Eur Respir J 2008; 32: 129–138
DOI: 10.1183/09031936.00097707
Copyright©ERS Journals Ltd 2008

Wood smoke exposure induces a pulmonary and systemic inflammatory response in firefighters

J.R. Swiston*, W. Davidson#, S. Attridge*, G.T. Li*, M. Brauer¶ and S.F. van Eeden*

ABSTRACT: Epidemiological studies report an association between exposure to biomass smoke and cardiopulmonary morbidity. The mechanisms for this association are unclear. The aim of the present study was to characterise the acute pulmonary and systemic inflammatory effects of exposure to forest fire smoke.

Seasonal forest firefighters (n=52) were recruited before and/or after a day of fire-fighting. Exposure was assessed by questionnaires and measurement of carbon monoxide levels (used to estimate respirable particulate matter exposure). The pulmonary response was assessed by questionnaires, spirometry and sputum induction. Peripheral blood cell counts and inflammatory cytokines were measured to define the systemic response.

Estimated respirable particulate matter exposure was high (peak levels >2 mg·m^{-3}) during fire-fighting activities. Respiratory symptoms were reported by 65% of the firefighters. The percentage sputum granulocytes increased significantly from 6.5 to 10.9% following fire-fighting shifts, with concurrent increases in circulating white blood cells (5.55×10^9 to 7.06×10^9 cells·L^{-1}) and band cells (0.11×10^9 to 0.16×10^9 cells·L^{-1}). Serum interleukin (IL)-6, IL-8 and monocyte chemotactic protein-1 levels significantly increased following fire-fighting. There were no changes in band cells, IL-6, and IL-8 following strenuous physical exertion without fire-fighting. There was a significant association between changes in sputum macrophages containing phagocytosed particles and circulating band cells.

In conclusion, acute exposure to air pollution from forest fire smoke elicits inflammation within the lungs, as well as a systemic inflammatory response.

KEYWORDS: Air pollution, cytokines, inflammation, leukocytes

Fine particles are thought to be the best single indicator of the health impacts of most combustion sources. Numerous epidemiological studies have documented a detrimental relationship between exposure to particulate matter in urban air pollution and cardiopulmonary morbidity and mortality with both short- and long-term exposure [1–5]. Although the mechanism by which exposure to particles in urban air induces adverse cardiopulmonary effects remains unclear, several studies from the present authors' laboratory and others support a mechanism in which exposure to fine particles promotes inflammation in the lung [6–11] *via* activation of alveolar macrophages and lung epithelial cells [12–16]. Such exposure is also associated with a systemic inflammatory response [17–20], which in turn is associated with an increased presence of several cytokines (interleukin (IL)-6, IL-1β) in the bloodstream [21] as well as increased production and release of polymorphonuclear leukocytes (PMN) and monocytes from the bone marrow [17–20].

In contrast to the large amount of information relating urban particulate matter to human health impacts, there are a limited number of studies directly evaluating the mechanisms of the health impacts of wood smoke. While wood smoke contains high concentrations of both particulate matter and gaseous compounds, wood smoke particles are similar in size to urban combustion-generated particles, and within the respirable size range that is thought to be most damaging to human health [22]. Epidemiological and toxicological evidence of the adverse effects of wood

AFFILIATIONS
*The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, Division of Respirology, St Paul's Hospital, University of British Columbia,
¶School of Occupational and Environmental Hygiene, University of British Columbia, Vancouver, BC, and
#Dept of Medicine, Division of Respirology, University of Calgary, Calgary, AB, Canada.

CORRESPONDENCE
S.F. van Eeden,
The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, University of British Columbia, St. Paul's Hospital, 1081 Burrard Street, Vancouver, BC, V6Z 1Y6, Canada. Fax: 1 6048069274
E-mail: svaneeden@mrl.ubc.ca

Received:
July 31 2007
Accepted after revision:
January 28 2008

SUPPORT STATEMENT
The present study was supported by grants from the Heart and Stroke Foundation of Canada, Wolfe and Gina Churg Foundation and the British Columbia Lung Association. S. F. van Eeden is an American Lung Association Career Investigator, the recipient of the William Thurlbeck Distinguish Research Award and the Michael Smith Foundation Senior Investigators Award.

