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Running title: PAH exposure and DNA damage after firefighting activity.

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ABSTRACT

This study investigated a number of biomarkers, associated with systemic inflammation as well as genotoxicity, in 53 young and healthy subjects participating in a course to become firefighters, while wearing personal protective equipment. The exposure period consisted of a three-day training course where the subjects participated in various live-fire training exercises in a constructed training building (burn house) and flashover container. The subjects were instructed to extinguish fires of either wood or wood with electrical cords and mattresses. The personal exposure was measured as dermal polycyclic aromatic hydrocarbon (PAH) concentrations and urinary excretion of 1-hydroxypyrene (1-OHP). The subjects were primarily exposed to particulate matter in by-stander positions, since the self-contained breathing apparatus effectively prevented pulmonary exposure. There was increased dermal exposure to pyrene (68.1%, 95% CI: 52.5%, 83.8%) and ΣPAH (79.5%, 95% CI: 52.5%, 106.6%), and increased urinary excretion of 1-OHP (70.4%, 95% CI: 52.5%; 106.6%) after the firefighting exercise compared to control measurements two weeks before and two weeks after the fire fighting course. The level of Fpg-sensitive sites in peripheral blood mononuclear cells (PBMCs) was increased by 8.0% (95% CI: 0.02%, 15.9%). The level of DNA strand breaks was positively associated with dermal exposure to pyrene and ΣPAHs, an urinary excretion of 1-OHP. Fpg-sensitive sites were only associated positively with PAHs. Biomarkers of inflammation showed no consistent response. In summary, the study demonstrated that PAH exposure during firefighting activity was associated with genotoxicity in PBMCs.

INTRODUCTION

Firefighters are potentially exposed to numerous types of carcinogens during fire suppression. The exposure depends on the nature and source of the fire, which the International Agency for Research on Cancer (IARC) has broadly categorized as municipal, wildland, industrial, aviation, military and oil well fire scenarios. IARC evaluated occupational firefighting activity as possibly carcinogenic to humans (Group 2B), based on increased risk of testicular and prostate cancer as well as non-Hodgkin's lymphoma (1). Pulmonary exposure to particles causes inflammation, which is considered to be an important mechanism for both lung cancer and systemic effects. Pulmonary inflammation and measures of acute toxicity has been described in firefighters and noted by IARC as a potential mechanism of carcinogenesis (1). Nevertheless, the IARC evaluation did not find evidence for increased risk of lung cancer among firefighters (1) and recently a pooled analysis of case-control studies, encompassing approximately 15,000 cases and 17,500 controls showed no association between firefighting and lung cancer risk with or without adjustment for smoking (2). The IARC expert committee noted an insufficient number of studies to evaluate genotoxic effects in firefighters.

Polycyclic aromatic hydrocarbons (PAHs) are an important group of genotoxic compounds in smoke that can form DNA adducts (3). Exposure to PAHs during firefighting can occur both by skin exposure to soot and by inhalation of combustion particles (1). The exposure to PAHs can be assessed by measurement of compounds such as pyrene and benzo[a]pyrene (BaP) on the skin or the urinary excretion of 1-hydroxypyrene (1-OHP). The latter is the excreted metabolite of pyrene and is commonly used as a biomarker for complex PAH exposure (4). It has been shown that firefighting activity was associated with dermal exposure to PAHs and increased urinary excretion of 1-OHP (5), indicating systemic exposure to PAHs by either dermal and possibly also inhalation routes.

There is a large body of literature on the association between inhalation of particulate matter (PM) and DNA damage in terms of DNA adducts, oxidatively damaged DNA and clastogenic effects in peripheral blood mononuclear cells (PBMCs) and leukocytes from humans (6). Such effects of air pollution particles on oxidation damage to DNA in PBMCs are typically observed within hours of controlled inhalation exposure (7). Likewise, particle-induced pulmonary inflammation has been shown to induce systemic inflammation and acute phase response (8,9). The aim of the present study was to investigate the effect of firefighting activities on lung function, systemic inflammation and DNA damage in circulating PBMCs. It is possible that pulmonary inflammation spills over to the circulation and causes low-grade systemic inflammation with elevated levels of C-reactive protein (CRP), serum amyloid protein (SAA), interleukin 6 and 8 (IL6 and IL8), intracellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) (8). We performed an exposure study of recruited healthy, young non-smoking volunteers from a rescue education course for firefighting. This three-day course involved various smoke diving exercises in a burn house and stays in flashover containers. The fuels for fires in the firehouse were EU wood pallets with or without the addition of electrical cords and mattresses to mimic a fire situation in a municipal setting. The study is part of a larger investigation on occupational exposure to combustion-derived compounds, cardiovascular risk factors and genotoxicity in firefighters.

