Acute Respiratory Effects in Firefighters

Frans E. Greven, PhD,[1,2]* Esmeralda J. Krop, PhD,[1] Jack J. Spithoven, BASc,[1] Nena Burger, BASc,[1] Jos M. Rooyackers, MD, PhD,[1,3] Huib A. Kerstjens, MD, PhD,[4] Sicco van der Heide, PhD,[5] and Dick J. Heederik, PhD[1,6]

Background *Associations between acute respiratory inflammatory responses, changes in bronchial hyperresponsiveness, serum pneumoprotein levels, and exposure to fire smoke were studied.*

Methods *The study comprised 51 firefighters. Blood samples were taken within 24 hr following exposure to fire smoke, and after a week and 3 months. Sputum was induced within 5 days post-exposure and subjects underwent spirometry and methacholine provocation one week post-exposure. Exposure was registered by a questionnaire.*

Results *No changes were observed following smoke exposure in bronchial hyperresponsiveness and serum pneumoprotein levels. Nevertheless, in a sizable proportion of the firefighters (44%) elevated sputum neutrophil levels (≥60%) were found. Serum IL-8 concentrations were higher 24 hr post-exposure compared to pre-exposure. Elevated neutrophil levels in sputum were associated with elevated serum IL-8 ($\beta = 0.010$, $P = 0.004$) and TNFα ($\beta = 0.005$, $P = 0.034$) levels within 24 hr post-exposure and IL-8 elevation lasted up to 3 months.*

Conclusions *Acute exposure to fire smoke induces acute neutrophilic airway and long-lasting systemic inflammation in healthy firefighters in the absence of bronchial hyperresponsiveness.* Am. J. Ind. Med. 55:54–62, 2012. © *2011 Wiley Periodicals, Inc.*

KEY WORDS: bronchial hyperresponsiveness; serum pneumoproteins; cytokines; inflammation; firefighters

INTRODUCTION

Fires produce a complex mixture of particulate matter, asphyxiants, and irritant gases. Occupational exposure to fire smoke amongst firefighters should be avoided by the use of self-contained breathing apparatuses (SCBA) but exposure remains a concern, because the devices are often not used in overhaul situations, or not during firefighting itself, especially owing to the visual impression of low smoke concentration [Brandt-Rauf et al., 1988; Burgess et al., 2001].

The evidence of respiratory effects of airway irritants, such as fire smoke, relies mostly on reported symptoms and lung function testing [Brandt-Rauf et al., 1989; Chia et al., 1990; Large et al., 1990]. Previous studies have indicated that smoke exposure may result in acute respiratory obstruction [Mustajbegovic et al., 2001; Miedinger et al., 2007] sometimes accompanied by an acute increase of airway responsiveness [Chia et al., 1990], or even reactive airways dysfunction syndrome (RADS) [Ribeiro et al., 2009]. We previously reported a positive association between the

[1]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands
[2]Department of Environmental Health, Municipal Health Services Groningen, Groningen, the Netherlands
[3]Division Heart & Lungs, University Medical Center Utrecht, Utrecht, the Netherlands
[4]Department of Pulmonary Diseases, University Medical Center Groningen, University of Groningen, the Netherlands
[5]Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, the Netherlands
[6]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands

Contract grant sponsor: The Netherlands Organisation for Health Research and Development (ZonMW, The Hague); Contract grant number: 7135.0005.
The authors declare that they have no financial competing interest.

*Correspondence to: Frans E. Greven, PhD, Department of Environmental Health, Municipal Health Services, Hulpverleningsdienst Groningen, PO Box 584, Groningen 9700 AN, the Netherlands. E-mail: f.greven@hvd.groningen.nl

Accepted 29 August 2011
DOI 10.1002/ajim.21012. Published online 29 September 2011 in Wiley Online Library (wileyonlinelibrary.com).

numbers of fires fought in the last 12 months and bronchial hyperresponsiveness, which we interpreted as an indication of an elevated irritant-induced asthma risk [Greven et al., 2011b]. Furthermore, studies have suggested that fire smoke inhalation leads to acute airway inflammation [Nordenhall et al., 2000; Swiston et al., 2008]. Fire smoke exposure has also been associated with a systemic inflammatory response [Goto et al., 2004; Swiston et al., 2008].

Recently, several lung-specific proteins, such as Clara cell protein (CC16) and surfactant protein A (SP-A), have been proposed as new biomarkers for lung epithelial injury following exposure to airway irritants [Hermans and Bernard, 1999]. Serum pneumoprotein levels have been used as biomarkers in studies of exposure to general air pollution [Berthoin et al., 2004], respiratory irritants like trichloramines [Carbonnelle et al., 2002] and fire smoke [Bernard et al., 1997; Burgess et al., 2001, 2002, 2003].

Many studies have focused specifically on high-risk subcategories such as forest firefighters [Betchley et al., 1997; Slaughter et al., 2004; Swiston et al., 2008], or firefighters in the 9/11 disaster [Prezant et al., 2002; Banauch et al., 2006]. Only a few studies with sufficient power have been conducted among common firefighters.

Few studies have explored pre- and post-exposure health status data in firefighters, and no study did link inflammation in sputum to changes in hyperresponsiveness. We hypothesized that changes in hyperresponsiveness after exposure would be associated with respiratory inflammation markers. Additionally, we explored pneumoprotein levels in blood as early markers of exposure and predictors of susceptibility to smoke exposure.

