaard et al., 2015; World Health Organization, 2013). Especially, fine particles have been identified as an important factor in these associations.

Barbecuing is a very popular year-round event throughout the world. It is experienced as a culinary and social get together. Johnson (2009) estimated that annually over 30.000 tons of charcoal is used as fuel for barbecues in England only (Johnson, 2009). However, by a large group of people, including elderly and people with respiratory diseases, the smoke is experienced as a source of nuisance. Nuisance may occur when

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people barbecue in public places such as parks, campsites or private gardens, with little natural ventilation or small distances to neighbours. Few studies have assessed air pollution exposures from barbecuing. A study executed by Wu et al. (2015) assessed multiple aerodynamic size fractions of particulate matter at various distances from an outdoor barbecue vendor stall in a Chinese city. They observed very high concentrations of both particulate matter smaller than 10 μm ($PM_{10}$) and 2.5 μm ($PM_{2.5}$) of around 2400 μg/m$^3$ at 2 m from the barbecue stall (Wu et al., 2015). Additionally, a study executed by Badyda et al. (2017) observed high average $PM_{2.5}$ levels in barbecue emissions 1.5 m above the barbecue, ranging between 1000 and 2700 μg/m$^3$ (Badyda et al., 2017).

While a small number of studies characterized air pollutant levels emitted from barbecue fumes, health effects of exposure to barbecue smoke have not been studied yet. Short-term health effects of wood smoke have been studied using human controlled exposure studies (Barregard et al., 2006; Bølling et al., 2009; Sällsten et al., 2006; Sehlstedt et al., 2010; Stockfelt et al., 2012). In these studies, humans are exposed in an exposure chamber, in which a comparison is typically made between a fixed exposure of several hundred μg/m$^3$ particulate matter and a filtered air exposure. Health effects investigated include respiratory and systemic effects such as changes in lung function and increases in biomarker levels. Overall, these wood smoke studies observed weak to mild irritations and relatively small increases in biomarkers of inflammation, including clara cell secretory protein-16, glutathione and malondialdehyde.

The immune system has been shown to respond to particulate matter in general, with increases of various so-called inflammatory markers, in particularly cytokines such as interleukin (IL)-8, −6, −1 and tumor necrosis factor alpha (TNFα) (Kelly and Fussell, 2015; Russell and Brunekreef, 2009). These cytokines and chemokines may subsequently recruit inflammatory cells that are considered to play a key role in particle-induced adverse health effects, which is predominantly based on animal-, in vitro and human volunteer studies (Kelly and Fussell, 2015). Air pollutants may induce adverse health effects indirectly, via inflammatory cells that participate in the generation of cellular stress, but particles may also directly induce cellular stress (N. Li et al., 2003; Møller et al., 2010; Steenhof et al., 2011). The objective of the current study was to investigate the acute changes in lung function and inflammatory biomarkers in healthy adults related to short-term exposure to barbecue fumes.

## 2. Methods

### 2.1. Study design

The current study used a repeated-measures design, which was built upon the design of the RAPTES (Risk of Airborne Particles: A Toxicological-Epidemiological Hybrid Study) project. RAPTES was designed to look at the short-term effects of particle composition on respiratory function in healthy, human volunteers (Strak et al., 2011). The present study included 16 healthy, non-smoking volunteers. Each subject participated in two sessions: one control- and one barbecue session. It was decided to investigate healthy subjects, because during a pilot study high air pollutant levels were monitored at 2 m downwind from the barbecue ($PM_{2.5}$ 320–1000 μg/m$^3$; Particle Number Concentrations ~190,000 pt/cm$^3$). It was considered unethical to expose subjects with chronic respiratory diseases to these high exposure levels, as we were not able to fully anticipate the size of potential health effects. The experiments were carried out in 8 sessions of 3–5 volunteers and the order of the sessions was randomized. Furthermore, the sessions were all performed at the same time-of-day and were separated by at least one week, to avoid potential carry-over effects. The sessions were performed at the outskirts of the University campus, located just outside of the city of Utrecht, several hundred meters away from the main campus roads. A priori it was decided that the sessions should take place with no rain, and a windspeed lower than 3 Beaufort (bft; 5.4 m/s). All 8 sessions were carried out in the months September and October 2019, between 12:00 and 13:30 p.m.

During the barbecue sessions, the volunteers sat at a distance of 2–2.5 m from the barbecue. The distance from the barbecue was based on the potential exposure levels measured during the pilot study. During all sessions the volunteers were seated around a small table in a meadow (Figure A). For 90 min the volunteers were exposed to the barbecue fumes, including the starting-up phase (lighting the charcoal) and the grilling phase (grilling three types of meat). During control sessions volunteers were not exposed to the barbecue fumes. The barbecue was placed downwind from the volunteers, at a minimum distance of 200 m. During all sessions, the volunteers were allowed to freely consume the grilled meat and food, including bread with garlic-butter, salad, water, soft-drinks and cocktail- and garlic sauce. The volunteers were instructed to note down how much and what kind of meat they ate during the first session. The volunteers were requested to eat the same amount and types of meat during the second session.

Both before and directly after each session, the volunteers filled out a questionnaire regarding basic health parameters and lung function measurements were performed. Furthermore, before, immediately after, 4 h and 18 h after each session, epithelial cells were collected using nasal swabs. Total RNA was extracted from these swabs and multiple inflammatory markers were examined using quantitative polymerase chain reaction (QPCR). Changes in the inflammatory marker levels were assessed from the nasal mucosa, because the consumption of food and drinks are known to influence potential analyses executed on saliva. More details regarding collection and measurement of the various health parameters, is described below ('Study Parameters'). Finally, from approximately 1 h before, until 1 h after the sessions, levels of relevant air pollutants were monitored on-site. To enable easy handling and translocation of the various monitors together with the volunteers, two sets of custom-made backpacks, henceforward referred to as measurement units, were filled with each three monitors, and located as close as possible to the volunteers. The monitors continuously measured $PM_{2.5}$, particle number concentrations (PNC), black- and brown carbon concentrations.

### 2.2. Study population

Sixteen non-smoking, healthy adults with ages ranging from 18 to 41 years participated in this study. They were recruited by means of flyers distributed at the Utrecht university campus, by word of mouth, social media and online platforms, including Intranet: a platform used to reach out to Utrecht university employees. Volunteers interested in participating were instructed to complete a screening questionnaire on the in- and exclusion criteria. Exclusion criteria were: being pregnant, living outside Utrecht city, being a smoker or living in a household with a smoker and having objections to eating meat.. Volunteers were offered the possibility to consume the grilled meat, because attendees of a barbecue would normally consume the meat as well. This also stimulated recruitment of subjects. Furthermore, individuals who had used anti-inflammatory medication in the last 12 months, or were diagnosed with atopy, asthma, cardiovascular disease or COPD, were excluded. Volunteers were provided with the necessary information regarding the objective and procedures of the study before signing the informed consent form. To ensure reliable measurements, volunteers were instructed not to participate in barbecues or campfires seven days before each session and not to engage in heavy physical activity or consume any alcohol the day before each session. The study was approved by the Utrecht Medical Ethics review committee (METC, protocol number: 19/237).

