Research Article

Assessment of Polycyclic Aromatic Hydrocarbon Exposure, Lung Function, Systemic Inflammation, and Genotoxicity in Peripheral Blood Mononuclear Cells from Firefighters before and after a Work Shift

Maria Helena Guerra Andersen,[1,2] Anne Thoustrup Saber,[2] Julie Elbæk Pedersen,[3] Peter Bøgh Pedersen,[4] Per Axel Clausen,[2] Mille Løhr,[1] Ali Kermanizadeh,[1] Steffen Loft,[1] Niels E Ebbehøj,[3] Åse Marie Hansen,[2,5] Ismo Kalevi Koponen,[2] Eva-Carina Nørskov,[4] Ulla Vogel,[2,6]* and Peter Møller[1]*

[1]Department of Public Health, Section of Environmental Health, University of Copenhagen, Øster Farimagsgade 5A, Copenhagen K DK-1014, Denmark
[2]The National Research Centre for the Working Environment, Lersø Parkalle 105, Copenhagen Ø DK-2100, Denmark
[3]Department of Occupational and Environmental Medicine, Bispebjerg and Frederiksberg University Hospital, Copenhagen, NV DK-2400, Denmark
[4]Danish Technological Institute, Teknologiparken, Kongsvang Allé 29, Aarhus C, DK-8000, Denmark
[5]Department of Public Health, Section of Social Medicine, University of Copenhagen, Øster Farimagsgade 5A, Copenhagen K DK-1014, Denmark
[6]Department of Micro- and Nanotechnology, Technical University of Denmark, Lyngby DK-2800 Kgs, Denmark

Firefighting is regarded as possibly carcinogenic, although there are few mechanistic studies on genotoxicity in humans. We investigated exposure to polycyclic aromatic hydrocarbons (PAH), lung function, systemic inflammation and genotoxicity in peripheral blood mononuclear cells (PBMC) of 22 professional firefighters before and after a 24-h work shift. Exposure was assessed by measurements of particulate matter (PM), PAH levels on skin, urinary 1-hydroxypyrene (1-OHP) and self-reported participation in fire extinguishing activities. PM measurements indicated that use of personal protective equipment (PPE) effectively prevented inhalation exposure, but exposure to PM occurred when the environment was perceived as safe and the self-contained breathing apparatuses were removed. The level of PAH on skin and urinary 1-OHP concentration were similar before and after the work shift, irrespective of self-reported participation in fire extinction activities. Post-shift, the subjects had reduced levels of oxidatively damaged DNA in PBMC, and increased plasma concentration of vascular cell adhesion molecule 1 (VCAM-1). The subjects reporting participation in fire extinction activities during the work shift had a slightly decreased lung function, increased plasma concentration of VCAM-1, and reduced levels of oxidatively damaged DNA in PBMC. Our results suggest that the firefighters were not exposed to PM while using PPE, but exposure occurred when PPE

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*Correspondence to: Peter Møller, Department of Public Health, Section of Environmental Health, University of Copenhagen, Øster Farimagsgade 5A, DK-1014 Copenhagen K, Denmark. E-mail: pemo@sund.ku.dk and Ulla Vogel, The National Research Centre for the Working Environment, Lersø Parkalle 105, DK-2100 Copenhagen Ø, Denmark. E-mail: ubv@nrcwe.dk

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was not used. The work shift was not associated with increased levels of genotoxicity. Increased levels of VCAM-1 in plasma were observed. Environ. Mol. Mutagen. 00:000–000, 2018. © 2018 Wiley Periodicals, Inc.

Key words: biomonitoring; ultrafine particles; 1-hydroxypyrene; oxidative DNA damage; comet assay

INTRODUCTION

The International Agency for Research on Cancer (IARC) has evaluated occupational firefighting activity as possibly carcinogenic to humans (Group 2B) [IARC, 2010], based on increased risk of testicular and prostate cancer as well as non-Hodgkin's lymphoma [Guidotti, 1993; LeMasters et al., 2006; Golka and Weistenhofer, 2008]. IARC segregated firefighting activities into different fire scenarios, including municipal, wildland, industrial, aviation, military, and oil well fires. Firefighters can be exposed to different types of carcinogens, depending on the type of fuel that is being burnt. As such, "smoke" is a complex mixture that contains soot and genotoxic compounds. IARC noted a lack of studies on genotoxicity in firefighters, which is a critical mechanism of carcinogenesis.

Previous studies on firefighters have focused on genotoxic effects of polycyclic aromatic hydrocarbons (PAH). This is a group of genotoxic compounds that can form PAH-DNA adducts in cells [Phillips, 2013]. Pioneer studies showed the same level of PAH-DNA adducts in peripheral blood mononuclear cells (PBMC) from municipal firefighters and matched controls [Liou et al., 1989]. Firefighting activity did not change PAH-DNA adduct levels in PBMC in wildland firefighters [Rothman et al., 1993]. Likewise, volunteer firefighters had the same level of PAH-DNA adducts in PBMC before and after a work shift where they were exposed to plumes of smoke from oil well fires in Kuwait [Darcey et al., 1992]. The collective results suggest a lack of PAH-DNA adducts, although this cannot be taken as evidence of lack of exposure or that smoke does not pose a genotoxic hazard to firefighters.