METHODS

Population and Design

In a previously conducted survey 1,249 active firefighters of 54 municipal fire brigades of 3 provinces of the Netherlands (Groningen, Friesland, and Drenthe) filled in a web-based version of the European Community Respiratory Health Survey questionnaire [Greven et al., 2011c]. Firefighters ($n = 402$) who participated in a subsequently conducted cross-sectional study [Greven et al., 2011b] were invited to contact the researchers within hours following accidental fire smoke exposure. In a telephone interview perceived exposure to fire smoke was assessed as being positive if a firefighter noticed inhalation of fire smoke or unprotected exposure to visible smoke. Exposed subjects ($n = 51$) were enrolled in the current study if a blood sample could be obtained within 24 hr following the exposure. Sputum was induced within 5 days following exposure, and after an interval of at least 24 hr more spirometry and bronchial responsiveness testing was carried out and a second blood sample was taken. After 3 months a third blood sample was taken. Sputum induction was carried out in the University Medical Center Groningen, and spirometry and bronchial responsiveness testing was carried out at the Municipal Health Services Groningen. All tests were executed between February 2009 and October 2009. Enrolment of subjects was stopped when the number of 51 firefighters was reached from whom a blood sample was taken within 24 hr. The institutional review board for human studies of the University Medical Centre Utrecht (Utrecht, the Netherlands) approved the protocol and written consent was obtained from all participants.

Questionnaire and Exposure Estimates

Questionnaire items involved the type and number of incidents, and the type, the onset and the duration of symptoms following the last fire. Several questionnaire items reflecting exposure were included involving questions on job history, working years, the use of SCBA, perceived exposure to fire smoke (discomforting as opposed to exclusively perceivable) during the last fire preceding the test, and the presence of acute symptoms following reported smoke inhalation.

Spirometry and Methacholine Challenge

Spirometry was obtained by experienced technicians according to European Respiratory Society standards [Pellegrino et al., 2005] and as described previously [Greven et al., 2011b].

Bronchial hyperresponsiveness (BHR_{20}) was considered to be present if the provocative dose of methacholine causing a 20% fall in FEV_1 (PD_{20}) $\leq$1.92 mg as described previously [Greven et al., 2011b]. To make optimal use of all available data, we also calculated the dose–response slope (DRS) as the % fall in FEV_1 per mg inhaled methacholine [O'Connor et al., 1987].

Serology

Blood samples were processed within 4 hr and serum aliquots were stored at $-80^{\circ}C$ until analysis. Atopy was defined as positive reaction to the specific IgE panel of house dust mite, cat, dog, grass pollen mixture (Phleum pratense and Lolium perenne, protein 1:1), and birch pollen (Allergon AB, Angelholm, Sweden), or total IgE exceeding 100 kU/L as described previously [Greven et al., 2011b].

Induced Sputum

Sputum was induced according to European Respiratory Society guidelines [Djukanovic et al., 2002]. Whole

sputum samples were processed for cell counts within 120 min, as described before [Rutgers et al., 2000]. A total cell count was performed on sputum samples after addition of 0.1% dithiothreitol equal to the sample's volume and filtration. Viability was checked by means of trypan blue exclusion. Two slides for differential cell counts were stained with May–Grunwald–Giemsa. Differential cell counts were performed by two technicians counting 300 non-squamous cells in a blinded fashion, and the mean was used for analysis. Significant eosinophil associated airway inflammation was defined if sputum eosinophil levels were $\geq$2% [Malo et al., 2009]. The percentage of neutrophils was also assessed and compared to normal values [Belda et al., 2000; Spanevello et al., 2000], and values above 60% were judged to be elevated. Samples with contamination of $>$80% squamous cells were excluded from analyses.

Sputum for Analysis of Smoke Particles in Sputum

Sputum was homogenized and analyzed by microscopic visualization in a 100 μm deep Bürker-Türk counting chamber (BT, Brand, Wertheim, Germany). Free floating particles with a crystalline appearance were visible. They were estimated to have a size of around 30 μm × 60 μm. Sometimes these particles clustered, forming rosettes. In this case the ''leafs'' of the rosette were counted separately. None of those containing the mentioned particles were seen in cells. After loading the counting chamber it was assessed whether the particles were distributed evenly within the whole counting area. In the Bürker-Türk counting chamber, the counted area in each side of the chamber was 0.4 mm^2 giving a total counted volume for each side of 4.0×10^{-5} ml in the first step. Counts were performed using 100× magnifications. Particle count was expressed as numbers/ml. Log-transformed particle count was used as an exposure variable.

Serum Pneumoproteins

Blood was obtained from each subject by venipuncture. Each sample was processed within 4 hr and serum aliquots were stored at −80°C until analysis.

Clara cell protein (CC16) was measured with a BioVendor Human Clara Cell Protein ELISA (enzyme-linked immunosorbent assay) kit (BioVendor Laboratorni Medicina a.s., Modrice, Czech Republic) as described in the manufacturer's protocol. Surfactant protein A (SP-A) was measured using a homemade sandwich ELISA technique as previously described [Greven et al., 2011a]. The optical density was read at 492 nm with a spectrophotometer (BioTek Instruments, Inc., Winooski). SP-A was expressed in units/ml (U/ml) with the highest point in our serum pool standard set at 100 U/ml. The detection limit was set at 5 U/ml.