### 2.3. Barbecue smoke generation and characterization

Barbecue smoke was generated identically in all experiments. The

experiments were performed outdoors. A charcoal barbecue, with a diameter of 47 cm, was used. All appliances were thoroughly cleaned before each session. The meat was purchased at a local supermarket and consisted of chicken blocks (2% fat), which were marinated in Conimex Ketjap Manis for 5 h before loading them onto skewers. Furthermore, beef burgers (20% fat) and seasoned pork lard-cutlets (28% fat) were used. These three types of meat represent typical barbecue meat choices in The Netherlands.

The barbecue sessions were divided into two phases: the starting up-phase and the 'grilling of the meat' phase. Starting-up phase: 1.5 kg charcoal was weighted and put into a briquettes-starter. Two firelighters were put in the barbecue and lighted using one match. Next, the briquettes-starter filled with the charcoal blocks were put over the firelighters. After 20 min the charcoal was transferred out of the briquettes-starter into the barbecue, after which the second phase started: the grilling of the meat. First the chicken skewers, next the hamburgers and lastly the lard cutlets were grilled. This particular order of increasing fat content was chosen to minimize air pollutant influences from one to the other meat, based on the expectation that higher fat content would contribute to higher air pollutant levels. Each round of meat was put on the barbecue for approximately 10–15 min and turned regularly by the researchers for even heating.

### 2.4. Air pollutant monitoring

Starting at least 1 h before, until at least 1 h after the sessions, $PM_{2.5}$, PNC and black carbon levels were measured using the following monitors respectively: SidePak-AM520 (TSI Incorporated, Minnesota, The United States of America (USA)), MiniDiSC Diffusion Charger (Testo, West Chester, USA) and microAeth®/MA200 aethalometer (Aethlabs, San Francisco, USA). The MiniDiSC is able to monitor particles with a size between 10 and 300 nm from $10^3$ – $10^6$ pt/cm$^3$. MA200 aethalometer detection limit is 50–100 ng/m$^3$. The SidePak is able to monitor particles with a size between 0.1 and 10 μm, from 0.001 to 100 mg/m$^3$. The $PM_{2.5}$ and PNC measurements were performed at a resolution of one per second, while black carbon levels were monitored with a time resolution of 60 s and a flow of 50 mL/min. The DualSpot® loading compensation method was used, which corrects for optical loading effects and enables sampling at various wavelengths: >880 nm (infrared radiation, IR) is interpreted as black carbon (BC) and <375 nm (ultraviolet, UV) as brown carbon (BrC) (Madueño et al., 2019). Two measurement units each contained these three monitors. The inlet tubes were placed close together to reduce measurement variation.

Before and after each session, zero checks were performed using a HEPA filter. The measurement units were placed at a height of 1.5 m, to correspond with the breathing height of the subjects. One measurement unit was placed in front of the volunteers, at 2 m from the barbecue, and one measurement unit at 2.5 m from the barbecue, behind the volunteers. Each volunteer was personally assigned to the values measured closest to him/her. Furthermore, hourly weather conditions were collected from 10 until 15 h from the weather station at the Bilt, the Royal Netherlands Meteorological Institute (Koninklijk Nederlands Meteorologisch Instituut, 2019), located about 3 km from the study site.

### 2.5. Health outcomes

#### 2.5.1. Lung function

Two trained technicians conducted lung function tests according to the American Thoracic Society/European Respiratory Society guidelines of 2014 (ATS/ERS) (Graham et al., 2019). The same technician conducted the lung function measurements both before and after each session, to prevent technician bias. One and the same spirometer (Easy-on-PC spirometer; NDD Medical Technologies; Zurich, Switzerland) was used throughout the study period. On each measuring day, calibration of the spirometer was checked using the 'linear calibration' method available via NDD's software (Lode Holding, ProCare). At least four forced expiratory manoeuvres were obtained per person, while subjects sat upright. The best values from the technically correct manoeuvres were selected according to the ATS/ERS criteria (2014). The manoeuvres were evaluated by a certified respiratory technician, with vast experience in pulmonary function testing. The parameters included in the final analyses were: Forced Expiratory Volume in 1-s ($FEV_1$), Forced Vital Capacity (FVC), Peak Expiratory Force (PEF) and Maximal Mid-Expiratory Flow (MMEF).

#### 2.5.2. Inflammatory marker analyses

*2.5.2.1. RNA isolation.* Nasal swabbing was executed four times in each session: before, immediately after, 4 h and 18 h post session. The nasal swab protocol was based upon procedures used in the PIAMA (prevention and incidence of asthma and mite allergy) birth cohort study (Wijga et al., 2013). A CytoSoft Brush (Cyto-Pak CytoSoft Brush, Medical Packaging, Camarillo USA) was used to collect epithelial cells from the inferior turbinate from the right nostril by trained personnel. However, if technical reasons (*e.g.* septal deviation or nose piercing) prevented swabbing the right nostril, the left nostril was consistently used instead. The brush was cut with scissors cleaned thoroughly with Ethanol-70% and RNAse ZAP and stored in an Eppendorf tube filed with 1 mL TRIzol RNA Isolation Reagent (Invitrogen, Thermo Fisher Scientific Inc., Massachusetts, USA). Subsequently, the brushes were stored at −80 °C until further processing. Total RNA was isolated using a phenol-chloroform extraction method using RNA Instapure (Eurogenetic, Liege, Belgium). Purity and concentration of the RNA samples were determined using a Nanodrop-1000 spectrophotometer (Invitrogen, Thermo Fisher Scientific Inc., Massachusetts, USA) at an absorbance wavelength of 260/280 nm and 230/260 nm. Next, the isolated RNA samples were stored at −80 °C for further analysis using QPCR (Desjardins and Conklin, 2010).

### 2.6. Gene expression with QPCR

Isolated RNA samples were first reverse-transcribed into 30 ng/μL complementary DNA (cDNA), with an iScript cDNA synthesis kit (Bio-Rad, Veenendaal, The Netherlands) and thermal cycler (Biometra, Westburg BV, Leusden) following manufacturer's instructions. Obtained cDNA was diluted 10 times and stored at −20 °C until further analysis. QPCR was performed for quantitative assessment of gene expression with a CFX-connector and iCycler Thermocycler (Bio-Rad, Veenendaal, The Netherlands) using SYBR Green (Invitrogen, Thermo Fisher Scientific Inc., Massachusetts, USA). All genes analysed and their corresponding primers are presented in Supplement Table A ('QPCR primers'). QPCR was performed in concordance to a 2-step-amplification protocol with melting curve analyses. QPCR reaction was initiated by heating at 95 °C for 3 min, after which 45 cycles with denaturation at 95 °C for 15 s followed and annealing/extension at 58 °C for 45 s. The specificity of amplification and absence of primer dimers was confirmed after each run by performing a melt curve analysis using MyiQ Software system (Bio-Rad Laboratories Inc., California, USA). A negative control sample was included in each individual run. Mean normalized expression values for each inflammatory gene were calculated as Ct values of genes of interest, relative to the average of the reference housekeeping genes Glyceraldehyde 3-phosphate dehydrogenase and β-Actin (fold change), using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008; Vandesompele, 2008).