STATEMENT OF INTEREST
A statement of interest for M. Brauer can be found at www.erj.ersjournals.com/misc/statements.shtml

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

This article has supplementary material accessible from www.erj.ersjournals.com

smoke have recently been reviewed by NAEHER *et al.* [21] and support several other studies indicating that increases in cardiopulmonary morbidity are related to smoke from forest fires and biomass burning [23–27]. For example, health impact surveillance during severe forest fires in Southeast Asia in 1997 (where particles were the main pollutant) showed a 30% increase in outpatient medical attendance for haze-related conditions in the general population of Singapore [25]. All-cause mortality increased by 19% in certain highly polluted regions, with the most prominent effect in the elderly population, in which deaths due to nontraumatic causes increased by 70% [27].

Wildland firefighters experience greater exposure from forest fire smoke than members of the general public. They frequently have prolonged and direct exposure to smoke, and elevated pulmonary ventilation rates can result in substantial doses of smoke to the respiratory tract. REINHARDT and OTTMAR [28] showed that firefighters were exposed to high levels of respirable particles (particulate matter $\leqslant 3.5$ μm ($PM_{3.5}$)) with peak concentrations ranging 2.3–2.9 $mg \cdot m^{-3}$.

Given the high smoke exposures experienced by firefighters and the epidemiological evidence indicating association between exposure to wood smoke and adverse health [23–27], the present authors postulated that acute exposure to smoke from forest fires provokes a local inflammatory response in the lungs and stimulates a measurable systemic inflammatory response. In order to test this hypothesis, healthy seasonal firefighters from the British Columbia Forest Service (BCFS) were recruited before the fire season, when relevant parameters of health (questionnaires), lung function (spirometry), lung inflammation (sputum induction) and systemic inflammation (blood cell counts and circulating cytokines) were collected. Collection of these parameters was repeated during the fire season immediately after a day of active duty within fire zones. Preliminary results of the present study have previously been published [29].

METHODS

Subjects recruitment and sampling

Seasonal BCFS forest firefighters were recruited during the 2004 and 2005 fire seasons (May–August). Healthy nonsmoking firefighters 17–60 yrs of age were eligible for inclusion. Exclusion criteria were: a prior diagnosis of, or ongoing evaluation for, any chronic medical condition; regular use of prescription medications; pregnancy or lactation; or any upper respiratory tract symptoms consistent with infection or inflammation in the 4 weeks prior to enrolment. The study was approved by the institutional ethics committee (St Paul's Hospital, University of British Columbia, Vancouver, BC, Canada) and written consent was obtained from all subjects.

Baseline data were collected in training camps before firefighters were dispatched to fire zones. Stress control data were collected after firefighters had spent a work shift (~8 h) engaged in strenuous physical activity (similar to physical activity during fire-fighting) with no exposure to forest fire smoke. Post-exposure measurements were made within 4 h after completing fire-fighting shifts. Both baseline (12:00–17:00 h) and post-exercise and fire-fighting samples (16:00–20:00 h) were collected in the afternoon or early evening. Subjects completed surveys detailing work shift activities and smoke exposure. Sputum induction, spirometry and blood sampling by peripheral venopuncture was performed. Selected participants were assigned to carry portable carbon monoxide monitors (Pac III; Dräger, Mississauga, ON, Canada) during their work shift. Carbon monoxide monitors were distributed by the crew leader to study participants performing different fire-fighting tasks in an attempt to obtain representative sampling. The monitors recorded ambient carbon monoxide levels every 5 min. Particulate matter was not directly measured due to BCFS regulations prohibiting access to fire zones to collect samples and the lack of sufficiently rugged, small and lightweight continuous personal particulate matter sampling equipment. Instead, carbon monoxide concentrations were measured as a surrogate based on the recommendations of REINHARDT and OTTMAR [30], who have previously shown that carbon monoxide exposures are highly correlated with respirable particle exposures of firefighters. Measured carbon monoxide exposures were then converted to estimated respirable particulate matter concentrations based upon the regression equations published by REINHARDT and OTTMAR [30].