Cytokines

Blood was obtained, processed and stored as described above. IL-1β, IL-6, IL-8, IL-10, INFγ, and TNFα were simultaneously quantitatively determined in serum with a High Sensitivity Human Cytokine Lincoplex kit (Millipore, Billerica, MA) as described in the manufacturer's protocol. Values below the detection limit were allotted the value of the detection limit. Prevalence of values below detection limit were 76.1% for IL-1β, 6.5% for IL-6, 0.0% for IL-8, 1.5% for IL-10, 51.7% for INFγ, and 0.0% for TNFα.

Statistical Analyses

SAS statistical software version 9.2 was used (SAS Institute, Cary, NC). Associations between (log-transformed) exposure variables and (log-transformed) continuous health outcome variables were calculated using a linear regression analysis. Associations with binary health outcome data were calculated using a logistic regression analysis. The level of statistical significance was set at $P < 0.05$.

RESULTS

Population Characteristics

In a previously conducted cross-sectional study in 21 fire brigades in the Netherlands, lung function, bronchial responsiveness, atopy, CC16, and SP-A were determined in 402 active firefighters [Greven et al., 2011b]. Afterwards 54 firefighters contacted us following acute exposure, of which 51 (94.4%) entered into this study. The three non-participating subjects were not able to complete testing within 24 hr following exposure. The exposure took place 1 day to 6 months after the cross-sectional tests were carried out.

General characteristics of the subgroup are shown in Table I. Of 51 firefighters 37 worked as volunteer, 8 as professional, and 6 as both. The subgroup was not different from the original cross-sectional population in sex distribution, smoking, working years, FEV_1, FVC, atopy, bronchial responsiveness, and age though the subgroup was slightly younger than the source population (41.3 ± 8.1 years; $P = 0.06$). For some parameters we did not obtain a complete dataset from all participants since not all subjects could fit in the time window allowed for the tests. From all 51 subjects (100%) post-exposure blood was obtained. In 4 subjects (7.8%) only post-exposure blood was obtained, in 13 subjects (25.5%) additionally only

TABLE I. Descriptive Characteristics of the Firefighters

	Total (n = 402)	Subgroup post-exposure (n = 51)
Age, years (mean, SD, range)	41.3 ± 8.1 (20–60)	39.1 ± 7.5 (20–55)
Working as firefighter, years (mean, SD, range)	12.5 ± 8.5 (<1–40)	11.1 ± 6.9 (1–27)
Male (no., %)	356 (88.6)	43 (84.3)
Current smoker (no., %)	111 (27.7)	10 (19.6)
Former smoker (no., %)	115 (28.7)	20 (39.2)
FEV_1 [% predicted] (mean, SD, range)	101.6 ± 12.8 (50.7, 138.7)	100.9 ± 12.0 (78.1, 130.1)
FVC [% predicted] (mean, SD, range)	107.9 ± 12.3 (70.6, 149.2)	107.8 ± 12.4 (82.5, 139.4)
FEV_1/FVC (mean, SD, range)	77.3 ± 6.3 (52.1, 97.1)	77.4 ± 5.0 (62.1, 86.9)
BHR_{20} (no., %)	63 (16.1)	7 (13.7)
Atopy (no., %)	126 (31.4)	14 (28.0)

BHR_{20}, bronchial hyperresponsiveness, $PD_{20} \leq 1.92$ mg methacholine causing a fall in forced expiratory volume in one second (FEV_1); FVC, forced vital capacity.

lung function tests and bronchial provocation tests were performed, in 4 subjects (7.8%) additionally only sputum induction was performed, and in 30 firefighters (58.8%) all tests were executed.

Symptoms

Exposure was reported as discomforting by 31 subjects (60.8%), and as just perceivable by 20 subjects (39.2%). Symptoms immediately following accidental exposure to fire smoke were reported by 34 firefighters (68%) and ranged from 2.0% (nausea) to 31.4% (coughing) (Table II). No wheezing, chest pain, dizziness, nose bleed, and weakness were reported. The presence of symptoms was clearly more frequent in the current study than in the cross-sectional analysis of the source population. Symptoms lasted less than an hour in 22 subjects (43.1%) while in 9 subjects (17.6%) symptoms were still present when the first blood sample was taken. No information was available about the persistence of symptoms beyond this moment.

TABLE II. Acute Symptoms

	Total with reported exposure following the last fire preceding the tests (n = 241)	Subgroup (n = 50)
Acute symptoms (no., %)	38 (15.8)	34 (68.0)
Coughing (no., %)	14 (5.8)	16 (31.4)
Itchy eyes (no., %)	13 (5.4)	15 (29.4)
Sore throat (no., %)	7 (2.9)	11 (2.2)
Headache (no., %)	2 (0.8)	5 (9.8)
Itchy nose (no., %)	6 (2.5)	2 (3.9)
Lung irritation (no., %)	1 (0.4)	2 (3.9)
Shortness of breath (no., %)	1 (0.4)	2 (3.9)
Nausea (no., %)	0 (0.0)	1 (2.0)
Wheeze (no., %)	0 (0.0)	0 (0.0)
Chest pain (no., %)	0 (0.0)	0 (0.0)

Bronchial Hyperresponsiveness, Spirometry

Baseline lung function and bronchial responsiveness parameters of the source population (n = 402) and the current population (n = 51) are provided in Table I.