METHODS

Study subjects and study design

The study design, study population, airborne PM concentrations and cardiovascular risk factors have previously been described (Andersen et al, submitted for publication). Briefly, we

recruited conscripts from a rescue specialist educational course, a nine-month residential education program under the Danish Emergency Management Agency. The subjects were healthy, self-reported non-smokers, not pregnant and with no history of drug or alcohol abuse. We used non-smokers in order to have maximum exposure contrast with respect to PAH because smokers have higher urinary levels of 1-OHP (4). Fifty-four subjects were enrolled from four different and consecutive training classes (campaigns) that took place in summer, autumn and winter of 2015 and spring of 2016: campaign 1 (9 conscripts), campaign 2 (11 conscripts), campaign 3 (17 conscripts) and campaign 4 (17 conscripts) (table I). One subject from campaign 1 dropped out of the training course hence was excluded from the study. The age of the 53 subjects varied from 18 to 26 years. Thirty-nine subjects had a body mass index (BMI) between 18.5 and 24.9 kg/m^2 and 14 had BMIs between 25.0 and 29.5 kg/m^2.

The design was a cross-over study, where the subjects served as their own controls, in three exposure scenarios separated by two week periods with the firefighting-related exposure in the middle. The subjects extinguished fires from either combustion of standard wooden EUR pallets in absence (campaign 1 and 2) or presence (campaign 3 and 4) of foam mattresses and electrical cords. Firefighting exercises were carried out during the 3 days using personal protective equipment (PPE), including self-contained breathing apparatus. The self-contained air supply was taken off outside the burn house during instruction, feedback and breaks in zones that were considered smoke-free by the instructors of the training course. The assessment of the personal exposure to ultrafine particles in two conscripts indicated that PM inhalation exposure occurred mainly during these bystander situations where the subjects were not actively extinguishing fires. The PPE including the self-contained breathing apparatus effectively prevented particle exposure during smoke-diving as documented by measurement of the personal particle exposure in the inhalation zone of two conscripts during smoke-diving

(Andersen et al, submitted for publication). However, dermal exposure occurred as a consequence of deposition of soot on the skin. The present study used skin concentrations of PAHs and urinary 1-OHP excretion as personal exposure markers. The 1-OHP measurements from 43 out of the 53 subjects in the present publication have been previously published (Andersen et al, submitted for publication).

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

Sample collection

Biological samples were collected on three occasions namely -14 days before the smoke-diving course, immediately after the 3-day course exercises and 14 days subsequent to the end of the training session.

Dermal wiping of the neck was carried out to assess the PAH exposure on the skin. A skin area of approximately 3 cm x 6 cm on the back of the neck was wiped with a "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 exchanged for each wipe. The wipes were placed in 15-ml screw cap glass vials with foil-lined lid (Wheaton), which were placed 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 expriments, 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 analysed for each series of wipe samples. None of the individual PAHs were above the limit of detection (LOD) in any of the blanks.

For the ELISA analysis of SAA, CRP, ICAM-1, VCAM-1, IL-6 and IL-8, plasma was prepared by 10 min centrifugation at 4000 rpm (1780 x g) of blood collected into EDTA-coated tubes. The plasma samples were prepared in a room adjacent to the fire house and were transported to the laboratory on ice on the same day. At arrival the plasma samples were stored at −80°C until ELISA analysis.

PBMCs were isolated using Vacutainer Cell Preparation Tubes (Vacutainer® CPT Becton Dickinson A/S, Brøndby, Denmark). PMBCs were separated by 20 min centrifugation at 1650 x g. The PBMCs were diluted in 3 ml ice-cold medium (RPMI with 10% foetal bovine serum and 1% Pen/Strep). The samples were transported to the laboratory on ice on the same day. At arrival, the PBMCs were separated by 15 min centrifugation at 300 x g at 5°C and re-suspended in 3 mL RPMI medium with 10% foetal bovine serum and 1% Pen/Strep. The same centrifugation procedure was repeated and the PBMCs were re-suspended in freezing medium (RPMI with 50% foetal bovine serum and 10% DMSO). The PBMCs were stored at −80°C until analysis of DNA damage by the comet assay.

For the determination of 1-OHP, urine samples were collected in the morning. The urine collected 2 weeks before and after exposure was used to assess the control background levels. For the exposure scenario, the urine was collected on the day after the 3-days fire extinguishing exercises. The urine samples were kept in cooling boxes until arrival at the laboratory and at −20°C until analysis. Urinary creatinine was used to standardise the result.