#### 2.6.1. Respiratory symptoms

Before and after the sessions, the volunteers were asked to fill out a short questionnaire regarding hours of physical activity (cycling), current health status, (passive) smoking and (on-demand) medication use of the day before, and the day of the barbecue session. Furthermore, information was obtained regarding the occurrence and severity of various symptoms including cough, runny nose, eye- and throat irritations

(Supplement Questionnaire C, 'Questionnaire basic health symptoms'). The researchers included severity on a more detailed scale than simple YES/NO, as subjects tend to under-report mild symptoms on a YES/NO scale. Therefore, a questionnaire was used to record the severity of the symptoms using a 0–3 scale, with 0 meaning 'no symptoms' and 3 meaning 'severe symptoms'.

### 2.7. Data analyses

#### 2.7.1. Air pollution exposures

All air pollutant measurement data were averaged to 1 min data. The MiniDiSC data were processed using a *java tool*, to obtain the PNCs according to the manufacturer's specifications (Fierz, 2010). Next, the average concentration before, during and after each session, per air pollutant was determined. The barbecue sessions were divided into start-up phase and the subsequent grilling of the three meat types (grilling phase). During some of the control sessions, negative values were monitored using the MA200-Aethalometer. The researchers could not find the reason, nor a solution for this problem. Smoothing or averaging the monitoring data did not solve the issue. Consequently, the original negative values were used to assess the associations between BC (UV and IR) and the various health parameters. Furthermore, during one control and one barbecue session no PNC and BC-IR were monitored, because of equipment failure. To execute data analyses taking the missing data into account, the method of mean substitution was used, substituting missing data by the average of PNC and BC-IR control or barbecue sessions. The rationale for this choice was the small number of observations and the large difference in air pollution between barbecue and control sessions.

#### 2.7.2. Air pollution and health

Only data from subjects who completed both sessions were included in the analyses. The various lung function parameters, the nasal inflammatory markers and air pollutants were all measured on a continuous scale. The averaged air pollutant levels were matched at a personal level with the health measurements of each person. Next, associations between the various air pollution concentrations (independent variable) and the differences in lung function parameters and inflammatory markers (normalized fold change) between post- and pre-session were determined using linear regression. Data of the inflammatory markers were right-skewed and transformed logarithmically before analyses, to reduce outliers and improve normality. We also analysed, as independent variable, whether a session was an exposed or control session, instead of the actual measured air pollution concentration during the session.

To account for the influence of repeated observations per volunteer, mixed linear regression models were used, by specifying the subjects ID-number as a random factor (clustering). To adjust for potential confounding, models were adjusted for confounding factors determined a priori, based on previous studies of short-term air pollution exposure (Analitis et al., 2018; Gualtieri et al., 2010; Steenhof et al., 2015; Strak et al., 2012). Both the lung function measurements and the inflammatory marker analyses were adjusted for continuous environmental factors including temperature and relative humidity.

Sensitivity analysis included assessing the impact of influential observations on the estimated association, by comparing effect estimates with and without observations determined using a Cook's Distance test, with a cut-off value of 1. Furthermore, for the lung function measurements, the models were additionally adjusted for potential technician effects, having a cold (YES/NO) and alcohol consumption the day before the session (YES/NO). For the inflammatory marker analyses, as additional confounding factors having a cold (YES/NO) and alcohol consumption (YES/NO) were assessed.

Effect estimates, their 95% confidence intervals (CI) and p values are presented as the differences in health for an increment approximately equal to the average exposure during the barbecue sessions. A p value equal or smaller than 0.05 was considered statistically significant. Analyses were performed using R software (version 3.6.2). Packages used during the analysis included lme 4, lmerTest, ggplot 2, tidyr, readxl, xlsx, xfun, readr, reshape, knitr, (r)markdown, reshape 2, aggregate, scales and gridExtra.

## 3. Results

### 3.1. Descriptive statistics

In total sixteen volunteers participated with both the exposure and the control barbecues and were consequently included in the final analyses. Study population characteristics are displayed in Table 1. The volunteers (10 females and 6 males) had a mean age of 29 years and included 3 former smokers, who quit smoking at least 2 years ago.

### 3.2. Exposure assessment

#### 3.2.1. Air pollutant measurements

The averaged air pollutant concentrations and weather parameters per control- and exposure session can be found in Table 2. A more detailed overview of the averaged monitored levels per time point (total, background, start-up phase and three different types of meat) can be found in Supplement Table B ('Overview air pollutant levels'). Fig. 1 displays the temporal variation of 1-min values of the air pollutants monitored during the first barbecue session at 2 and 2.5 m from the barbecue.

Individual session average $PM_{2.5}$ levels and PNCs during the four control sessions ranged from 2 to 40 μg/m$^3$ and from 4000 to 10,000 pt/cm$^3$, whereas during the exposure sessions the $PM_{2.5}$ levels and PNCs ranged from 401 to 1062 μg/m$^3$ and 92,000 to 463,000 pt/cm$^3$, respectively (Table 2). BC-IR and BC-UV control levels varied from −0.1 to 2.6 μg/m$^3$ and -0.8 to 0.4 μg/m$^3$, compared with levels ranging between 3.2 and 13.0 μg/m$^3$ and 4.6–17.5 μg/m$^3$, respectively, during the exposure sessions. The average exposure levels monitored during the four barbecue sessions were substantially higher compared with the levels measured during the control sessions. The ratio of exposure to background levels was for $PM_{2.5}$, PNC and BC (IR and UV) approximately 21.0–32.0x, 27.9–51.3x and 22.0–32.3x. The air pollutant levels during each exposure session were much higher compared with the background levels measured, before and after each session, whereas the levels monitored during the control sessions were similar to values measured before and after. The 0.5 m extra distance from the barbecue (comparing in front and at the back of the volunteers) resulted in substantially lower air pollutant levels, *i.e.* a drop of about 40%. High variability was observed between the average exposure levels across the

**Table 1**
*Descriptive characteristics of study population at baseline (t = 0).*

| Subjects characteristics | n (%) or mean (min, max) Baseline (t = 0) |
|---|---|
| Subjects (% women) | 16 (63%) |
| Age (yrs) | 29 (19, 41) |
| BMI (kg/m$^2$) | 25 (20, 36) |
| Former smokers >2 yrs | 3 (19%) |
| Nasal allergy (incl. hay fever) | 2 (13%) |
| Education (HBO/WO) | 16 (100%) |
| Lung function | |
| FEV1 (L) | 3.79 (2.35, 4.92) |
| FVC (L) | 4.69 (2.72, 6.51) |
| MMEF (Ls$^{-1}$) | 3.78 (2.05, 5.34) |
| PEF (Ls$^{-1}$) | 9.39 (5.46, 12.21) |
| Nasal Inflammatory markers | |
| IL8 (ΔCq) | 2.35 (−2.16, 7.57) |
| IL6 (ΔCq) | 13.02 (8.63, 16.05) |
| TNFα (ΔCq) | 11.05 (12.83, 8.94) |

Note: Lung function and nasal markers were average of the two pre-exposure measurements. Abbreviations: BMI, body mass index, Cq, quantification cycle.