Spirometry and methacholine challenge testing were carried out 6.7 ± 1.7 (min 2, max 12) days following exposure. No significant changes were found in FEV_1 (−24.9 ± 290.0 ml; $P = 0.87$), FVC (−27.6 ± 345.9 ml; $P = 0.90$), and bronchial responsiveness (Table III).

Induced Sputum

Induced sputum was assessed in 31 subjects between 1 and 5 days following exposure. Sputum contained >80% squamous cells in 6 subjects while cell differential parameters were interpretable in the other 25 (Table IV). Two subjects (8%) had eosinophils ≥2%, and 11 subjects (44%) had neutrophils ≥60%.

We found a relation between neutrophils and particle count assessed in sputum (Fig. 1). For each individual with particle count above 100,000/ml, the percentage of neutrophils was >58.8%. Below the threshold of 100,000 particles /ml, the distribution of neutrophils was unrelated to the particle count. There was a borderline positive association between neutrophils (expressed as quartiles) and particle count (expressed as quartiles) ($\beta = 0.430$, $P = 0.051$). When the population was restricted to current non-smokers the association became significant ($\beta = 0.449$, $P = 0.036$). Symptomatic non-smokers had higher neutrophil levels than non-smokers without acute

TABLE III. Lung Function and Bronchial Hyperresponsiveness (n = 43)

	Pre-exposure	Post-exposure (2–12 days)
FEV_1 [% predicted] (mean, SD, range)	102.5 ± 11.6 (79.8, 130.1)	101.6 ± 11.1 (79.3, 128.2)
FVC [% predicted] (mean, SD, range)	109.1 ± 12.4 (83.3, 139.4)	108.1 ± 12.3 (87.0, 142.5)
FEV_1/FVC [%] (mean, SD, range)	77.7 ± 4.6 (62.1, 86.9)	77.8 ± 4.7 (58.8, 85.3)
DRS (median, 25th, 75th percentile)	5.1 (2.9, 7.0)	3.9 (2.5, 6.8)
BHR_{20} (no., %)	6 (15.0)	6 (15.0)

DRS, dose–response slope of the methacholine challenge test: a higher number signifies more hyperresponsiveness; BHR_{20}, bronchial hyperresponsiveness, $PD_{20} \leq 1.92$ mg methacholine causing a fall in forced expiratory volume in 1 s (FEV_1); FVC, forced vital capacity.

symptoms ($\beta = 32.5$, $P = 0.067$). This association was not found when all subjects were included ($\beta = 10.1$, $P = 0.298$).

Serum Pneumoproteins and Cytokines

Pneumoprotein levels in serum of the source population did not differ from the pre-exposure levels of the current study population (Table V). No changes were found within 24 hr post-exposure in log-transformed serum pneumoproteins (Table V). No associations were found between serum pneumoproteins and acute symptoms, perceived exposure, or particle cell count.

Log-transformed serum IL-8 concentrations were significantly higher 24 hr post-exposure ($\beta = 0.127$, $P = 0.031$), 1 week post-exposure ($\beta = 0.085$, $P = 0.0007$), and 3 months post-exposure ($\beta = 0.112$, $P < 0.0001$) compared to pre-exposure.

The presence of elevated neutrophils in sputum was positively associated with IL-8 ($\beta = 0.431$, $P = 0.0023$), IL-10 ($\beta = 0.383$, $P = 0.023$), and TNFα ($\beta = 0.227$, $P = 0.011$) in serum within 24 hr following exposure. Associations were also found between the percentage of neutrophils in sputum, and IL-8 ($\beta = 0.010$, $P = 0.0044$) and TNFα ($\beta = 0.005$, $P = 0.034$) in serum within 24 hr following exposure. No associations were found between sputum cell differentials parameters and serum pneumoproteins, IL-1β, IL-6 and INFγ, lung function parameters, and DRS.

TABLE IV. Sputum Inflammatory Markers (n = 25) and Particle Count (n = 27)

Total cell count [$\times 10^6$ cells/ml] (mean, SD, range)	3.0 ± 2.5 [0.1, 11.2]
Eosinophils [%] (mean, SD, range)	0.7 ± 1.3 (0.0, 5.7)
Lymphocytes [%] (mean, SD, range)	2.5 ± 1.2 (0.8, 5.3)
Macrophages [%] (mean, SD, range)	35.7 ± 17.2 (8.0, 72.8)
Neutrophils [%] (mean, SD, range)	53.5 ± 20.1 (15.0, 87.7)
Basophils [%] (mean, SD, range)	0.0 ± 0.0 (0.0, 0.2)
Bronchial epithelial cells [%] (mean, SD, range)	7.5 ± 8.1 (1.8, 41.0)
Particle count [$\times 10^3$ particles/ml] (mean, SD, range)	81.8 ± 83.1 [2.7, 385.5]

Perceived exposure (just perceivable versus discomforting) was positively associated with a change in concentration in IL-8 after a week ($\beta = 3.437$, $P = 0.001$). The same pattern was seen when having acute symptoms was used as exposure variable ($\beta = 2.476$, $P = 0.03$).

DISCUSSION

In a sizable proportion of the firefighters elevated sputum neutrophils were found following fire smoke exposure. Additionally, sputum neutrophils and serum IL-8 levels were positively associated with exposure. Remarkably, we found (neutrophilic) inflammation without bronchial hyperresponsiveness following smoke exposure.