Oxidatively generated nucleobase lesions constitute a group of DNA lesions formed by oxidative stress and inflammation in cells or tissues [Moller et al., 2013]. The most widely investigated lesion has been 8-oxoguanine, which is a pre-mutagenic nucleobase lesion [Cadet et al., 2012]. Traditionally, 8-oxoguanine levels have been measured with chromatographic assays, whereas the formamidopyrimidine DNA glycosylase (Fpg)-modified comet assay has become the method of choice for this type of oxidatively damaged DNA. The comet assay can also be used to detect non-specific DNA lesions; these are typically called DNA strand breaks in order to distinguish them from the Fpg-sensitive sites. In a recent biomonitoring study of 53 conscripts under education as firefighters, firefighting was associated with increased levels of PAH on the skin, increased urinary excretion of 1-OHP and increased levels of oxidatively damaged DNA in PBMC [Andersen et al., 2018]. Municipal firefighters had increased levels of DNA strand breaks in PBMC after a firefighting episode at a chemical plant where they did not wear protective clothing or other safety provisions [Hengstler et al., 1995]. Another study showed higher levels of DNA strand breaks in PBMC from firefighters as compared to controls, whereas the levels of Fpg-sensitive sites were similar in the two groups of subjects [Abreu et al., 2017]. However, a controlled wood smoke exposure cross-over study showed unaltered levels of DNA strand breaks and Fpg-sensitive sites in PBMC [Forchhammer et al., 2012], whereas a sequential study actually showed decreased levels of Fpg-sensitive sites and a tendency toward increased DNA repair activity after wood smoke exposure [Danielsen et al., 2008].

Inhalation of smoke particles may elicit pulmonary inflammation, which is considered to be an important mechanism for both lung cancer and systemic effects [IARC, 2010]. Low-grade systemic inflammation, that is, elevated levels of C-reactive protein (CRP), serum amyloid protein (SAA), interleukin (IL), intercellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) might occur from pulmonary inflammation [Stone et al., 2017]. In a recent biomonitoring study of 53 conscripts under education as firefighters, firefighting did not affect markers of systemic inflammation (IL-6, IL-8, CRP, SAA, ICAM-1 or VCAM-1) but lowered heart rate variability and affected vascular function [Andersen et al., 2017, 2018], indicating effects of firefighting on the cardiovascular system.

The aim of the present study was to investigate PAH exposure, lung function, systemic inflammation and DNA damage in circulating PBMC of firefighters after a day of work.

MATERIALS AND METHODS

Subjects and Study Design

Twenty-two male subjects were enrolled from a firefighter station in Greater Copenhagen Fire Department. In this station, firefighters had 24-h work shifts (from 8 A.M. to 8 A.M. the following day), followed by 3 days off prior to the next work shift. We included firefighters from 3 consecutive 24-h work shift days in December (these are described as day 1, 2, and 3 in the results, Table I). The age of the subjects ranged from 39 to 59 years. The body mass index (BMI) was determined using self-reported information on weight and height and ranged from 22.2 to 31.3 kg/m^2 (seven subjects between 22.2 and 24.6, twelve subjects between 25.1 and 28.3 and three subjects between 30.4 and 31.3). From the 22 subjects, nine self-reported as never smokers, nine had a history of smoking and four reported being current smokers.

The design was a sequential study, where the subjects served as their own controls and were measured at two different time points: start of the work shift (between 8 and 9 A.M.) and at the end of the shift around 24-h

TABLE I. Characteristics of the Subjects

Characteristic	Day 1 ($n = 7$)	Day 2 ($n = 8$)	Day 3 ($n = 7$)	Total ($n = 22$)
Age (years)	50.9 ± 6.8	51.9 ± 5.6	52.4 ± 6.9	51.7 ± 6.2
BMI (kg/m^2)[a]	24.2 ± 0.9	27.0 ± 3.3	26.8 ± 2.1	26.8 ± 2.6
Smoker (yes/no)[a]	1/6	1/7	2/5	4/18
ETS (yes/no)[a,b]	1/5	1/6	1/4	3/15
Fire smoke exposure (yes/no)[a]	7/0	1/7	6/1	14/8

BMI, body mass index; ETS, environmental tobacco smoke. Values are number or mean and standard deviation.
[a]Self-reported information.
[b]ETS among non-smokers.

later (between 7 and 8 A.M.), termed "before" and "after," respectively. All subjects had emergency calls related to firefighting on their work shift.

The Danish Committee on Health Research Ethics of the Capital Region (H-15003862) approved the study and the subjects provided written informed consent.

Exposure Assessment

The exposure assessment had certain limitations, as individual emergency calls are all different and impossible to predict. Thus, the assessment only provides a description of the exposure situation rather than a detailed assessment of the exposure. The study was carried out at the time of year when fires are most frequent in the greater Copenhagen area. The exposure to particulate matter (PM) was evaluated by a skilled researcher on call with the firefighter team during the three work shifts where biological samples were obtained from the firefighters. The assessment included measurements of the particle number concentration in the inhalation zone of the firefighter, whose responsibility was to extinguish the fire, and other measurements at the scene of the emergency. The equipment encompassed a handheld ultrafine particle (UFP) counter DiSCmini (Testo, Titisee-Neustadt, Germany) with a tube into the mask (as illustrated in Supporting Information Fig. S1) or just in the breathing area when the mask was not used, an UFP counter P-Trak (model 8525, TSI, USA) and an aerosol monitor DustTrak (DRX, model 8533, TSI, USA). PAH exposure was assessed by personal monitoring of 1-OHP in urine and PAH levels on the skin. The subjects completed a questionnaire where they were asked whether they actively participated in fire extinguishing activities during their shift and about their responsibilities at the fire.