1-OHP analysis

Reverse-phase HPLC was used for the quantitative measurement of 1-OHP in urine using a previously published method (10). We standardised for diuresis with the concentration of creatinine as used in other studies (11).

Determination of PAH from skin wipes by GC-MS analysis

The extraction of PAH were carried out by covering the wipes with 6 mL cyclohexane in 10 mL glass vials and sonicate for 30 min in an ultra-sonic bath (Branson 5200, output power 120 W at extraction of 25 samples at one time). One millilitre 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 an Bruker CP-8400 auto sampler to a programmable temperature vaporising (PTV) injector at 280° into the column with a He flow of 1 mL/min. The column was 30 m x 0.25 mm with 0.25 µm film thickness (VF-5MS, Agilent Technologies, US). 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 16 PAH (analytical details are shown in the Supplement).

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). A single spirometer was used in all tests to ensure standardization of the measurements, with the equipment calibrated before each testing session. All measurements were performed with the conscripts 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.

Inflammation markers analysis

The concentrations of sICAM-1 (Cat. No. 560269) and sVCAM-1 (Cat. No: 560427) was assessed in plasma with BD cytometric bead array system, utilising Accuri CFlow®Plus software (BD Bioscience) according to methods described previously (12). Plasma levels of SAA and CRP were determined by Enzyme-linked Immunosorbant assay (ELISA) from Invitrogen (CA, USA) and IBL International GMBH (Hamburg, Germany), respectively, as described previously (13). 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

The levels of DNA strand breaks and Fpg-sensitive sites were detected by the comet assay as described previously (14). Briefly, PBMCs 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

minutes 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 3 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 scored using an Olympus fluorescence microscope at 40x magnification with visual inspection after staining with YOYO-1 in PBS (Molecular Probes, Eugene, OR, USA). All samples from one subject were coded and analysed simultaneously in order to minimise inter-assay variation. In addition, each batch of comet assay experiments included samples from the four campaigns in order to control for inter-day experimental variation. We analysed 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 (15). We had assay control samples in each experiments (corresponding to each electrophoresis) that consisted of $KBrO_3$ exposed THP-1 cells (5 mM for 1 hour 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 (14), based on the assumption that an

average molecular weight of a DNA bp is 650 Dalton and one Gy yields 0.29 breaks per 10^9 Dalton DNA (16).

Statistical analysis

We used R statistical language and the package *lme4* (17) to perform a linear mixed effects analysis of the relationship between the biomarkers and exposure. As fixed effect we used factorial variable of exposure (before/exposure/after). The exposure term in the statistical analysis was either exposure period (i.e. one exposure and two non-exposure periods within each campaign) or type of fire (i.e. wood or wood with mattresses and electrical cords). As random effects, we used by-subject intercepts. P-values were obtained with the function *glht* from *multcomp* (18). The results on urinary excretion of 1-OHP were cubic root transformed and DNA strand breaks, CRP and SAA were logarithmic transformed because of a skewed distribution of residuals for the normal data. A few outlier values were eliminated from the dataset (1-OHP: 3.48 µmol/mol creatinine, control before; VCAM-1: 13210.3 ng/mL, exposure; CRP: 11.0 and 35.5 mg/L, control before and exposure, respectively; SAA: 572.4 and 596.2 mg/L, control before and exposure, respectively). The percent changes were obtained by dividing the estimate change with the intercept value from the mixed model graph line and multiplying with 100, except for CRP, SAA and DNA strand breaks where the percent change was obtained directly from the effect estimate using the expression: $(\exp^{estimate}-1)*100$. Welch t-test was used to compare the difference in means of effect change between exposed and unexposed scenarios for the different types of fire. A few subjects were eliminated from the dataset for the t-test analysis due to missing values in the control or exposure measurements (1 subject for DNA strand breaks and Fpg-sensitive sites, 3 subjects for Pyrene and ΣPAH and 10 subjects for 1-OHP).

RESULTS

PAH exposure assessment

Skin exposure to soot occurred both by handling contaminated equipment and during firefighting. In a pilot study, PAH content of wipes of different skin areas were assessed immediately before (i.e. after putting on the PPE, but before smoke diving) and after smoke diving (figure 1). The PAH content on the skin wipes from the neck was high before and after smoke diving, whereas there were substantial differences in the ΣPAH concentrations from different parts of the body. For the main study, we selected skin swipes from the back of the neck. During the main study, firefighting increased skin exposure to PAH by 79.5% (95% CI: 52.5, 106.6) and pyrene by 68.1% (95% CI: 52.5, 83.8) compared to control situations (figure 2, table II).