**Table 2**
*Averaged air pollutant concentrations and weather parameters* per *exposure- and control session (Exposure YES) at 2 and 2.5 (barbecue session 1) and 3 and 3.5 m (barbecue sessions 2 – 4) from the barbecue.*

| Exposure (YES/NO) | Session | Date | $PM_{2.5}$ (μg/m$^3$) ±SD | | PNC (*pt**10$^3$/*cm*$^3$) ±SD | | BC IR (μg/m$^3$) ±SD | | BC UV (μg/m$^3$) ±SD | | Wind-speed (m/s) ±SD | Temp (°C) ±SD | RH (%) ±SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2/3 Meters | 2.5/3.5 Meters | 2/3 Meters | 2.5/3.5 Meters | 2/3 Meters | 2.5/3.5 Meters | 2/3 Meters | 2.5/3.5 Meters | | | |
| NO | 1 | 09/09/'19 | 40 ± 4 | 40 ± 4 | 4 ± 0 | 5 ± 1 | 2.4 ± 1.3 | −0.1 ± 0.8 | 0.4 ± 0.9 | −0.8 ± 0.6 | 4.0 ± 0.0 | 18.2 ± 0.5 | 73 ± 4 |
| YES | | 23/09/'19 | 1062 ± 43 | 401 ± 5 | 383 ± 149 | 92 ± 40 | 13.0 ± 1.5 | 4.7 ± 0.7 | 17.5 ± 1.5 | 6.1 ± 0.4 | 2.3 ± 5.8 | 17.1 ± 0.3 | 53 ± 2 |
| NO | 2 | 11/09/'19 | 40 ± 4 | 39 ± 4 | 4 ± 0 | 5 ± 1 | 2.6 ± 1.4 | −0.1 ± 0.8 | 0.6 ± 1.3 | −0.8 ± 0.6 | 5.0 ± 0.0 | 15.6 ± 0.4 | 88 ± 8 |
| YES | | 25/09/'19 | 710 ± 55 | 428 ± 42 | 278 ± 29 | 151 ± 15 | 4.1 ± 1.5 | 3.2 ± 0.4 | 5.0 ± 0.9 | 4.6 ± 0.8 | 4.7 ± 5.8 | 16.7 ± 0.2 | 75 ± 2 |
| NO | 3 | 18/09/'19 | 18 ± 8 | 16 ± 8 | 10 ± 5 | 10 ± 5 | NA | 0.5 ± 0.4 | 0.5 ± 0.5 | 0.3 ± 0.4 | 2.7 ± 5.8 | 15.7 ± 0.8 | 56 ± 7 |
| YES | | 09/10/'19 | 553 ± 66 | 532 ± 25 | 109 ± 49 | 109 ± 71 | NA | NA | NA | NA | 5.0 ± 0.0 | 13.1 ± 0.4 | 72 ± 3 |
| NO | 4 | 03/10/'19 | 5 ± 1 | 4 ± 1 | NA | NA | 0.5 ± 0.7 | 0.2 ± 0.4 | 0.2 ± 0.5 | 0.1 ± 0.5 | 1.7 ± 5.8 | 13.6 ± 1.0 | 73 ± 2 |
| YES | | 18/10/'19 | 1044 ± 45 | 730 ± 39 | 463 ± 32 | 425 ± 158 | NA | NA | 16.2 ± 1.0 | 9.0 ± 0.4 | 8.3 ± 5.8 | 13.8 ± 0.6 | 67 ± 3 |
| NO | Avg. | | 26 ± 17 | 25 ± 18 | 6 ± 4 | 7 ± 3 | 1.8 ± 1.1 | 0.1 ± 0.3 | 0.4 ± 0.2 | −0.3 ± 0.6 | 3.9 ± 1.4 | 15.1 ± 2.2 | 76 ± 8 |
| YES | | | 842 ± 252 | 523 ± 149 | 308 ± 153 | 195 ± 156 | 8.6 ± 6.3 | 4.0 ± 1.0 | 12.9 ± 6.9 | 6.6 ± 2.2 | 4.5 ± 2.5 | 15.8 ± 1.4 | 63 ± 10 |

Note: BC IR reflects black, and BC UV brown carbon. Abbreviations: SD = Standard Deviation of 1-min measurements; Avg. = Average of four sessions; NA = not available.

various sessions (Table 2).

After the first barbecue session was performed, we decided to increase the distance between the volunteers and the barbecue with one extra meter, because of even higher $PM_{2.5}$ concentrations and PNCs (~740–1200 μg/m$^3$) than expected based upon the pilot. The measurement units were also installed at a distance of 3 and 3.5 m from the barbecue in sessions 2–4. Because the monitors measured the exposure close to the volunteers, we could combine the exposure and health observations from the eight sessions in one analysis. Exposures differed from day to day not only because of the distance, but also because of weather circumstances.

Overall, the grilling of the meat phase resulted in higher $PM_{2.5}$ levels, compared with the start-up phase (Fig. 1A). Grilling chicken resulted in the lowest $PM_{2.5}$ exposure levels, but in the highest ultrafine particle number concentrations (Fig. 1A – B). Fig. 1C presents the changes in BC levels at wavelength >880 nm (IR, BC) and <375 nm (UV, BrC). Overall, the start-up phase resulted in the highest peak and average BC concentrations compared with the other time points. The BC-UV concentrations were generally higher than the BC-IR concentrations during the start-up phase and during grilling of the chicken. The $PM_{2.5}$ mass constituted of 1.0% and 1.5% BC and BrC, respectively.

### 3.3. Weather conditions

The wind speed and temperature did not differ between exposure and control sessions, whereas relative humidity was higher during control sessions compared with exposure sessions (Table 2). Six of eight sessions were executed following the beforehand agreed upon weather conditions (<5.4 m/s, no rain). During 1 exposure session the wind speed was higher than 5.5 m/s and during 2 control sessions (sessions 1 and 2), it was raining moderately, resulting in the re-location of the volunteers to a second location, near a small parking lot used primarily for employees (Figure B). This may have resulted in slightly higher control $PM_{2.5}$ levels, PNCs and BC air pollutant levels, that were, however, much lower than the exposure levels measured during the barbecue sessions.

### 3.4. Associations air pollutants and lung function

Associations between the difference in lung function (post – pre sessions) and the air pollutants $PM_{2.5}$, PNC, BC-UV and BC-IR are presented in Table 3. No statistically significant associations were observed between the air pollutant exposures and the four lung function parameters. Effect estimates were small and both positive and negative in the various models. Mixed linear regression analyses with varying adjustments for potential confounding factors resulted in highly insignificant associations. When influential points were removed, associations remained highly non-significant (Supplement Tables C-F, 'Overview estimates air pollutants and lung function parameters'). Predominantly, subjects had responded with a 0 on the respiratory symptoms using the questionnaire, meaning 'no symptoms'. No significant differences were observed for each symptom between the control- and exposure sessions (Supplement Table G).

### 3.5. Associations air pollutants and nasal inflammatory markers

Associations, between the air pollution exposures and changes in the nasal inflammatory markers of the various time points are displayed in Table 4. The normalized inflammatory marker expression levels of IL8, IL6 and TNFα per time point of both the control and exposure sessions, can be found in Supplement Table H ('Normalized inflammatory marker expressions').