Sample Collection

Lung function measurements and samples of blood were collected before and after the work shift at the fire station (between 7 and 9 A.M.). Dermal wipes were collected at the same time as blood at the start of the work shift, and just after the emergency call for post samples. Urine morning samples were collected between 5 and 9 A.M. and were used for determination of 1-OHP. Urine was kept in cooling boxes until arrival at the laboratory and at −20°C until analysis. Blood sampling was carried out to assess inflammatory markers (SAA, CRP, ICAM-1, VCAM-1, IL-6 and IL-8) and DNA damage in PBMC. Plasma for the ELISA analysis of the inflammatory markers was prepared by 10 min centrifugation at 4000 rpm (1780× g) of blood collected into EDTA-coated tubes (Becton Dickinson, 10 mL Vacutainer containing 18.0 mg K_2EDTA). The plasma samples were prepared at the firefighter station and transported to the laboratory on ice on the same day. Upon arrival, the plasma samples were stored at −80°C. At the fire station, PBMC were isolated using Vacutainer Cell Preparation Tubes (Vacutainer® CPT Becton Dickinson A/S, Brøndby, Denmark) before a 20 min centrifugation at 1650× g. The PBMC were resuspended in 3 mL ice-cold medium (RPMI with 10% fetal bovine serum and 1% Pen/Strep) and the samples were transported to the laboratory on ice on the same day where further sample preparation was performed. At arrival at the laboratory, the PBMC were separated by 15 min centrifugation at 300× g at 5°C and re-suspended in 3 mL RPMI medium with 10% fetal bovine serum and 1% Pen/Strep. The centrifugation procedure was repeated and the PBMC re-suspended in freezing medium (RPMI with 50% fetal bovine serum and 10% DMSO). The PBMC were stored at −80°C until analysis of DNA damage by the comet assay.

Dermal wiping of the neck was carried out to assess the PAH exposure on the skin. A skin area of approximately 3 × 6 cm^2 on the back of the neck was wiped with an "Alkoholswab" (70% isopropanol/water, Mediq Danmark A/S). The skin was wiped twice with the same wipe, first with one side of the wipe and then the other side. The operator wore nitrile gloves (TouchNTuff, 92–600, Ansell), which were changed for each wipe. The wipes were placed in 15 mL screw cap glass vials with foil-lined lid (Wheaton), kept in the dark and transported to the laboratory on the same day. The samples were stored at −18°C until extraction and analysis. In these experiments, a skin area (back of the neck) of nominally 18 cm^2 was used in all calculations. During the campaigns, extracts of two blank wipes in screw cap glasses were analyzed for each series of wipe samples. None of the individual PAH were above the limit of detection in any of the blanks.

Determination of PAH from Skin Wipes

The extraction of PAH was carried out by covering the wipes with 6 mL cyclohexane in 10 mL glass vials and sonication for 30 min in an ultrasonic bath (Branson 5200, output power 120 W at extraction of 25 samples at one time). One milliliter of the supernatant was transferred into a small glass vial and 30 μL of internal standard solution (10 ng/μL) added. The extracts were stored at −18°C until analysis. The extracts were analyzed by gas chromatography and mass spectrometry (GC-MS) using a Bruker SCION TQ (Bruker Daltonics, Bremen, Germany). The analysis was carried out by injection of 1 μL of the sample extract with a Bruker CP-8400 auto sampler to a programmable temperature vaporising (PTV) injector at 280°C into the column with a He flow of 1 mL/min. The column was 30 m × 0.25 mm with 0.25 μm film thickness (VF-5MS, Agilent Technologies, USA). The GC oven program was set at 70°C for 4 min, ramp 1, 10°C min^{-1} to 30°C, ramp 2, 45°C min^{-1} to 325° hold for 7 min and transfer line and the source were kept at 275°C. The MS was operated in scan mode (mass range m/z 50–500) in Electron Ionization (EI) and in Selected Ion Monitoring (SIM) for each PAH. ΣPAH is the sum of the concentrations of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)-fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, indeno(1,2,3-cd)pyrene, and benzo(g,h,i)perylene. The applied identification ions and the estimated limits of detection (LOD) of the individual PAH have been published in the supplementary material of previous study [Andersen et al., 2018].

Urinary 1-OHP Analysis

Reverse-phase HPLC with fluorescence detection was used for the measurement of 1-OHP in urine as previously described [Hansen et al., 1993]. Urinary creatinine was used to standardise for diuresis as performed in other studies [Hansen et al., 2008]. We analyzed a low (9.23 nmol/L) and a high (29.14 nmol/L) reference sample together with the samples to assess day-to-day variation. The limit of detection was 1.1 nmol/L.

Inflammatory Markers Analysis in Plasma

The concentrations of soluble ICAM-1 (Cat. No. 560269) and VCAM-1 (Cat. No: 560427) were assessed in plasma with BD cytometric bead array system, utilising Accuri CFlow®Plus software (BD Bioscience) as described previously [Jensen et al., 2014]. Plasma levels of SAA and CRP were determined by Enzyme-linked Immunosorbant assay (ELISA) from Invitrogen (Carlsbad, CA, USA) and IBL International GMBH (Hamburg, Germany), respectively, as described previously [Madsen et al., 2016]. Plasma levels of IL-6 and IL-8 were determined by ELISA from BD Biosciences (Cat. No. 555244 and Cat. No.555220) according to the manufacturer's specifications.