The internal dose of PAH was assessed using 1-OHP excretion in urine. Thefirefighting increased 1-OHP concentration in urine by 70.4% (95% CI: 33.4, 113.5) compared to control period (figure 3). Urinary 1-OHP concentrations were positively associated with ΣPAH and pyrene content on the skin (table III).

A stratification of the exposure markers according to the type of fuel for the fires is outlined in table IV. The firefighting exercises using only wood pallets was associated with higher exposure in terms of pyrene ($p < 0.001$) and ΣPAH ($p < 0.001$) on the skin as well as 1-OHP ($p < 0.001$) in urine as compared to the fire that was supplemented with electrical cords and mattresses.

Systemic inflammation

SAA, CRP, ICAM-1 and VCAM-1 levels in plasma were unaffected by exposure (figure 4, table II) and type of fire (table IV). The levels of IL-6 and IL-8 were below the limit of detection (only 11 and 7 observations were detectable respectively, from a total of 158).

Lung function

The conscripts had unaltered lung function after firefighting as compared to the control periods (table II). There was statistically significant differences between the types of fire and lung function tests; however, these biological responses are regarded as ambiguous as no discerning pattern is recognised (table IV).

Levels of DNA damage in PBMCs

Firefighting was not statistically significantly associated with increased levels of DNA strand breaks, whereas there was a positive association between firefighting activity and levels of Fpg-sensitive sites (figure 5 and table II). The levels of DNA strand breaks were increased after the fighter training course especially for campaign 2 (see supplement). DNA strand break levels correlated positively with ΣPAH levels on the skin ($p < 0.001$) and with 1-OHP levels in urine ($p < 0.001$) (table III). The levels of Fpg-sensitive sites were positively correlated with PAH levels on skin ($p < 0.01$), but not with 1-OHP levels in urine. The induction of DNA strand breaks was stronger after the firefighting exercise with wool pallet fuel as compared to mixed fuels ($p < 0.001$; table IV). A similar pattern was observed for Fpg-sensitive sites, although it did not reach statistical significance ($p = 0.09$, table IV).

DISCUSSION

This study showed that participation in a three-day fire-fighting training with smoke diving exercises was associated with elevated levels of ΣPAH on the skin, 1-OHP in urine and Fpg-sensitive sites in PBMCs. No effect of exposure on lung function (FEV1, FVC or FEV1/FVC) or markers of systemic inflammation was observed.

The subjects were exposed to soot on the skin. Potential inhalation exposure to PM occurred in situations when the subjects were not using the self-contained breathing apparatus. The inhalation exposure typically occurred at bystander locations where the subjects were gathered for instructions or waiting for their turn of smoke diving. The skin exposure to ΣPAH and pyrene was considerably increased after the smoke diving exercises on wood fires. Previously, it has been shown that training firefighting exercises with wood fires are associated with markedly increased whole-body PAH exposure (5). We used 1-OHP as biomarker of the internal PAH dose since 1-OHP is the metabolite of pyrene (4). Pyrene is not a carcinogen, but the carcinogenic potential of the PAH mixture can be estimated from the amount of pyrene and the ratio between pyrene and BaP (4). In previously studied occupational exposures such as in coke oven workers, the ratio between pyrene and the carcinogenic BaP was approximately 2.5 (4). In the current study, the pyrene/BaP ratio was estimated to be 0.1, indicating that the soot had high carcinogenic potential. Urinary concentrations of 1-OHP were increased after firefighting training with wood and mixed fuel fires, indicating systemic PAH exposure. The 1-OHP concentrations in control measurements were similar to previous reported background levels (<0.1 µmol/mol creatinine) (4). The urinary excretion of 1-OHP increased especially in the subjects who participated in the firefighting exercises on wood pallets (0.6 µmol/mol creatinine) although the levels did not exceed the level that has been defined as a “no observed genotoxicity effect level for occupational exposure” (1.0 µmol/mol creatinine) (4).

The firefighting training is a multifaceted situation with elevated PAH exposure, heat and physical exercise. Each of these conditions or their combinational effects may be genotoxic. Nevertheless, PM, PAHs, certain volatile organic and semi-quinone compounds are well-described genotoxic carcinogens (19). PAHs are able to penetrate the skin, and it has been shown that a single application of pharmacological coal tar on the skin of hairless mice induces PAH-DNA adduct formation in skin and liver as well as increased levels of DNA strand breaks and increased mutation frequency in epidermal cells (20). Previous observations did not indicate higher levels of PAH-DNA adducts in leukocytes from municipal firefighters as compared to matched controls (21). Likewise, there was no association between levels of PAH-DNA adducts in leukocytes and fire-fighting activity in wildland firefighters in California, USA (22). A study on volunteer firefighters demonstrated no difference in levels of PAH-DNA adducts in lymphocytes between pre- and post-shift samples, although the subjects stated that they had been exposed to plumes of smoke from oil well fires (23).