We found high neutrophil percentages in sputum (53.5 ± 20.1%) when compared to earlier studies in healthy volunteers by Spanevello et al. [2000] (27.3 ± 13.0%) and Belda et al. [2000] (37.5 ± 20.1%). The high percentages could not be explained by smoking habits, because when we restricted the population to current nonsmokers, the percentage of individuals with high neutrophil levels did not diminish, but slightly increased (56.9 ± 18.4%). Further exclusion of asthmatics hardly changed the percentage of individuals with high neutrophil

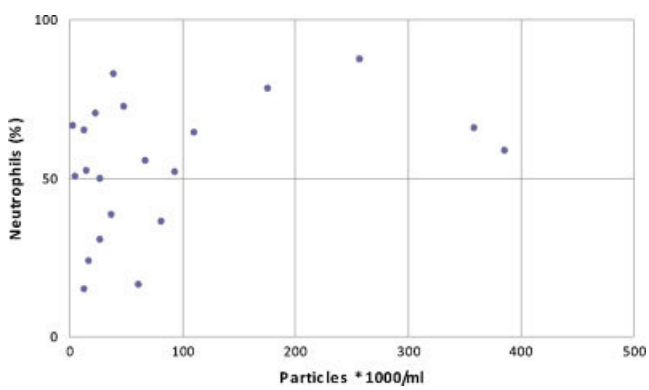

FIGURE 1. Association between post-exposure neutrophil levels (%) and particle counts in induced sputum. Neutrophils (expressed as quartiles) were weakly associated with particle count (expressed as quartiles) ($\beta = 0.430$, $P = 0.05$).

TABLE V. Mean Serum Pneumoprotein and Cytokine Levels

	Source population (n = 396)	Pre-exposure (n = 50)	Post-exposure (24 hr)	Post-exposure (1 week)	Post-exposure (3 months)
Serum pneumoproteins					
CC16 [ng/ml] (mean, SD, range)	5.80 ± 2.40 (0.99, 18.62)	5.87 ± 2.74 (2.00, 18.62)	5.98 ± 2.29 (2.24, 12.15)	5.89 ± 2.40 (2.14, 13.40)	4.72 ± 1.75 (1.16, 8.85)*
SP-A [U/ml] (mean, SD, range)	80.64 ± 265.79 (5.0, 3217.41)	85.92 ± 213.55 (5.0, 872.53)	84.12 ± 204.74 (5.0, 826.31)	96.99 ± 245.90 (5.0, 1109.75)	90.02 ± 206.45 (5.0, 851.63)
Serum cytokines					
IL-1β [pg/ml] (mean, SD, range)	n.a.	0.37 ± 0.83 (0.16, 5.45)	0.29 ± 0.26 (0.12, 1.31)	0.48 ± 1.24 (0.17, 8.00)	0.87 ± 2.46 (0.14, 11.58)
IL-6 [pg/ml] (mean, SD, range)	n.a.	25.64 ± 58.41 (0.21, 250.15)	27.97 ± 67.22 (0.21, 302.87)	27.46 ± 70.04 (0.21, 364.16)	29.53 ± 58.63 (0.80, 259.43)*
IL-8 [pg/ml] (mean, SD, range)	n.a.	4.98 ± 2.68 (1.28, 13.89)	8.16 ± 7.86 (1.81, 35.82)*	7.68 ± 4.88 (1.14, 27.24)*	16.18 ± 21.51 (2.56, 91.43)*
IL-10 [pg/ml] (mean, SD, range)	n.a.	14.23 ± 17.41 (1.32, 95.97)	13.40 ± 11.89 (0.61, 66.40)	14.64 ± 17.80 (1.43, 88.66)	20.20 ± 27.97 (0.61, 138.78)
IFNγ [pg/ml] (mean, SD, range)	n.a.	9.10 ± 26.81 (0.29, 168.98)	5.79 ± 9.38 (1.44, 47.67)	12.57 ± 43.88 (1.16, 274.51)	23.41 ± 78.95 (0.60, 400.89)
TNFα [pg/ml] (mean, SD, range)	n.a.	7.00 ± 3.26 (1.89, 15.27)	7.61 ± 3.68 (0.92, 20.67)	7.68 ± 4.49 (0.94, 19.30)	9.52 ± 10.07 (1.40, 63.88)

U, units; n.a., not assessed.
*$P < 0.05$ compared to pre-exposure.