DNA Damage Analysis in PBMC

The levels of DNA strand breaks and Fpg-sensitive sites were detected by the comet assay as described previously [Lohr et al., 2015]. Briefly, PBMC were embedded in 0.75% low-melting point agarose (Sigma-Aldrich A/S, Brøndby, Denmark) on GelBond films (Lonza Copenhagen Aps, Vallenbæk Strand, Denmark) and lysed (1% Triton X-100, 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH = 10) overnight at 4°C. The Gelbond films were washed three times for 5 min in endonuclease buffer (40 mM HEPES, 0.1 M KCl, 0.5 mM Na_2EDTA, 200 μg/mL bovine serum albumin, pH = 8). Subsequently, the nuclei were incubated for 45 min with Fpg at 37°C. The Fpg enzyme was a gift from Professor Andrew Collins (University of Oslo, Norway). Thereafter, the Gelbond films were immersed in an alkaline solution (300 mM NaOH, 1 mM Na_2EDTA, pH > 13) for 40 min and subsequently subjected to electrophoresis for 20 min at 0.83 V/cm (cathode to anode) and 300 mA. After electrophoresis the Gelbonds were washed three times for 5 min in Tris buffer (0.4 M Tris-HCl, pH = 7.5), rinsed with milliQ water and dried in 96% ethanol. The nuclei were viewed using an Olympus fluorescence microscope at 40x magnification with visual inspection after staining with YOYO-1 in PBS (Molecular Probes, Eugene, OR, USA). The two samples from each subject were coded and analyzed simultaneously in order to minimize inter-assay variation. In addition, each batch of comet assay experiments included samples from the three workday-shifts in order to control for inter-day experimental variation. We analyzed 100 comets per slide and there were four slides for each sample, corresponding to a total number of 400 nuclei. The slides had been prepared in duplicates on two different assay runs (including different electrophoresis). The nuclei were scored by visual classification based on a five-class scoring system (arbitrary score range: 0–400) as previously described [Moller et al., 2004]. Additionally, assay control samples were added in each experiment (corresponding to each electrophoresis) that consisted of $KBrO_3$ exposed THP-1 cells (5 mM for 1 h at 37°C). The number of Fpg-sensitive sites was obtained as the difference in scores of parallel slides incubated with and without Fpg. These scores were transformed to lesions per 10^6 base pairs (bp) by means of a calibration curve based on induction of DNA strand breaks by ionising radiation, which has a known yield. We used an investigator-specific conversion factor of 0.01989 lesions/10^6 bp per score in 0–100 range [Lohr et al., 2015], based on the assumption that an average molecular weight of a DNA bp is 650 Dalton and 1 Gy yields 0.29 breaks per 10^9 Dalton DNA [Forchhammer et al., 2010].

Lung Function Measurement

The lung function was assessed using a Vitalograph S spirometer (Buckingham, United Kingdom) measuring forced vital capacity (FVC) and forced expiratory volume after 1 second (FEV1). The equipment was calibrated before each testing session. All measurements were performed with the subjects standing and using a nose clip. Up to three measurements were taken to obtain reproducible tracings with the two highest FVC, FEV1 and FEV1/FVC.

Statistical Analysis

The initial assessment of exposure showed that only some of the firefighters had been actively engaged in fire extinction activities where the exposure to smoke is presumed to occur. It means that the effect would be diluted in paired Student's T-test. Therefore, the results were analyzed by linear mixed-effects model using the *lme4* package [Bates et al., 2015] in R statistical environment. Subjects were included as random factor and as fixed factor we used factorial variables of exposure. The exposure term (i.e., independent variable or predictor) was either work shift or self-reported participation in fire extinguishing activities. The statistical model with "work shift" as fixed factor is equivalent to repeated measures ANOVA, whereas the model with "self-reported participation in fire extinction activities" has a design equivalent to a panel study. *P*-values were obtained with the function *glht* from *multcomp* package [Hothorn et al., 2008]. The results on 1-OHP, FEV1/FVC, CRP, SAA and ICAM-1 were logarithmically transformed because of a skewed distribution of residuals for the normal data. Effects are reported as percent changes and 95% confidence interval (95% CI) based on coefficient and intercepts in the mixed effects models, except for the logarithmically transformed variables where the percent change was obtained directly from the effect estimate using the expression: $(\exp^{estimate}-1)*100$.

RESULTS

Exposure Assessment

During all three monitoring days, a number of the emergency calls were false alarms or incidences that did not entail suppression of fires (e.g., flooding of a basement on day 3). A description of situations that entailed particle exposure is provided below. The supplement material includes a more detailed narrative of the observations at the fire scene as well as time-series measurements of the PM exposure during the fire suppression activities. It should be noted that each firefighter in a team has specific responsibilities at the scene of fire and specific tasks may or may not entail exposure to smoke.

All firefighters reported exposure to smoke on day 1, when they were called to both an outdoor fire in a car and a fire in an electrical installation in a basement (≈7 P.M., according to the firefighters report). On the second day, only one firefighter reported participation in fire extinguishing activities (≈8.30 P.M.). The observer noted that the firefighters did not wear personal protective equipment (PPE) with self-contained breathing apparatus during the inspection of an indoor suspected fire scene. The on-site measurements of UFPs peaked at number concentration of ≈90.000 #/cm^3 (Supporting Information Fig. S4). Day 3 included a small fire in a waste container (≈6 P.M.) and a large fire that

TABLE II. Levels of Outcome Markers before and after the 24-h Work Shift (Mean ± Standard Deviation) in 22 Firefighters