Here, we assessed both DNA strand breaks and the Fpg-sensitive sites in PBMCs. Previous studies on traffic-related air pollution exposures or wood smoke are summarised in table V. Exposure to air with mixed pollution from busy streets appear to increase levels of in particular Fpg-sensitive sites whereas diesel exhaust alone or wood burning for heating in a stove or a fire place showed no apparent effects on these biomarkers. The levels of DNA strand breaks (15.6%, 95% CI: -0.60%; 34.4%) and Fpg-sensitive sites (8.0%, 95% CI: 0.02%; 15.9%) are not overtly different from levels reported in other studies using the same protocol for assessment of DNA damage in PBMCs. Another study has shown increased levels of DNA strand breaks in lymphocytes from a municipal firefighting episode at a chemical plant where the firefighters had not worn PPE (24). Studies on controlled exposure to wood smoke

indicated a lack of genotoxicity in terms of DNA strand breaks and oxidatively damaged DNA in PBMCs (25,26). However, a recent study showed increased level of DNA damage in wideland firefighters (27). Interestingly, we found positive associations between DNA strand breaks levels and all measures of PAH exposure (pyrene, ΣPAH and 1-OHP), whereas the level of Fpg-sensitive sites was associated with dermal ΣPAH levels. DNA strand breaks measured by the alkaline comet assay represent strand breaks in the DNA, alkaline labile sites and breaks that occur as a consequence of base and nucleotide excision repair of DNA lesions. The Fpg-modified comet assay detects oxidatively damaged DNA and has been used widely in studies on air pollution exposure in biomonitoring studies (28,29). The association between heavy PAHs such as BaP and levels of oxidatively damaged DNA is in keeping with observations that metabolites of BaP can generate superoxide anion radicals via redox cycling (30). The strong associations between PAHs including pyrene on skin, 1-OHP and DNA strand breaks in PBMCs as well as the high BaP content in the soot indicate that skin exposure to PAH contributes to the increased DNA strand break levels. Importantly, these associations were found in the same concentration range of urinary 1-OHP as the recommended exposure limit of 1.0 μmol/mol creatinine.

There have been numerous studies on associations between exposure to physical exercise and levels of DNA damage in leukocytes or lymphocytes. Physical activity typically entails a short-term increase in the body-temperature due to the increased energy expenditure. Our previous studies demonstrated no effect on DNA strand breaks or Fpg-sensitive sites in PBMCs after an exhaustive exercise test (31). However, other studies have demonstrated elevated levels of DNA strand breaks and oxidatively damaged DNA in PBMCs or leukocytes after long-term and exhaustive exercise such as completing a marathon race, which is associated with tissue damage and inflammation (32). Heat exposure is a well-known inducer of heat shock genes and

DNA repair pathways in cell cultures, whereas the mechanisms of genotoxicity, including DNA strand breaks, are not elucidated (33). The effect of physical hyperthermia or intermittent heat exposures on DNA damage levels in PBMCs remains to be investigated in controlled studies. Associations between fever-associated diseases and genotoxicity are most likely confounded by the direct effect of the infectious agent on the genome.

We did not observe any consistent effect on lung function, plasma levels of cytokines and acute phase proteins, although CRP levels were statistically significantly increased after firefighting training as compared to the control samples collected 2 weeks after the firefighting training (table II). In a controlled study, inhalation exposure to wood combustion particles increased levels of SAA and IL6 in human volunteers (34). However, low level exposures did not increase markers of systemic inflammation (35). A meta-analysis indicated little association between controlled exposure to combustion-derived PM and systemic low-grade inflammation in humans (36). It should be emphasized that inflammation is not a pre-requisite for generation of DNA strand breaks and oxidatively damaged DNA in either lung tissue or the blood compartment (8,37).

CONCLUSION

In conclusion, exposure of young, healthy non-smoking subjects to a 3-day firefighting course resulted in increased skin exposure to PAH including pyrene, increased urinary 1-OHP levels and increased levels of Fpg-sensitive sites in PBMCs. ΣPAH and pyrene levels on skin were strongly associated with 1-OHP. ΣPAH on skin and 1-OHP in urine both correlated with DNA strand break levels in PBMCs.