levels. High neutrophil levels in sputum or bronchoalveolar lavage have also been found after exposure to several other irritants such as particulate matter [Rudell et al., 1999; Salvi et al., 1999; Nordenhall et al., 2000; Ghio and Devlin, 2001], isocyanates [Park et al., 1999; Lemiere et al., 2002], high molecular and low molecular weight agents [Lemiere et al., 2001]. Also in firefighters caught in the dust cloud during the morning of the collapse of the World Trade Center towers, neutrophil levels in induced sputum were increased till at least 10 months after the exposure [Fireman et al., 2004]. Recently it has been described that in a high percentage of subjects with a diagnosed irritant-induced asthma elevated neutrophils were found in induced sputum [Malo et al., 2009] and in bronchoalveolar lavage fluid [Takeda et al., 2009] even more than 10 years following the onset of the irritant-induced asthma. Additionally, neutrophils in sputum were positively associated with particle count in current non-smokers following exposure to fire smoke; this relation was slightly weaker in the whole population. We found a remarkable distribution between particles and neutrophils in sputum: above 100,000 particles/ml sputum all neutrophil percentages were 60% or higher. Subjects with these high particle counts comprised three never-smokers and two ex-smokers, who quitted smoking 14 and 16 years before, respectively. Additionally, neutrophil levels tended to be higher when acute symptoms were present. This relation is interesting since sputum was induced several days after exposure, while the mentioned symptoms generally lasted less than an hour (and were rather mild). Furthermore, in this study neutrophil levels were associated with the rise in IL-8 and TNFα. This is not unexpected, because these are pro-inflammatory cytokines which play a role in neutrophil recruitment. Moreover, the significant rise in IL-8 lasted for at least 3 months, and was accompanied by a rise in TNFα that was even higher at 3 months, but no longer statistically significant. We cannot rule out that additional exposures might have occurred between these measurements. Nevertheless, recently, increases in IL-8 following exposure to wood fire smoke [Swiston et al., 2008] and diesel exhaust [Salvi et al., 2000] have been described. These results support the observation that exposure to fire smoke triggers neutrophil recruitment in the airways and that inhalation of fire smoke evokes both pulmonary and long-lasting systemic inflammatory responses [Swiston et al., 2008]. Furthermore, these results are in line with toxicological studies in which IL-8 increased after exposure of human macrophages [Karlsson et al., 2006] or a co-culture of monocytes and pneumocytes with wood combustion particles [Kocbach et al., 2008]. Blood samples were stored between 6 and 16 months at -80°C until analysis. Although IL-8 post-exposure levels were associated with blood storage time, associations between exposure variables and changes in IL-8 levels were hardly

affected when adjusted for blood storage time, making it unlikely that blood storage time confounded results.

Unexpectedly we found no post-exposure associations between sputum inflammatory cells and bronchial responsiveness. Within our study, this was probably explained by the lack of change in airway responsiveness following exposure. Although the number of subjects with bronchial hyperresponsiveness rose from four preceding current exposure to six post-exposure when we excluded asthmatics (data not shown), and the maximum DRS was found post-exposure, no significant changes in bronchial hyperresponsiveness were found for our study group as a whole. This is in contrast to studies in which acute increases in hyperresponsiveness following exposure to fire smoke were described [Sherman et al., 1989; Chia et al., 1990]. Park et al. [2003] found that victims of smoke inhalation with persisting inflammatory responses were still hyperresponsive at least 6 months post-exposure. In the majority of subjects with a diagnosed irritant induced asthma, BHR remained present for at least 10 years and a third also had neutrophils $\geq$60% [Malo et al., 2009; Takeda et al., 2009]. As we expected an association of inflammation with bronchial hyperresponsiveness, we performed several sensitivity analyses in which asthmatics and firefighters who happened to have been exposed to fire smoke within 7 days preceding the baseline tests and asthmatics were excluded, but this made no difference.

The higher neutrophil levels in sputum in the absence of bronchial hyperresponsiveness in this study might be explained by a possible higher exposure in irritant-induced asthma cases and victims of smoke inhalation in former studies [Park et al., 2003; Malo et al., 2009; Takeda et al., 2009] compared to our study. In general, it is difficult to compare studies, as the specter of causative agents is wide, and exposure levels are mostly not quantified. Our findings are comparable to the findings described by Swiston et al. [2008] who also described associations between wood smoke exposure, neutrophils, and IL-8 levels, whereas the majority of acute symptoms were relatively mild and were not associated with exposure and lung function and bronchial hyperresponsiveness. Changes in cytokine levels and neutrophil recruitment could be more sensitive to smoke exposure, than lung function and bronchial hyperresponsiveness.

Contrary to expectations, no clear associations were found between exposure and serum pneumoprotein levels. Serum pneumoproteins have been used as biomarkers in studies on exposure to respiratory irritants such as tobacco-smoke [Bernard et al., 1994; Robin et al., 2002], trichloramines [Carbonnelle et al., 2002], and fire smoke [Bernard et al., 1997; Burgess et al., 2001, 2003]. Previously, we found that CC16 and SP-A levels in serum are very stable within persons, whereas differences between individuals can be large [Greven et al., 2011a]. This might imply that serum pneumoproteins are less applicable as an easy assessment of monitoring smoke exposure within individuals. Furthermore, the first post-exposure blood samples were obtained after a mean of 16 $\pm$ 6.5 (3, 23) hr. Both serum CC16 and SP-A levels were lower when the first blood sample was taken later within 24 hr. These associations were not found for the second and third post-exposure blood samples. This might be a further explanation why no associations were found between exposure and the within 24 hr post-exposure serum protein levels.

In conclusion, we have found acute pulmonary and long-lasting systemic inflammatory responses to inhalation of fire smoke, in the absence of bronchial hyperresponsiveness. These health effects do not seem fully or quickly transitory in these healthy individuals, since the elevation in blood IL-8 lasted for at least 3 months. Given these adverse health effects we recommend more strictly reducing or avoiding exposure to fire smoke even in the absence of visible smoke in order to attenuate possible health consequences.