Outcome	Day 1 ($n = 7$)	Day 2 ($n = 8$)	Day 3 ($n = 7$)	Total ($n = 22$)
ΣPAH before (μg/m^2)	132.8 ± 17.0	301.8 ± 37.6	253.2 ± 32.4	232.5 ± 78.4
ΣPAH after[a] (μg/m^2)	155.6 ± 16.8	329.0	213.0	181.2 ± 60.4
1-OHP before (μmol/mol creatinine)	0.72 ± 0.62	0.24 ± 0.10	0.66 ± 0.58	0.52 ± 0.51
1-OHP after (μmol/mol creatinine)	0.47 ± 0.22	0.28 ± 0.20	0.98 ± 0.76	0.56 ± 0.53
FVC before (L)	5.02 ± 0.49	5.25 ± 0.73	5.19 ± 0.90	5.16 ± 0.70
FVC after (L)	5.06 ± 0.47	5.28 ± 0.88	5.17 ± 1.02	5.18 ± 0.79
FEV1 before (L)	4.09 ± 0.36	4.05 ± 0.69	4.12 ± 0.76	4.08 ± 0.60
FEV1 after (L)	4.02 ± 0.34	4.04 ± 0.76	4.03 ± 0.83	4.03 ± 0.65
ICAM-1 before (ng/mL)	38.1 ± 5.4	34.9 ± 5.6	30.0 ± 6.1	34.4 ± 6.4
ICAM-1 after (ng/mL)	41.7 ± 9.1	37.9 ± 7.4	30.7 ± 9.1	36.8 ± 9.3
VCAM-1 before (ng/mL)	101.4 ± 11.8	90.5 ± 25.7	82.2 ± 16.9	91.3 ± 20.1
VCAM-1 after (ng/mL)	114.5 ± 20.6	104.3 ± 36.1	89.9 ± 24.3	103.0 ± 28.7
DNA strand breaks before (lesions/10^6 bp)	0.13 ± 0.03	0.14 ± 0.05	0.09 ± 0.02	0.12 ± 0.04
DNA strand breaks after (lesions/10^6 bp)	0.14 ± 0.04	0.12 ± 0.05	0.13 ± 0.04	0.13 ± 0.04
Fpg-sensitive sites before (lesions/10^6 bp)	0.47 ± 0.07	0.44 ± 0.05	0.49 ± 0.08	0.47 ± 0.07
Fpg-sensitive sites after (lesions/10^6 bp)	0.40 ± 0.07	0.43 ± 0.08	0.41 ± 0.05	0.41 ± 0.07

ΣPAH, total polycyclic aromatic hydrocarbons; 1-OHP, 1-hydroxypyrene; ICAM-1, intercellular cell adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.
[a]Levels were only measured in 9 firefighters (7 from day 1, 1 from day 2, and 1 from day 3).

was initiated in a container and later spread to an apartment building (≈7.30 P.M.). Unfortunately, for logistic reasons, we do not have on-site measurements for the last event. The majority of firefighters reported exposure to smoke on day 3 (6 out of 7 firefighters, Table I). In addition to particle exposure from fires, our measurements suggested that a significant UFP exposure resulted from a call to a flooding incident on day 3 in the home of a heavy smoker (i.e., the objective number concentration measurements picked up secondary or tertiary smoke exposure).

Measurements of UFP inside the mask of one firefighter per work shift showed that use of PPE prevented inhalation exposure to PM during firefighting, and that exposure occurred when firefighters had taken off the mask (Supporting Information Figs. S3 and S6). Moreover, the exposure measurements did not demonstrate high exposure to PM on the assessed work shift events.

The skin concentration of ΣPAH before work shift was measured on all 22 subjects and was on average 233 μg/m^2 ± 78 (SD). For logistic reasons, we only succeeded in obtaining skin wipes immediately after returning to the station after the emergency call for 7 subjects from day 1, 1 from day 2, and 1 from day 3. For these 9 subjects, all with self-reported participation in fire extinction activities during the shift, the skin concentration of ΣPAH before the work shift was on average 179 μg/m^2 ± 95 (SD), and 181 μg/m^2 ± 60 (SD) after the shift.

The concentrations of 1-OHP in urine for each measurement day are reported in Table II. The results were further stratified according to self-reported participation in fire extinction activities (Table III). On day 2, the subjects had somewhat lower 1-OHP excretion levels as compared to the other days. The reason for this difference is not clear. However, pre-samples for subjects on day 2 were obtained at the same time as post-samples for subjects on day 1. Likewise, post-samples for subjects on day 2 were obtained at the same time as pre-samples from subjects on day 3. The highest concentrations of 1-OHP from the subjects on day 2 were higher than the lowest concentrations from the subjects on days 1 and 3. Thus, the difference in 1-OHP concentrations between days does not appear to be due to the sampling procedure. The levels of 1-OHP on days 1–3 contributable to smoking status (never smoker, history as smoker and current smoker) were not different. In general, there was no difference in levels of urinary 1-OHP before and after the work shift and self-reported participation in fire extinction activities (Table IV).

Biological Measurements

Table II shows the mean levels of biomarkers in samples from all firefighters obtained before and after the work shifts. The results were further stratified according to self-reported participation in fire extinction activities (Table III). Effect sizes are reported in Table IV as the difference related to the work period (i.e. difference in biomarkers between before and after the work shift) or self-reported fire extinction activities as proxy-measure for smoke exposure. The subjects had lower levels of Fpg-sensitive sites in PBMC after work shift compared to before work shift, whereas the levels of VCAM-1 were increased. No effects on ICAM-1, SAA, and CRP were observed. The self-reported participation in fire extinction activities during the work shift was associated with decreased levels of FEV1/FVC and Fpg-sensitive sites, whereas the VCAM-1 levels were increased. The levels of FVC and FEV1 were not altered after the work shift, irrespectively of participation in fire extinction activities.