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Table I. The characteristics of the subjects in the control measurements

Characteristics	Females (n=12)	Males (n=41)	All (n=53)
Age (years)	21.9 (2.4)	21.2 (1.6)	21.4 (1.8)
Height (cm)	172.2 (3.1)	181.4 (6.5)	179.3 (7.0)
Weight (kg)	67.3 (9.6)	77.5 (11.3)	75.2 (11.7)
BMI (kg/m^2)	22.7 (2.9)	23.5 (2.6)	23.3 (2.7)
Allergies (n)	3	10	13
1-OHP (µmol/mol creatinine)[a]	0.4 (0.3)	0.4 (0.3)[b]	0.4 (0.3)
Pyrene ($µg/m^2$)[a]	26.2 (13.3)	27.8 (12.9)	27.5 (12.9)
ΣPAH ($µg/m^2$)[a]	176.1 (146.7)	143.5 (81.1)	150.8 (99.0)
FEV1 (L)[a]	3.6 (0.5)	4.7 (0.6)	4.4 (0.7)
FVC (L)[a]	4.2 (0.6)	5.6 (0.8)	5.3 (0.9)
FEV1/FVC[a]	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)
DNA strand breaks (lesions/10^6 bp)[a]	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
Fpg-sensitive sites (lesions/10^6 bp)[a]	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)
ICAM-1 (ng/mL)[a]	29.5 (7.9)	30.0 (6.3)	29.9 (6.6)
VCAM-1(ng/mL)[a]	126.2 (25.8)	139.3 (33.8)	136.3 (32.4)
CRP (mg/mL)[a]	1.4 (1.3)	0.9 (0.9)	1.0 (1.0)
SAA (mg/mL)[a]	16.1 (12.5)	18.0 (19.1)	17.6 (17.8)

BMI, body mass index; 1-OHP, 1-hydroxypyrene; ΣPAH, polycyclic aromatic hydrocarbons; FEV1, forced expiratory volume after 1 second; FVC, forced vital capacity; ICAM-1, intercellular cell adhesion molecule 1; VCAM-1, vascular cell adhesion molecule; CRP, C-reactive protein; SAA, serum amyloid protein. Values are number or mean and standard deviation. [a] Values are mean of the means from both control measurements (before and after)

Table II. Percent changes (95% confidence interval) in outcome levels estimated by mixed effects model

	Exposure vs Before	Exposure vs After	After vs Before	Exposure vs Unexposed[a]
Pyrene	36.7 (23.2, 50.2)***	117.4 (96.1, 138.7)***	-37.1 (-50.4, -23.9)***	68.1 (52.5, 83.8)***
ΣPAH	74.6 (50.8, 98.2)***	84.6 (59.8, 109.5)***	-5.5 (-28.7, 17.7)	79.5 (52.5, 106.6)***
1-OHP[b]	124.8 (73.1, 185.7)***	49.7 (14.7, 91.1)**	50.4 (11.6, 97.3)**	70.4 (33.4, 113.5)***
ICAM-1	1.9 (-4.6, 8.4)	5.1 (-1.6, 11.8)	-3.1 (-9.6, 3.5)	3.5 (-2.0, 9.0)
VCAM-1	0.4 (-8.1, 9.0)	-1.1 (-9.5, 7.3)	1.5 (-7.0, 10.1)	-0.4 (-6.5, 5.7)
CRP	33.8 (-4.8, 88.2)	56.1 (11.3, 119.0)**	-14.3 (39.0, 20.6)	24.4 (-7.7, 67.7)
SAA	11.7 (-15.1, 47.0)	16.4 (-11.4, 52.9)	-4.0 (-27.1, 26.3)	2.5 (-19.9, 31.2)
FEV1	-0.9 (-2.0, 1.0)	0.5 (-1.4, 2.4)	-1.5 (-3.4, 0.5)	-0.2 (-1.9, 1.5)
FVC	-0.03 (-1.6, 1.6)	-0.4 (-2.0, 1.1)	0.4 (-1.2, 1.2)	-0.2 (-1.7, 1.2)
FEV1/FVC	-0.8 (-2.1, 0.4)	0.9 (-0.4, 2.1)	-1.7 (-2.9, -0.4)**	0.1 (-0.8, 0.9)
DNA strand breaks	11.4 (-3.2, 28.2)	21.2 (5.4, 39.4)**	-8.1 (-20.1, 5.7)	15.6 (-0.6, 34.4)
Fpg-sensitive sites	10.2 (1.4, 18.9)*	5.4 (-3.0, 13.7)	4.6 (-4.1, 13.3)	8.0 (0.02, 15.9)*

ΣPAH, sum of 16 polycyclic aromatic hydrocarbons; 1-OHP, urinary excretion of 1-hydroxypyrene adjusted for excreted creatinine concentration; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; CRP, C reactive protein; SAA, serum amyloid A protein; FEV1, forced expiratory volume after 1 second; FVC, forced vital capacity. Results are percent change from the mixed effect model in figures 2-5. The data is based on 53 individuals. *,**,*** Significantly different ($p<0.05$, $p<0.01$ and $p<0.001$ respectively)

[a] Unexposed corresponds to the mean between "Before" and "After" for each subject.