Consistent, positive associations were observed between $PM_{2.5}$, PNC, BC-UV and BC-IR and the nasal inflammatory marker IL8, at all time-

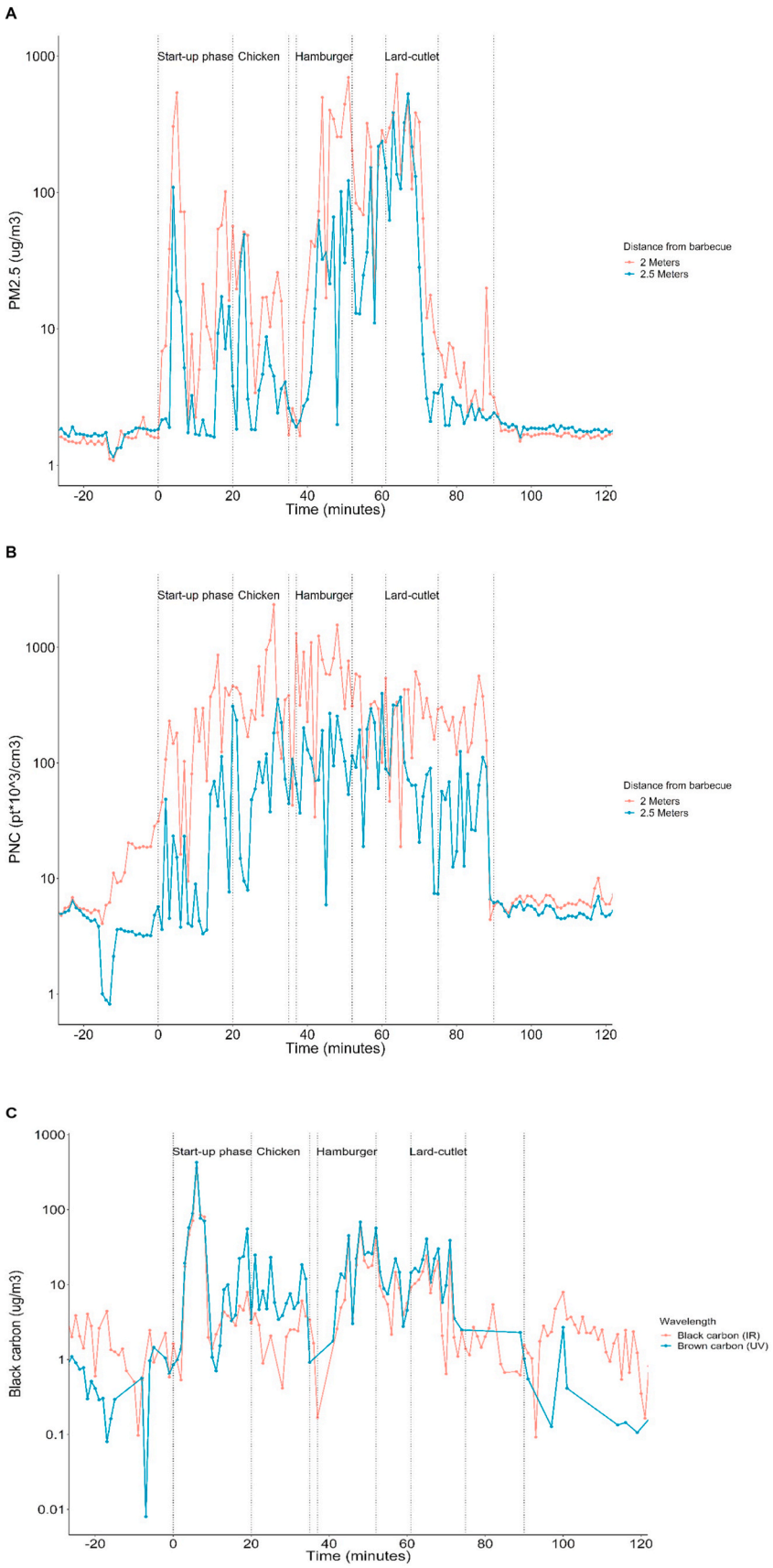


**Fig. 1.** Temporal variation of 1-min concentrations of A) $PM_{2.5}$; B) UFP; and C) BC, IR and UV wavelengths, monitored during barbecue session 1 at 2 and 2.5 m from the barbecue. Please note different concentration logarithmic scales and distances from the barbecue used in the graphs. Before time 0 and after time 90 min, background concentrations..

**Table 3**
Associations between air pollution exposure and lung function (post-pre) parameters.

| | | Model 1 Estimate (95% CI)[a] | p value | Model 2 Estimate (95% CI)[a] | p value |
|---|---|---|---|---|---|
| $PM_{2.5}$ 1000 μg/$m^3$ | $FEV_1$ | −8.8 (−65.6, 48.0) | 0.76 | 1.9 (−56.7, 60.5) | 0.95 |
| | FVC | −10.5 (−52.1, 31.1) | 0.63 | −8.0 (−53.1, 37.2) | 0.73 |
| | MMEF | −7.3 (−145.8, 131.2) | 0.92 | 37.7 (−97.3, 172.7) | 0.59 |
| | PEF | 32.1 (−172.2, 236.4) | 0.76 | 68.3 (−157.8, 294.4) | 0.56 |
| PNC 200.000 pt/$cm^3$ | $FEV_1$ | 18.3 (−27.3, 63.9) | 0.44 | 9.0 (−37.7, 55.7) | 0.71 |
| | FVC | 8.0 (−25.8, 41.7) | 0.65 | −0.1 (−37.6, 37.3) | 0.99 |
| | MMEF | 52.3 (−57.6, 162.1) | 0.36 | 38.6 (−71.9, 149.2) | 0.50 |
| | PEF | 55.4 (−111.0, 221.9) | 0.52 | 65.1 (−109.6, 239.8) | 0.47 |
| BC-UV 10 μg/$m^3$ | $FEV_1$ | 1.7 (−4.3, 7.8) | 0.58 | 1.1 (−5.7, 7.8) | 0.76 |
| | FVC | 1.8 (−2.6, 6.2) | 0.43 | 1.8 (−3.6, 7.1) | 0.52 |
| | MMEF | 1.4 (−13.2, 16.0) | 0.85 | 1.5 (−14.4, 17.4) | 0.86 |
| | PEF | 5.5 (−16.5, 27.5) | 0.63 | 8.7 (−16.7, 34.1) | 0.51 |
| BC-IR 5 μg/$m^3$ | $FEV_1$ | 4.5 (−5.8, 14.9) | 0.40 | 2.1 (−9.0, 13.2) | 0.71 |
| | FVC | 4.6 (−3.0, 12.2) | 0.25 | 4.1 (−4.8, 13.0) | 0.38 |
| | MMEF | 4.5 (−20.8, 29.8) | 0.73 | 0.6 (−26.0, 27.2) | 0.97 |
| | PEF | 10.0 (−27.1, 47.1) | 0.60 | 12.1 (−29.3, 53.4) | 0.57 |