TABLE III. Levels of Outcome Markers before and after the 24-h Work Shift (Mean ± Standard Deviation) in 22 Firefighters Stratified by Self-Reported Participation in Fire Extinction Activities During the Work Shift

	Outcome	Before work shift	After work shift
Self-reported participation in fire extinction activities ($n = 14$)	ΣPAH (μg/m^2)	205.3 ± 83.9	181.2 ± 60.4[a]
	1-OHP (μmol/mol creatinine)	0.66 ± 0.59	0.67 ± 0.57
	FVC (L)	5.04 ± 0.62	5.06 ± 0.71
	FEV1 (L)	4.02 ± 0.48	3.96 ± 0.55
	FEV1/FVC (%)	80.0 ± 4.4	78.3 ± 3.2
	CRP (mg/mL)	1.04 ± 0.68	1.79 ± 3.50
	SAA (mg/mL)	38.3 ± 33.0	54.4 ± 91.2
	ICAM-1 (ng/mL)	34.0 ± 7.0	36.8 ± 9.8
	VCAM-1 (ng/mL)	91.3 ± 18.1	103.3 ± 22.5
	DNA strand breaks (lesions/10^6 bp)	0.11 ± 0.04	0.14 ± 0.04
	Fpg-sensitive sites (lesions/10^6 bp)	0.48 ± 0.07	0.42 ± 0.06
Self-reported lack of participation in fire extinction activities ($n = 8$)	ΣPAH (μg/m^2)	280.2 ± 35.4	NA
	1-OHP (μmol/mol creatinine)	0.29 ± 0.18	0.36 ± 0.42
	FVC (L)	5.38 ± 0.81	5.38 ± 0.93
	FEV1 (L)	4.19 ± 0.80	4.17 ± 0.82
	FEV1/FVC (%)	77.6 ± 6.5	77.4 ± 7.3
	CRP (mg/mL)	1.81 ± 3.29	1.59 ± 2.41
	SAA (mg/mL)	46.6 ± 59.7	29.3 ± 31.4
	ICAM-1 (ng/mL)	35.1 ± 5.5	36.9 ± 9.1
	VCAM-1 (ng/mL)	91.3 ± 24.5	102.4 ± 39.2
	DNA strand breaks (lesions/10^6 bp)	0.13 ± 0.05	0.12 ± 0.04
	Fpg-sensitive sites (lesions/10^6 bp)	0.44 ± 0.05	0.41 ± 0.08

ΣPAH, total polycyclic aromatic hydrocarbons on back of the neck skin; 1-OHP, 1-hydroxypyrene; FVC, forced vital capacity; FEV1, forced expiratory volume after 1 second; CRP, C-reactive protein; SAA, serum amyloid protein; ICAM-1, intercellular cell adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; NA, not analyzed.

[a]Levels were only measured in 9 firefighters, all 9 with self-reported participation in fire extinction activities (7 from day 1, 1 from day 2, and 1 from day3).

The levels of IL-6 and IL-8 were generally below the limits of detection of the assays (2.34 and 1.56 pg/mL, respectively).

DISCUSSION

Use of PPE effectively prevented inhalation exposure, but exposure to PM occurred when the self-contained breathing apparatuses were removed. The firefighters had increased plasma levels of VCAM-1 and lowered levels of genotoxicity in PBMC after the work shift compared to pre-shift values. Slightly decreased lung function measurements were seen in statistical models with self-reported participation in fire extinguishing activities as predictor.

Firefighting encompasses many different jobs, which result in varying type and intensity of exposure, as it has been observed in previous studies [Fernando et al., 2016; Keir et al., 2017]. Thus, it is not surprising that the subjects reported differences in participation in fire extinguishing activities. In narrative, the firefighters described their tasks during a fire emergency as "fire suppression," "surveillance of the fire after first suppression," "in charge of the ladder," "water supply," and "team leader." The limited number of subjects precludes the possibility to distinguish between the tasks with respect to smoke exposure. In a recent Canadian biomonitoring study of 16 firefighters and 17 office workers, higher urinary levels of PAH metabolites were found for firefighters not using PPE or being engaged in vertical ventilation [Keir et al., 2017]. In the present study, PM measurements indicated that use of PPE effectively prevented inhalation exposure, but exposure to PM occurred when the environment was perceived as safe and the self-contained breathing apparatuses were removed. Similar patterns of exposure were observed previously for conscripts undergoing training as firefighters [Andersen et al., 2017, 2018]. Skin exposure level of ΣPAH before and after work shift was not essentially different (179–181 μg/m^2) and was on the same average level as observed previously for conscripts in control periods (151 μg/m^2 ± 99 [SD]) but in the lower end of the range after exposure (88–832 μg/m^2) [Andersen et al., 2018]. In the present study, the subjects had three days off prior to the work shift. On the three different biomonitoring days, pre-shift 1-OHP average values varied from 0.24 to 0.72 μmol/mol creatinine. Background levels of 1-OHP are influenced by various exposure pathways of PAH including inhalation (smoking, air pollution), skin exposure, and dietary intake of PAH [Jongeneelen, 2001]. Keir et al reported geometric means of the urinary 1-OHP level of firefighters as 0.1 μg/g creatinine pre-fire suppression and 0.27 μg/g creatinine after

TABLE IV. Percent Changes (95% Confidence Interval) in Outcome Levels Estimated by Linear Mixed Effects Model Using Work Shift or Self-Reported Participation in Fire Extinction Activities as Fixed Effects

Outcome	Work shift	Participation in fire extinction activities
1-OHP	3.0 (–25.7; 42.7)	6.1 (–34.1; 70.7)
FVC	0.3 (–1.4; 1.9)	0.5 (–1.1; 2.0)
FEV1	−1.2 (–2.9; 0.5)	−1.6 (–3.7; 0.5)
FEV1/FVC	−1.5 (–3.0; 0.004)	**–2.0 (–3.9; –0.1)**
CRP	6.2 (–15.5; 33.4)	2.9 (–24.4 39.8)
SAA	−6.8 (–27.4; 19.8)	3.9 (–22.8; 39.8)
ICAM-1	5.4 (–1.7; 13.1)	6.8 (–1.1; 15.3)
VCAM-1	**12.8 (4.1; 21.5)**	**13.2 (3.9; 22.4)**
DNA strand breaks	11.7 (–6.5; 30.0)	20.4 (–1.6; 42.4)
Fpg-sensitive sites	**–11.1 (–18.9; −3.2)**	**–13.8 (–22.1; −5.5)**