Table III. The associations between biomarkers of external PAH exposure, internal PAH dose (i.e. 1-OHP) and genotoxicity in PBMCs assessed at 3 different time-points for 53 volunteers.

Predictor	Dependent variable	Estimate ± SE
1-OHP (urine)	Pyrene (skin)	0.00295 ± 0.00116*
1-OHP (urine)	PAH (skin)	0.00061 ± 0.000148***
DNA strand breaks (PBMCs)	1-OHP (urine)	0.6492 ± 0.1679***
DNA strand breaks (PBMCs)	PAH (skin)	0.0015 ± 0.0003***
DNA strand breaks (PBMCs)	Pyrene (skin)	0.0033712 ± 0.00059***
Fpg-sensitive sites (PBMCs)	1- OHP (urine)	-0.3656 ± 3.4941
Fpg-sensitive sites (PBMCs)	PAH (skin)	0.02595 ± 0.01004**
Fpg-sensitive sites (PBMCs)	Pyrene (skin)	0.0001584 ± 0.0003691
Pyrene (skin)	1-OHP (urine)	35.331 ± 6.685***
ΣPAH (skin)	1-OHP (urine)	269.866 ± 51.441***
ΣPAH (skin)	Pyrene (skin)	5.3257 ± 0.3961***

PBMCs, peripheral mononuclear blood cells. The estimates are based on non-transformed (pyrene, ΣPAH and Fpg-sensitive sites), cubic root transformed (1-OHP) and logarithmically transformed (DNA strand breaks) data. *,**,*** Significantly different ($p<0.05$, $p<0.01$ and $p<0.001$ respectively).

Table IV. Average effect change between exposure and unexposed situations for two different types of fires: wood pallets and wood pallets with mattresses and electrical cords (mixed fuel)

	Net effect of exposure[a]		
	Wood pallet fuel	Mixed fuel	Difference between wood pallet and mixed fuel[b]
Pyrene (µg/m^2)	30.4 (14.3)	12.3 (12.4)	18.1 [9.6; 26.6]***
ΣPAH (µg/m^2)	292.5 (144.7)	35.1 (35.2)	257.4 [179.6; 335.2]***
1-OHP (µmol/mol creatinine)	0.6 (0.5)	0.1 (0.5)	0.5 [0.1; 0.8]***
ICAM-1 (ng/mL)	0.05 (5.44)	1.59 (6.44)	1.54 [-4.91;1.82]
VCAM-1 (ng/mL)	-5.35 (30.47)	2.21 (31.08)	7.55 [-25.45; 10.34]
CRP (mg/L)	0.11 (1.2)	0.25 (1.6)	0.14 [-0.92; 0.65]
SAA (mg/L)	5.85 (21.3)	4.04 (24.9)	1.81 [-11.47; 15.09]
FEV1 (L)	0.19 (0.21)	-0.12 (0.25)	0.30 [0.17; 0.43]***
FVC (L)	0.21 (0.25)	-0.13 (0.21)	0.34 [0.20; 0.48]***
FEV1/FVC	0.003 (0.02)	-0.001 (0.03)	0.004 [-0.01; 0.02]
DNA strand breaks (lesions/10^6 bp)	0.213 (0.23)	-0.021 (0.05)	0.23 [0.12; 0.35]***
Fpg-sensitive sites (lesions/106 bp)	0.058 (0.11)	0.008 (0.08)	0.05 [-0.008, 0.107]

ΣPAH, sum of 16 polycyclic aromatic hydrocarbons; 1-OHP, urinary excretion of 1-hydroxypyrene adjusted for excreted creatinine concentration; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; CRP, C reactive protein; SAA, serum amyloid A protein; FEV1, forced expiratory volume after 1 second; FVC, forced vital capacity. *,**,*** Significantly different ($p<0.05$, $p<0.01$ and $p<0.001$ respectively). [a] Average difference (and SD) between between exposure and the mean of control measurements (i.e. periods before and after the exposure period). [b] Welch t-test (mean difference and 95% CI)

Table V. Percent difference in DNA strand breaks and Fpg-sensitive sites in PBMCs between particle-rich (E) and control (C) exposures