*P value: *p < 0.1; **p < 0.05; ***p < 0.01*

Model explanation.
LMM 1: linear mixed effects model. Random effects: (1|ID).
LMM 2: linear mixed effects model. Random effects: (1|ID) and fixed effects temperature and relative humidity.
Abbreviations: CI, confidence interval; FEV1, Forced Expiratory Volume in 1-s; FVC, Forced Vital Capacity; MMEF, Maximal Mid-Expiratory Flow; PEF, Peak Expiratory Force; PM2.5, particulate matter with an aerodynamic size <2.5 μm; PNC, particle number concentration; BC, black carbon; IR, infrared radiation; UV, ultraviolet.
[a] Estimates represent the change in lung function (post-pre) parameters (mL ($s^{-1}$)) associated with air pollutant $PM_{2.5}$ (1000 μg/$m^3$), PNC (200.000 pt/$cm^3$), BC-UV (10 μg/$m^3$) and BC-IR (5 μg/$m^3$) calculated by multiplying the slope with increment. Air pollution exposure represent the average measured exposure during each barbecue and control session.

points (Table 4, Supplement Table I – L, 'Overview estimates air pollutants and respiratory biomarkers'). In the model adjusted for potential confounding factors temperature and relative humidity, the effect estimates decreased with ≥20% compared to the unadjusted model for $PM_{2.5}$ (Table 4). The confounding effects of temperature and relative humidity (difference between model 1 and 2) were smaller for the other pollutants, especially BC (IR and UV). The BC-IR effect estimates with IL8 were overall much smaller, compared with the other air pollutants. No significant associations were observed between air pollutants and inflammatory markers IL6 and TNFα regarding all statistical models (Table 4, Supplement Tables I – L). Furthermore, no consistent patterns of predominantly positive or negative associations were found. Adjusting for the additional potential confounding factors having a cold and alcohol consumption, did not result in changes in the effect estimates of more than 10% compared with the estimates of the model adjusted for temperature and relative humidity (Table 4). After adjusting for confounding factors, the associations between $PM_{2.5}$ and IL8 remained borderline significant for time points 1 and 3.

Finally, the same models were applied to examine the associations between the various nasal inflammatory markers and whether the session was an exposure session YES or NO. Consistent, borderline significant, positive associations were observed between barbecue exposure YES/NO and inflammatory marker IL8, whereas no significant associations were observed regarding the inflammatory markers IL6 and TNFα (Supplement Table M).

## 4. Discussion

The aim of the current study was to investigate the acute changes in lung function and inflammatory biomarkers in healthy humans, related to short-term exposure to barbecue fumes, using a repeated-measures design. We found very high particulate air pollution concentrations at distances of 2–3 m downwind from the barbecue. We observed positive associations between air pollutants from barbecue smoke and the nasal inflammatory marker IL8, that were still increased 18-h post exposure. We did not find any associations with lung function and the nasal inflammatory markers IL6 and TNFα.

### 4.1. Air pollution

During the barbecue sessions we observed high $PM_{2.5}$ levels, PNC and BC concentrations compared with both the background and control sessions. Overall, $PM_{2.5}$ concentrations were in the same order of magnitude as $PM_{2.5}$ exposure levels observed by Wu et al. (2015) and Badyda et al. (2017) (~1000–2700 μg/$m^3$) (Badyda et al., 2017; Wu et al., 2015). Large variability of particulate air pollution concentrations was monitored both within and between the various exposure sessions. Differences in exposure levels both between, and within sessions, might largely be explained by fluctuations in wind speed, wind direction, (outdoor) temperature and relative humidity. (Hu et al., 2012; Y.-C. Li et al., 2015).

The MiniDiSC does not specifically measure ultrafine particles (UFP), which are defined as particles <100 nm, but PNCs. Nonetheless, multiple studies have illustrated that PNCs are dominated by UFPs (Kumar et al., 2013; Morawska et al., 2008). Therefore, we included studies reporting UFP levels, as well as studies reporting PNCs. PNCs were high compared to UFP concentrations measured at sites heavily impacted by traffic emissions (HEI, 2013). In a large review of studies published prior to 2008, average concentrations across studies were 7,300, 48,000, 71,000 and 168,000 pt/$cm^3$ at urban background, road-side, on-road and tunnel measurement sites respectively (HEI, 2013). More recent studies in the Netherlands, based on three repeated road-site measurements for 30 min at 160 sites in major cities, reported a maximum site-average concentration of 70,000 pt/$cm^3$ (Kerckhoffs et al., 2016, 2017). A study executed by Sinharay et al. (2018), monitoring traffic related air pollution in Oxford Street London, found UFPs of on average 25,000 pt/cm3 and 64,000 pt/cm3 in an earlier study in Oxford Street (McCreanor et al., 2007). $PM_{2.5}$ concentrations were much higher than measured at traffic sites. In the recent Oxford Street study, the $PM_{2.5}$ concentration measured with the same monitor as in our study was below 20 μg/$m^3$ (Sinharay et al., 2018). Consequently, the contribution of $PM_{2.5}$ and PNC to ambient background levels due to barbecue smoke was very high compared with the contribution of traffic related air pollution. BC concentrations were comparable to the average BC concentrations in Oxford Street and the traffic site with the highest BC concentrations in short-term monitoring Dutch studies (Kerckhoffs et al., 2016, 2017; Sinharay et al., 2018). BrC is composed of organic aerosols, which is associated with biomass burning (Tian et al., 2019; Yan et al., 2017). Therefore, it was hypothesized a priori that the charcoal briquettes would attribute predominantly to increased BrC levels, compared with BC levels. BrC levels were indeed overall higher than BC levels, but only during the start-up phase and while grilling chicken. Overall, however, BC and BrC levels contributed very little to the $PM_{2.5}$ levels monitored during barbecue sessions. The low carbon contribution to the $PM_{2.5}$ levels (mass closure) could partly be explained by overestimation of the $PM_{2.5}$ levels because of unidentified water vapor (Shi et al., 2017). In the current study, using an optical technique,