ACKNOWLEDGMENTS

The authors wish to thank the personnel of the fire brigades of the provinces Groningen, Friesland, and Drenthe for their cooperation in this study. In addition, the authors would like to thank Elzo Gankema, Maria Smith, Roelina Smid, Walburga Gulker, Harriëtte Smidt, Jacqueline Aalfs, Els Greven, and Ali Karimi for their contribution to the field work; the Lung Function Department of the University Medical Center Groningen for the Sputum Inductions; and Brigitte Dijkhuizen and Ibolya Sloots-Barta of the Department of Laboratory Medicine of the University Medical Center Groningen for all the sputum measurements. This study was supported in part by grant for project number 7135.0005 from the Netherlands Organisation for Health Research and Development (ZonMW, The Hague).

REFERENCES

Banauch GI, Hall C, Weiden M, Cohen HW, Aldrich TK, Christodoulou V, Arcentales N, Kelly KJ, Prezant DJ. 2006. Pulmonary function after exposure to the World Trade Center collapse in the New York City Fire Department. Am J Respir Crit Care Med 174:312–319.

Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. 2000. Induced sputum cell counts in healthy adults. Am J Respir Crit Care Med 161:475–478.

Bernard AM, Roels HA, Buchet JP, Lauwerys RR. 1994. Serum Clara cell protein: An indicator of bronchial cell dysfunction caused by tobacco smoking. Environ Res 66:96–104.

Bernard A, Hermans C, Van Houte G. 1997. Transient increase of serum Clara cell protein (CC16) after exposure to smoke. Occup Environ Med 54:63–65.

Berthoin K, Broeckaert F, Robin M, Haufroid V, De Burbure C, Bernard A. 2004. Serum pneumoproteins and biomarkers of exposure to urban air pollution: A cross-sectional comparison of policemen and foresters. Biomarkers 9:341–352.

Betchley C, Koenig JQ, van Belle G, Checkoway H, Reinhardt T. 1997. Pulmonary function and respiratory symptoms in forest firefighters. Am J Ind Med 31:503–509.

Brandt-Rauf PW, Fallon LF, Jr., Tarantini T, Idema C, Andrews L. 1988. Health hazards of fire fighters: Exposure assessment. Br J Ind Med 45:606–612.

Brandt-Rauf PW, Cosman B, Fallon LF, Jr., Tarantini T, Idema C. 1989. Health hazards of firefighters: Acute pulmonary effects after toxic exposures. Br J Ind Med 46:209–211.

Burgess JL, Nanson CJ, Bolstad-Johnson DM, Gerkin R, Hysong TA, Lantz RC, Sherrill DL, Crutchfield CD, Quan SF, Bernard AM, Witten ML. 2001. Adverse respiratory effects following overhaul in firefighters. J Occup Environ Med 43:467–473.

Burgess JL, Nanson CJ, Hysong TA, Gerkin R, Witten ML, Lantz RC. 2002. Rapid decline in sputum IL-10 concentration following occupational smoke exposure. Inhal Toxicol 14:133–140.

Burgess JL, Witten ML, Nanson CJ, Hysong TA, Sherrill DL, Quan SF, Gerkin R, Bernard AM. 2003. Serum pneumoproteins: A cross-sectional comparison of firefighters and police. Am J Ind Med 44: 246–253.

Carbonnelle S, Francaux M, Doyle I, Dumont X, De Burbure C, Morel G, Michel O, Bernard A. 2002. Changes in serum pneumoproteins caused by short-term exposures to nitrogen trichloride in indoor chlorinated swimming pools. Biomarkers 7: 464–478.

Chia KS, Jeyaratnam J, Chan TB, Lim TK. 1990. Airway responsiveness of firefighters after smoke exposure. Br J Ind Med 47:524–527.

Djukanovic R, Sterk PJ, Fahy JV, Hargreave FE. 2002. Standardised methodology of sputum induction and processing. Eur Respir J Suppl 37:1s–2s.

Fireman EM, Lerman Y, Ganor E, Greif J, Fireman-Shoresh S, Lioy PJ, Banauch GI, Weiden M, Kelly KJ, Prezant DJ. 2004. Induced sputum assessment in New York City firefighters exposed to World Trade Center dust. Environ Health Perspect 112: 1564–1569.

Ghio AJ, Devlin RB. 2001. Inflammatory lung injury after bronchial instillation of air pollution particles. Am J Respir Crit Care Med 164:704–708.

Goto Y, Ishii H, Hogg JC, Shih CH, Yatera K, Vincent R, van Eeden SF. 2004. Particulate matter air pollution stimulates monocyte release from the bone marrow. Am J Respir Crit Care Med 170:891–897.

Greven F, Krop E, Burger N, Kerstjens H, Heederik D. 2011a. Serum pneumoproteins in firefighters. Biomarkers 16:364–371.

Greven F, Krop E, Spithoven J, Rooyackers J, Kerstjens H, Heederik D. 2011b. Lung function, bronchial hyperresponsiveness, and atopy among firefighters. Scand J Work Environ Health 37:325–331.

Greven FE, Rooyackers JM, Kerstjens HA, Heederik DJ. 2011c. Respiratory symptoms in firefighters. Am J Ind Med 54:350–355.

Hermans C, Bernard A. 1999. Lung epithelium-specific proteins: Characteristics and potential applications as markers. Am J Respir Crit Care Med 159:646–678.

Karlsson HL, Ljungman AG, Lindbom J, Moller L. 2006. Comparison of genotoxic and inflammatory effects of particles generated by wood combustion, a road simulator and collected from street and subway. Toxicol Lett 165:203–211.