Description	Exposure contrast	DNA strand breaks (E/C)[a]	Fpg-sensitive sites (E/C)[a]	Ref
Subjects in Cotonou, Rep Benin (cross-sectional study)	>250,000 versus <10,000 UFP/cm^3 (during midday)	**33% (18, 49)** 0.16 ± 0.10 0.12 ± 0.05 (P<0.05)	**145% (136, 155)** 0.27 ± 0.05 0.11 ± 0.03 (P<0.001)	(38)
Cycling in Copenhagen Denmark	32,400 versus 13,400 UFP/cm^3	**0% (-56, 56)** 0.06 ± 0.08 (IQR) 0.06 ± 0.10 (IQR) (NS)	**300% (188, 412)** 0.08 ± 0.08 (IQR) 0.02 ± 0.04 (IQR) (P<0.001)	(7)
Controlled exposure to street air	10,067 versus 235 UFP/cm^3	**50% (20, 80)** 0.24 ± 0.21 (IQR) 0.16 ± 0.16 (IQR) (P<0.05)	**39% (18, 61)** 0.53 ± 0.28 (IQR) 0.38 ± 0.33 (IQR) P<0.05	(39)
Controlled wood smoke exposure (3 h)	>95,000 versus <10,000 UFP/cm^3	**-51% (-65, 31)** 0.04 ± 0.03 0.08 ± 0.08 (NS)	**-15% (-31, 4.9)** 0.23 ± 0.13 0.25 ± 0.12 (NS)	(25)
Controlled wood smoke exposure (3 h)	71,036 versus 222 UFP/cm^3	**50% (-2.0, 102)** 0.06 ± 0.08 0.04 ± 0.05 (NS)	**-17% (-37, 4.0)** 0.40 ± 0.32 0.48 ± 0.30 (NS)	(26)
Controlled exposure to diesel exhaust (3 h)	(276 versus 2 µg/cm^3 of PM_1)	**3% (-17, 15)** (NS)	**-13% (-69, 4.6)** (NS)	(40)
Controlled exposure to street air (5 h)	23,000 versus 1,800 UFP/cm^3	**4% (-8, 15)** 0.59 ± 0.39 0.57 ± 0.31 (NS)	**9% (-8.1, 27)** 0.35 ± 0.31 0.32 ± 0.31 (NS)	(41)
One-week stay in a reconstructed Viking age house (with indoor fire)	657 µg/m^3 of $PM_{2.5}$	**13% (-44, 132)** 0.06 ± 0.01 0.05 ± 0.01 (NS)	**-5% (-34, 25)** 0.11 ± 0.01 0.13 ± 0.02 (NS)	(12)
This study	0.7 versus 0.4 µm/mol	**15.6% (-0.6, 34.4)** 0.30 ± 0.20	**8.0% (0.02, 15.9)** 0.34 ± 0.11	Not applicable

	creatinine og 1-OHP	0.23 ± 0.10 **(NS)**	0.32 ± 0.08 **(P<0.05)**	

UFP, ultrafine particles; IQR, inter-quartile range; 1-OHP, 1-hydroxypyrene.

[a] The effect is reported as percent change (95% confidence) intervals per exposure unit in bold text. The exposure units differ between studies, indicating that it is not a stoichiometric measure. The second and third line represents the particle-rich exposure and reference, respectively. The values are lesions/10^6 bp. The fourth line reports the statistical outcome of the study as either a P-value or not statistically significant (NS).

Figure 1. Mean levels of ΣPAH (16 compounds) on the skin before and after a single episode of firefighting measured by skin wipes. Error bars are standard error of mean (N = 4-6). The subjects were wearing the personal protection aquipment before the first skin wiping (pilot study). The same skin area was wiped before and after firefighting. The recovery of the wiping is unknown but non-exhaustive, thus values may be considered minimum levels.

Figure 2. Levels of pyrene (a) and ΣPAH (b) on the back of the neck skin. Each symbol represents one measurement. The solid lines have been obtained from mixed effects linear regression.

Figure 3. Concentration of 1-OHP in urine after firefighting activity (exposure) and control periods (before and after). Each symbol represents one measurement. The solid line have been obtained from mixed effects linear regression. The dashed line represents the “no observed genotoxicity effect level for occupational exposure to PAH”.

Figure 4. Concentrations of ICAM-1 (a), VCAM-1 (b), CRP (c) and SAA (d) in plasma after firefighting activity (exposure) and control periods (before and after). Each symbol represents one measurement. The solid lines have been obtained from mixed effects linear regression.

Figure 5. Levels of DNA strand breaks (a) and Fpg-sensitive sites (b) in peripheral blood mononuclear cells after firefighting activity (exposure) and control periods (before and after). Each symbol represents one measurement. The solid lines have been obtained from mixed effects linear regression.