**Table 4**
Associations between air pollution exposure and changes in the nasal inflammatory markers (post-pre).

| | | | Model 1 Estimate (95% CI) [a] | p value | Model 2 Estimate (95% CI) | p value |
|---|---|---|---|---|---|---|
| $PM_{2.5}$ 1000 μg/$m^3$ | IL8 | Post | 2.93 (1.34, 6.43) | 0.013** | 2.13 (0.97, 4.67) | 0.075* |
| | | Post 4 h. | 3.14 (1.17, 8.46) | 0.030** | 2.01 (0.73, 5.49) | 0.184 |
| | | Post 18 h. | 2.29 (0.98, 5.32) | 0.071* | 2.21 (0.98, 5.00) | 0.075* |
| | IL6 | Post | 1.54 (0.71, 3.34) | 0.283 | 1.29 (0.62, 2.65) | 0.504 |
| | | Post 4 h. | 1.10 (0.56, 2.16) | 0.793 | 0.93 (0.41, 2.09) | 0.863 |
| | | Post 18 h. | 1.24 (0.56, 2.71) | 0.603 | 1.26 (0.53, 3.02) | 0.608 |
| | TNFα | Post | 0.84 (0.52, 1.37) | 0.498 | 0.87 (0.53, 1.43) | 0.594 |
| | | Post 4 h. | 0.78 (0.47, 1.32) | 0.371 | 0.72 (0.46, 1.10) | 0.147 |
| | | Post 18 h. | 1.04 (0.61, 1.76) | 0.898 | 1.17 (0.65, 2.12) | 0.599 |
| PNC 200.000 pt/$cm^3$ - | IL8 | Post | 1.64 (0.84, 3.20) | 0.166 | 1.79 (0.97, 3.33) | 0.078* |
| | | Post 4 h.- | 1.76 (0.80, 3.89) | 0.177 | 1.90 (0.90, 4.03) | 0.104 |
| | | Post 18 h. | 2.19 (1.11, 4.33) | 0.037** | 2.14 (1.11, 4.10) | 0.035** |
| | IL6 | Post | 1.31 (0.68, 2.51) | 0.422 | 1.27 (0.73, 2.19) | 0.406 |
| | | Post 4 h.- | 1.04 (0.60, 1.79) | 0.892 | 1.03 (0.56, 1.90) | 0.922 |
| | | Post 18 h. | 1.33 (0.71, 2.49) | 0.382 | 1.13 (0.58, 2.21) | 0.714 |
| | TNFα | Post | 1.17 (0.78, 1.75) | 0.458 | 1.13 (0.78, 1.62) | 0.529 |
| | | Post 4 h. | 0.76 (0.51, 1.15) | 0.210 | 0.74 (0.53, 1.02) | 0.081* |
| | | Post 18 h. | 1.26 (0.81, 1.95) | 0.309 | 1.24 (0.80, 1.91) | 0.341 |
| BC-UV 10 μg/$m^3$ | IL8 | Post | 1.89 (0.81, 4.39) | 0.155 | 1.89 (0.86, 4.16) | 0.126 |
| | | Post 4 h.- | 1.84 (0.69, 4.88) | 0.236 | 1.72 (0.66, 4.48) | 0.279 |
| | | Post 18 h. | 2.17 (0.95, 4.96) | 0.081* | 2.16 (0.95, 4.91) | 0.080* |
| | IL6 | Post | 1.11 (0.48, 2.58) | 0.811 | 1.09 (054, 2.18) | 0.813 |
| | | Post 4 h. | 1.01 (0.51, 2.01) | 0.973 | 0.93 (0.43, 2.01) | 0.850 |
| | | Post 18 h. | 1.06 (0.48, 2.36) | 0.884 | 0.89 (0.39, 2.04) | 0.785 |
| | TNFα | Post | 1.01 (0.60, 1.70) | 0.972 | 0.97 (.061, 1.56) | 0.915 |
| | | Post 4 h. | 0.72 (0.42, 1.22) | 0.241 | 0.68 (0.45, 1.04) | 0.094 |
| | | Post 18 h. | 1.07 (0.61, 1.93) | 0.806 | 1.05 (0.60, 1.83) | 0.877 |
| BC-IR 5 μg/$m^3$ | IL8 | Post | 1.07 (0.93, 1.24) | 0.399 | 1.12 (0.98, 1.29) | 0.117 |
| | | Post 4 h. | 1.07 (0.91, 1.27) | 0.406 | 1.09 (0.93, 1.29) | 0.308 |
| | | Post 18 h. | 1.14 (0.98, 1.31) | 0.096* | 1.15 (0.99, 1.33) | 0.086* |
| | IL6 | Post | 0.98 (0.85, 1.13) | 0.801 | 1.01 (0.89, 1.14) | 0.931 |
| | | Post 4 h. | 0.99 (0.88, 1.11) | 0.869 | 0.98 (0.85, 1.12) | 0.743 |
| | | Post 18 h. | 1.00 (0.87, 1.15) | 0.957 | 0.97 (0.83, 1.12) | 0.655 |
| | TNFα | Post | 1.00 (0.92, 1.09) | 0.952 | 0.99 (0.91, 1.07) | 0.791 |
| | | Post 4 h. | 0.94 (0.86, 1.03) | 0.212 | 0.93 (0.86, 1.00) | 0.078* |
| | | Post 18 h. | 1.01 (0.92, 1.11) | 0.837 | 1.00 (0.90, 1.10) | 0.935 |

*P value: *p < 0.1; **p < 0.05; ***p < 0.01*

Model explanation.
Model 1: linear mixed effects model. Random effects: (1|ID).
Model 2: linear mixed effects model. Random effects: (1|ID) and fixed effects temperature and relative humidity.
$PM_{2.5}$ (1000 μg/$m^3$), PNC (200.000 pt/$cm^3$), BC-UV (10 μg/$m^3$) and BC-IR (5 μg/$m^3$) calculated by exponentiating slope with increment.
Abbreviations: LMM, linear mixed effects model; CI, confidence interval; IL8, interleukin-8; IL6, interleukin-6; TNFα, tumor necrosis factor alpha; PM2.5, particulate matter with an aerodynamic size <2.5 μm; PNC, particle number concentration; BC, black carbon; IR, infrared radiation; UV, ultraviolet.
[a] Estimates represent the change in inflammatory markers (post-pre) associated with air pollutant.

overestimation of $PM_{2.5}$ might have occurred as well. No gravimetric monitoring was performed, which would have enabled quantification of the potential overestimation. In a study comparing Sidepak and RTI MicroPEM V3.2 nefelometer $PM_{2.5}$ measurements, $PM_{2.5}$ were about 50% higher outdoors for the Sidepak measurements (Sloan et al., 2016). Inside restaurants, Sidepak $PM_{2.5}$ was three times higher than nefelometer $PM_{2.5}$. Moreover, the BrC levels optically measured only assess the C-content and thus not the total particulate organic mass (Abdeen et al., 2014; Cappa et al., 2019).

During grilling of the hamburgers and lard-cutlets, fat melted due to the heat and dripped onto the charcoal, and consequently burnt and created substantial amounts of visible smoke. When comparing the fat percentage in each meat type and the subsequent exposure levels, it was noticed that meat with a higher fat percentage predominantly contributed to $PM_{2.5}$ levels. In our study, grilling hamburgers and lard cutlets resulted in relatively high $PM_{2.5}$ levels, but low PNCs, whereas grilling chicken resulted in modestly higher PNCs and much lower $PM_{2.5}$ concentrations. The increased contribution of high-fat meat to PNCs, compared with low-fat meat, was likewise observed by other studies investigating $PM_{2.5}$ and PNC emission rates during cooking, boiling, frying and roasting meat (Buonanno et al., 2009; Y.-C. Li et al., 2015; McDonald et al., 2003). Experimental research suggests that particles in the UFP size fraction have a more pronounced capacity to induce oxidative stress and po-inflammatory effects, compared with fine particles. This may be because UFP can deposit deeper in the lungs, potentially cross the alveolar barrier and in addition have a higher surface-to-mass ratio, enabling the particles to carry larger loads of hazardous additives, including heavy metals or PAHs (Barregard et al., 2006; Downward et al., 2018; Gualtieri et al., 2010; N. Li et al., 2016; Saleh et al., 2019). Therefore, our data suggest that frying chicken meat instead of meat types with a higher fat percentage may not be associated with smaller health effects as proposed previously (Buonanno et al., 2009; Y.-C. Li et al., 2015; McDonald et al., 2003).