Kocbach A, Herseth JI, Lag M, Refsnes M, Schwarze PE. 2008. Particles from wood smoke and traffic induce differential pro-inflammatory response patterns in co-cultures. Toxicol Appl Pharmacol 232:317–326.

Large AA, Owens GR, Hoffman LA. 1990. The short-term effects of smoke exposure on the pulmonary function of firefighters. Chest 97: 806–809.

Lemiere C, Chaboillez S, Malo JL, Cartier A. 2001. Changes in sputum cell counts after exposure to occupational agents: What do they mean? J Allergy Clin Immunol 107:1063–1068.

Lemiere C, Romeo P, Chaboillez S, Tremblay C, Malo JL. 2002. Airway inflammation and functional changes after exposure to different concentrations of isocyanates. J Allergy Clin Immunol 110:641–646.

Malo JL, L'Archeveque J, Castellanos L, Lavoie K, Ghezzo H, Maghni K. 2009. Long-term outcomes of acute irritant-induced asthma. Am J Respir Crit Care Med 179:923–928.

Miedinger D, Chhajed PN, Stolz D, Gysin C, Wanzenried AB, Schindler C, Surber C, Bucher HC, Tamm M, Leuppi JD. 2007. Respiratory symptoms, atopy and bronchial hyperreactivity in professional firefighters. Eur Respir J 30:538–544.

Mustajbegovic J, Zuskin E, Schachter EN, Kern J, Vrcic-Keglevic M, Heimer S, Vitale K, Nada T. 2001. Respiratory function in active firefighters. Am J Ind Med 40:55–62.

Nordenhall C, Pourazar J, Blomberg A, Levin JO, Sandstrom T, Adelroth E. 2000. Airway inflammation following exposure to diesel exhaust: A study of time kinetics using induced sputum. Eur Respir J 15:1046–1051.

O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. 1987. Analysis of dose-response curves to methacholine. An approach suitable for population studies. Am Rev Respir Dis 136:1412–1417.

Park H, Jung K, Kim H, Nahm D, Kang K. 1999. Neutrophil activation following TDI bronchial challenges to the airway secretion from subjects with TDI-induced asthma. Clin Exp Allergy 29:1395–1401.

Park GY, Park JW, Jeong DH, Jeong SH. 2003. Prolonged airway and systemic inflammatory reactions after smoke inhalation. Chest 123:475–480.

Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. 2005. Interpretative strategies for lung function tests. Eur Respir J 26:948–968.

Prezant DJ, Weiden M, Banauch GI, McGuinness G, Rom WN, Aldrich TK, Kelly KJ. 2002. Cough and bronchial responsiveness in firefighters at the World Trade Center site. N Engl J Med 347:806–815.

Ribeiro M, de Paula SU, Bussacos MA, Terra-Filho M. 2009. Prevalence and risk of asthma symptoms among firefighters in Sao Paulo, Brazil: A population-based study. Am J Ind Med 52:261–269.

Robin M, Dong P, Hermans C, Bernard A, Bersten AD, Doyle IR. 2002. Serum levels of CC16, SP-A and SP-B reflect tobacco-smoke exposure in asymptomatic subjects. Eur Respir J 20:1152–1161.

Rudell B, Blomberg A, Helleday R, Ledin MC, Lundback B, Stjernberg N, Horstedt P, Sandstrom T. 1999. Bronchoalveolar inflammation after exposure to diesel exhaust: Comparison between unfiltered and particle trap filtered exhaust. Occup Environ Med 56: 527–534.

Rutgers SR, Timens W, Kaufmann HF, van Der Mark TW, Koeter GH, Postma DS. 2000. Comparison of induced sputum with

bronchial wash, bronchoalveolar lavage and bronchial biopsies in COPD. Eur Respir J 15:109–115.

Salvi S, Blomberg A, Rudell B, Kelly F, Sandstrom T, Holgate ST, Frew A. 1999. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am J Respir Crit Care Med 159:702–709.

Salvi SS, Nordenhall C, Blomberg A, Rudell B, Pourazar J, Kelly FJ, Wilson S, Sandstrom T, Holgate ST, Frew AJ. 2000. Acute exposure to diesel exhaust increases IL-8 and GRO-alpha production in healthy human airways. Am J Respir Crit Care Med 161: 550–557.

Sherman CB, Barnhart S, Miller MF, Segal MR, Aitken M, Schoene R, Daniell W, Rosenstock L. 1989. Firefighting acutely increases airway responsiveness. Am Rev Respir Dis 140:185–190.

Slaughter JC, Koenig JQ, Reinhardt TE. 2004. Association between lung function and exposure to smoke among firefighters at prescribed burns. J Occup Environ Hyg 1:45–49.

Spanevello A, Confalonieri M, Sulotto F, Romano F, Balzano G, Migliori GB, Bianchi A, Michetti G. 2000. Induced sputum cellularity. Reference values and distribution in normal volunteers. Am J Respir Crit Care Med 162:1172–1174.

Swiston JR, Davidson W, Attridge S, Li GT, Brauer M, van Eeden SF. 2008. Wood smoke exposure induces a pulmonary and systemic inflammatory response in firefighters. Eur Respir J 32:129–138.

Takeda N, Maghni K, Daigle S, L'Archeveque J, Castellanos L, Al-Ramli W, Malo JL, Hamid Q. 2009. Long-term pathologic consequences of acute irritant-induced asthma. J Allergy Clin Immunol 124:975–981.