The percent change in outcome level (i.e., dependent variable in the linear mixed model) is the difference in biomarker levels "after" compared to "before" the work shift in 22 firefighters. The column "Participating in fire extinction activities" is the effect of smoke exposure at work (14 out of the 22 subjects participated in fire extinction activity). The percent change is the difference in the 14 "exposed" firefighters as compared to the eight "unexposed" firefighters.

1-OHP, 1-hydroxypyrene; FVC, forced vital capacity; FEV1, forced expiratory volume after 1 second; CRP, C-reactive protein; SAA, serum amyloid protein; ICAM-1, intercellular cell adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.

fire suppression [Keir et al., 2017]. This corresponds to 0.05 and 0.14 μmol/mol creatinine, respectively. In the current study, the average pre- and post-shift 1-OHP level was 0.52 ± 0.51 and 0.56 ± 0.53 μmol/mol creatinine, respectively. Thus, both pre- and post-shift 1-OHP levels tended generally to be higher for the Danish firefighters compared to the Canadian firefighters. In our study, 1-OHP was measured using reverse phase HPLC with fluorescence detection, whereas gas-chromatography-tandem mass spectrometry was used in the Canadian study. The differences in detection methods used and in lifestyle-dependent creatinine levels may complicate comparison between different studies. In a previous Danish study, using reverse phase HPLC with fluorescence detection as in the current study, non-smoking mail carriers had a urinary 1-OHP concentration of 0.1 μmol/mol creatinine (CI: 0.07; 0.13) and non-smoking bus drivers had 0.19 μmol/mol creatinine (CI: 0.16; 0.21) [Hansen et al., 2004]. This indicates that the Danish firefighters had higher urinary 1-OHP excretion levels than Danish non-smoking mail carriers. Background or reference levels of 0.24 μmol/mol creatinine for non-smokers and 0.76 μmol/mol creatinine for smokers have been suggested [Jongeneelen, 2001]. The observed post-shift 1-OHP average concentration of 0.56 ± 0.53 μmol/mol creatinine is below the proposed no-observed genotoxic effect level of 1.0 μmol/mol creatinine [Jongeneelen, 2014]. We did not restrict the dietary intake of PAH. However, in the previously mentioned Danish study of non-smoking bus drivers and mail carriers, no significant effects were detected for self-reported exposure to cooked food mutagens even though the study was relatively well-powered (ca. 100 participants per group) and 1-OHP levels were lower than in the present study [Hansen et al., 2004].

We observed slightly decreased levels of FEV1/FVC after the work shift, which may indicate a modest constriction of the conductive airways after exposure to smoke. The FEV1/FVC ratio is the proportion of the vital capacity that a person is able to expire in the first second of forced expiration. Levels lower than 80% might be an indication of obstructive lung disease. We regard the lung function measurements as demonstrative of subclinical and reversible acute alterations.

Interestingly, we observed a lower level of Fpg-sensitive sites in PBMC in the post-shift samples as compared to pre-shift samples. This is at odds with the expected effect, i.e. increased level of DNA damage, although it is not an unprecedented response after exposure to wood smoke. In a previous study we observed a decreased level of Fpg-sensitive sites in PBMC at 21-h after a short-term (3 h) exposure to wood smoke [Danielsen et al., 2008]. Fpg-sensitive sites are typically repaired with a half-life of approximately 6 h in cell cultures after exposure to PM [Danielsen et al., 2009]. A similar removal rate of Fpg-sensitive sites was observed in lung tissue after topical X-ray irradiation [Risom et al., 2003]. We have recently performed a similar human exposure study on 53 non-smoking conscripts undergoing a 3-day training course to become firefighters [Andersen et al., 2018]. Among these conscripts, firefighting was associated with increased PAH levels on skin, increased urinary 1-OHP excretion and increased levels of Fpg-sensitive sites in PBMC. A previous study reported similar levels of urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) for unexposed workers and firefighters who had been exposed at work [Hong et al., 2000]. However, the analysis was based on antibody-based detection of 8-oxodG, which is a highly unspecific and unreliable measurement of this lesion [Barregard et al., 2013]. Nevertheless, a study on wildland firefighters also showed no difference in urinary levels of 8-oxodG between pre- and post-shift samples after an episode of prescribed burns [Adetona et al., 2013]. It is generally acknowledged that 8-oxodG in urine does not originate from OGG1-mediated repair processes, whereas it is considered to be a biomarker of whole-body oxidative stress that has predictive value of lung and breast cancer risk [Loft and Moller, 2006; Loft et al., 2006, 2013].

A recent pooled analysis of case-control studies of firefighters, encompassing approximately 15,000 cases and 17,500 controls found no association with lung cancer risk with or without adjustment for smoking [Bigert et al., 2016]. IARC has classified exposures to diesel engine exhaust and PM in outdoor air as carcinogenic [IARC, 2014, 2016]. In contrast to this study in which no

genotoxicity is noted in the firefighters, there are numerous studies that have demonstrated associations between air pollution exposure and elevated levels of DNA adducts and oxidatively damaged DNA in PBMC and leukocytes from humans [Moller and Loft, 2010; Demetriou et al., 2012]. PM samples from different locations have shown concentration-dependent increases DNA damage in cell cultures [Moller et al., 2014]. These effects of air pollution particles on oxidation damage to DNA in PBMC are typically observed within hours of controlled inhalation exposure [Vinzents et al., 2005].