### 4.2. Lung function and air pollution associations

No associations were observed between the air pollutants and changes in lung function (FEV1, FVC, MMEF and PEF) directly after exposure to barbecue fumes. As there are no other studies that evaluated health effects of barbecue fumes, the associations observed in the current study were compared with changes in respiratory function measured in studies on other short-term biomass combustion sources. Controlled human exposure studies investigating potential adverse effects of exposure to wood smoke, similarly observed no significant associations between wood smoke and respiratory function (Muala et al., 2015; Stockfelt et al., 2012; Swiston et al., 2008).

Studies that did find a significant negative association between FVC or $FEV_1$ and short-term exposure to air pollutants from various

combustion sources, focused predominantly on effects in children or people with respiratory symptoms (*e.g.* asthma or COPD) (Chen et al., 2018; Heinzerling et al., 2016; Lagorio et al., 2006; Svartengren et al., 2000). It is likely that these people had a higher sensitivity and consequently require lower exposure levels for effects to be noticeable. In the studies in which researchers observed a significant negative association with lung function in healthy people, subjects were exposed to various levels of air pollutants while doing exercise (walking or cycling) (Panis et al., 2017; Rom et al., 2013; Sinharay et al., 2018; Strak et al., 2012). In our study subjects were exposed at rest, to reflect the normal daily life exposure of barbecuing.

Sixteen subjects were included in our study. The CI of the lung function analyses indicated that a difference of ~1% in lung function between barbecue and control sessions could have been detected with the current number of subjects and exposure level. The high exposure levels and the repeated-measures design with observations within the same individual contributed to the adequate precision despite the small number of observations. Our study size was comparable to human controlled exposure studies (Barregard et al., 2006; Stockfelt et al., 2012), also comparing a large contrast between exposed and unexposed scenario's.

### 4.3. Nasal inflammatory markers and air pollution associations

We found consistent and positive associations between all measured air pollutants and the nasal inflammatory marker IL8, but not IL6 or TNFα. As all air pollutants were substantially increased during the barbecue session, we could not separate the effects of $PM_{2.5}$, BC, BrC and PNC. Consistently, we observed very similar effect estimates for a simple dummy variable indicating whether a session was an exposed or a control session. IL8 is produced by a range of cells including macrophages, fibroblasts, endothelial- and epithelial cells and is a key chemoattractant for neutrophils (Gualtieri et al., 2010; Stieb et al., 2002). Neutrophils play a key role in the protection against various pathogens, but an excessive or prolonged activation of IL8 and subsequent neutrophil recruitment, is associated with perpetuation of the inflammatory state (Kolaczkowska and Kubes, 2013). It is unclear whether the IL8 response reached a plateau-phase 18 h after exposing the subjects to the barbecue smoke. Future studies should include even longer time points to investigate whether the nasal IL8 levels return to baseline, the expression of markers IL6 and TNFα as well changed, and in addition, whether other inflammatory markers, or biomarkers of inflammation, do increase after short-term exposure to barbecue smoke (Barregard et al., 2006; Stockfelt et al., 2012).

A short, incomplete overview of studies investigating effects of various combustion sources (biomass exposure and diesel exhaust) and the most important outcomes, can be found in Supplement Table N ('Overview controlled exposure studies'). The current paradigm is that airborne particles can activate the respiratory immune system both directly and indirectly, induce lung inflammatory responses and impair host defence (Kim et al., 2013). This is observed for both exposure to wood smoke and diesel exhaust particles, by an increase in IL8 and, dependent on air pollutant levels or duration of exposure, IL6 (Corsini et al., 2017; Gualtieri et al., 2010; Riddervold et al., 2012; Steenhof et al., 2011; Swiston et al., 2008). The exact health consequences of a prolonged increase in cytokine levels in humans remain unclear. However, studies in mice have shown that local cytokine production, including IL8, immediately after exposure to carbon black particles, is predictive of allergic airway inflammation and sensitization (De Haar et al., 2005). In humans, researchers who found an increase in inflammatory markers, also observed an associated increase in various oxidative stress markers (HMOX, $HO_1$), white blood cells (neutrophils, eosinophils) and antioxidant levels (GSH, GSSG) (Barregard et al., 2006; Becker et al., 2005; Bølling et al., 2009; Corsini et al., 2017; Daniel et al., 2021; Gualtieri et al., 2010; Lauer et al., 2009; Nightingale et al., 2000; Steenhof et al., 2011, 2015; Stockfelt et al., 2012; Swiston et al., 2008).

We did not find a consistent association between air pollutants and IL6. Lack of homogeneity observed for IL6 could at least partly be due to the area of sampling, the time point at which the biomarkers were measured, timing and levels of exposure and differences in combustion sources (Hurst et al., 2006; Nightingale et al., 2000).

### 4.4. Strengths, limitations and future perspectives

An important strength of this study was the repeated-measures experimental design, which enabled an informative study using a relatively small number of volunteers. The design ensures a decrease in inter-individual variation, which is especially important when looking at changes in inflammatory markers. Furthermore, the influence of the circadian rhythm on spirometry and inflammatory biomarkers was eliminated by executing the sessions and measuring the health effects at the same time-of-day. The experimental design reduced the uncertainty in exposure assessment compared to more common observational studies in environmental epidemiology (Steenhof et al., 2011; Strak et al., 2012).

Nasal swabs are a relatively easy sampling method to assess biomarker levels related to upper airway inflammation. However, no literature is available yet on the relationship with the more distal airways or the serum inflammatory status. The primary function of the nasal mucosa is to serve as a physicochemical barrier for the lungs. Therefore, whether inflammatory markers from the nasal area indeed reflects the inflammatory status of the distal lungs or serum, remains questionable (Purokivi et al., 2002; Xia et al., 2018).

The present study measured higher air pollutant levels compared with other human controlled exposure studies investigating effects of air pollutants on respiratory health, which vary normally for $PM_{2.5}$ between 100 and 450 $\mu g/m^3$. The current study might not be fully representative of exposure to barbecue fumes. During outdoor grilling, people tend to walk in and out of the barbecue smoke constantly, because they are (unconsciously) avoiding the most direct fumes. However, subjects in the current study were exposed for a shorter period of time, *i.e.* 90 min. Instead of 2–4 h, and did not move around during exposure. Furthermore, the air pollutant levels measured in the current study did not differ substantially from previous studies monitoring pollutant levels at barbecue vendor stalls (Badyda et al., 2017; Wu et al., 2015). Furthermore, Badyda et al. (2017) remarks that a barbecue session can take between 3.9 and 5.6 h, depending on the country (Badyda et al., 2017). The current research offers an interesting starting point to further investigate whether these relatively short term high air pollutant levels result in adverse health effects.

## 5. Conclusion

Barbecuing resulted in high $PM_{2.5}$, UFP and BC concentrations. Short-term exposure to air pollutants emitted from barbecuing was associated with a mild respiratory response in healthy young adults, including a sustained prolonged increase in nasal IL8 without a change in lung function and other measured inflammatory markers.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111868.

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