We found that plasma levels of soluble VCAM-1 were increased post-shift compared to pre-shift. ICAM-1 and VCAM-1 are considered to be markers of endothelial cell activation [Ballantyne and Entman, 2002]. Endothelial cells express these cell adhesion molecules on the membrane to bind inflammatory cells and assist the migration through the endothelium. ICAM-1 and VCAM-1 can be sequestered from plasma or translocated to the membrane from the cytoplasm. Thus, decreased levels of ICAM-1 and VCAM-1 in plasma may indicate increased binding to endothelial cells, whereas high plasma concentrations may indicate a higher turn-over of soluble cell adhesion molecules due to endothelial cell damage. Thus, soluble ICAM-1 and VCAM-1 levels may be increased (typically long-term exposures) or decreased (typically short-term exposures) in plasma, depending on the time-window between exposure (and endothelial cell activation) and blood sampling. Epidemiological studies have shown positive associations between long-term air pollution exposure and soluble ICAM-1 and VCAM-1 levels [Madrigano et al., 2010; Alexeeff et al., 2011; Chiu et al., 2016; Pope et al., 2016]. We recently reported no effect on ICAM-1 and VCAM-1 levels of a 3-day firefighting training course for conscripts [Andersen et al., 2018]. Short-term controlled exposure to wood smoke was associated with decreased levels of soluble ICAM-1 in plasma [Muala et al., 2015]. A one-week exposure also decreased plasma levels of ICAM-1 and VCAM-1 [Jensen et al., 2014]. However, increased levels of ICAM-1 and VCAM-1 were observed in plasma from wildland firefighters after exposure to wood smoke during prescribed burns [Hejl et al., 2013]. The differences in response to urban air pollution and wood smoke may be related to the physico-chemical characteristics of PM, although the time period between exposure and blood sampling is also important.

This study has several limitations. First, the study subjects only had 3 days without work prior to the biomonitoring day. This may result in elevated levels of some biomarkers in the pre-shift samples, due to potential effects from previous shifts. For instance, the half-life of the DNA damage, lung function measures, acute phase proteins, and cell adhesion molecules are not as well characterized as urinary 1-OHP. Levels of Fpg-sensitive sites are typically repaired with a half-live of 6 h in cell cultures, whereas there is more uncertainty about repair rates in humans and animal organs after exposure to PM with slow clearance. Second, there is a risk of exposure misclassification. It was not possible to objectively stratify the subjects according to exposure status as we only assessed personal exposure of a few selected firefighters and only during part of their work day, although we assessed urinary excretion of 1-OHP in all subjects of the study. All subjects may to some extent have been exposed to PM as the exposure measurements in the inhalation zone of a few firefighters showed exposure to PM under conditions the team leader considered sufficiently safe to not use mask. The measurement of 1-OHP is an objective measurement, but it only captures pyrene exposure and may also reflect other exposures, namely by ingestion route such as intake of grilled or smoked food. A non-differential exposure misclassification tends to drive effect estimates toward null. Third, the lack of restriction of PAH-rich food items and exposure to environmental tobacco smoke may have been important sources of 1-OHP levels and thus diluting the exposure gradient from occupational smoke exposure. Another limitation is the sequential study design with samples obtained before and after the work shift. This design does not control for period effects, which are unrelated to the exposure. For instance, we have not controlled for pre-work exposure to PAH from food or indoor combustion sources such as candle lights. Fourth, the number of subjects was small, thus limiting the statistical power. Lastly, the biological effective dose may not have been sufficiently large in the present study. This may be related to insufficient exposure gradient because the fires were not sufficiently large or complex to cause real exposure to the firefighters. This may partly depend on the PPE and training in avoidance of smoke plumes. In general, the PPE has high quality in the Danish fire departments. This limitation in exposure gradient can be overcome by conducting long-term panel studies, which increases the chance of catching situations of severe fires. However, the exposure assessment would probably have to rely on biological samples (e.g., urinary excretion of 1-OHP) and questionnaire data because it is costly to obtain 24-h ambient air measurements of PM with involvement of scientific personal.

In conclusion, this study indicates that firefighters are not exposed to PM when firefighting while wearing PPE, but exposure may occur in situations that are considered as safe. Firefighting did not increase the PAH levels on the skin (back of the neck) or 1-OHP concentrations in urine. The work shift was not associated with increased levels of genotoxicity or decreased lung function, while increased level of VCAM-1 in plasma was observed.

STATEMENT OF AUTHOR CONTRIBUTIONS

MHGA analyzed the results and wrote the draft of the manuscript. ATS designed and coordinated the study, supervised the data analysis and the writing of the

manuscript. JEP collected and reported the lung function and questionnaires data. PBP measured and reported the exposure. PAC supervised the collection, analysis and report of the dermal PAH data. ML performed the DNA damage analysis. AK supervised and reported on the ICAM-1 and VCAM-1 analysis. SL designed the study and was a major contributor in the analysis and interpretation of results. NE designed the study and contributed to the analysis and interpretation of results. AMH supervised the analysis and report of 1-OHP. IKK assisted in the exposure assessment. ECN contributed to the interpretation of results. UV designed and supervised the study and was a major contributor in the writing of the manuscript. PM designed and supervised the study and was a major contributor to the analysis, interpretation and writing of the manuscript. All authors have read and approved the final manuscript.

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COMPETING FINANCIAL INTERESTS

The authors claim no competing financial interests.

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P. White