Sputum induction and spirometry

Spirometry was performed according to American Thoracic Society criteria [31] using a single spirometer (EasyOne Spirometer; ndd Medical Technologies, Zurich, Switzerland). Induced sputum was performed using techniques described previously [32]. Samples were stored and processed using methods described by EFTHIMIADIS *et al.* [33] and modified by KELLY *et al.* [34]. Sputum induction and sample preparation are described in detail in the supplementary data. Under a light microscope at 630× magnification cells were classified as granulocytes, monocytes, lymphocytes, macrophages or bronchial epithelial cells. Macrophages were subdivided into those with no visible inclusions and those with less than, or greater than, 5% of their cytoplasm containing dust particles, labelled as negative, low-positive or high-positive macrophages, respectively, as described in detail previously [8]. Sputum cell counts were assessed by a trained technician blinded to the clinical details of the subjects.

Peripheral blood analysis

Complete blood counts were performed on whole blood samples using a Cell-Dyn 3700 Hematology Analyzer (Abbott Diagnostics, Mississauga, ON, Canada). Band cell counts were manually performed by a trained technician blinded to the clinical details of the subject, on thin blood smears that were air dried, fixed with methanol and stained with Wright stain. Serum IL-6 and IL-8 concentrations were measured using commercial ELISA assays (R&D Systems, Minneapolis MN, USA and Linco Research, St. Charles, MO, USA, respectively).

Statistical analysis

Data are expressed as mean ± SEM. Differences between baseline and fire-fighting values were evaluated using paired t-tests. Differences between multiple groups (baseline, exercise and fire-fighting) were compared by one-way ANOVA. The *post hoc* test for a multiple comparison was the Fisher's protected least significant difference test. Spearman rank correlations were performed to assess the relationship between

lung and systemic circulation. A p-value of 0.05 was considered to be statistically significant.

RESULTS

Subject recruitment and sampling

A total of 52 firefighters were enrolled in the study and underwent sampling either in training camps (baseline), after a day of fire-fighting (exposure) or after a day of physical activity without smoke exposure (exercise). Both baseline and exposure samples were obtained in 34 subjects (paired sampling). In nine subjects baseline, exercise and exposure samples were obtained. Basic demographic characteristics of firefighters enrolled in the study are shown in table 1. The majority of firefighters were young males of either Caucasian or Canadian First Nations descent. All subjects were free from acute or chronic medical comorbidities (including any history of allergies or asthma) and all were nonsmokers or ex-smokers (defined as both: 1) quit >6 months; and 2) not more than 5 pack-yrs of smoking) at the time of enrolment.

Smoke exposure and respiratory symptoms

Forest fires from which samples were obtained were in remote regions away from major roadways. Figure 1 shows fire-fighters' qualitative perception of smoke exposure during their work shift on the day of sampling. The majority report light or medium smoke exposure and, on average, <10% of the work shift was spent in conditions of heavy smoke. Carbon monoxide monitors carried by 17 firefighters during two separate forest fires showed workshift carbon monoxide levels to fluctuate predominately in the range 5–20 ppm with a background carbon monoxide level in the fire zone base camps of 1–5 ppm (fig. 2). Figure 2 shows the estimated respirable $PM_{3.5}$ exposure of firefighters, with peak levels of 2.8 $mg \cdot m^{-3}$ and 6 h of levels >1 $mg \cdot m^{-3}$ during a work shift. Post-exposure questionnaires were completed by 45 firefighters. In response to smoke exposure, 65% of firefighters reported one or more respiratory symptom. The most common complaints were cough (24.4%), sputum production (28.9%) and nasal congestion (20.0%; table 2). All reported symptoms were minor and no symptoms required acute medical intervention.

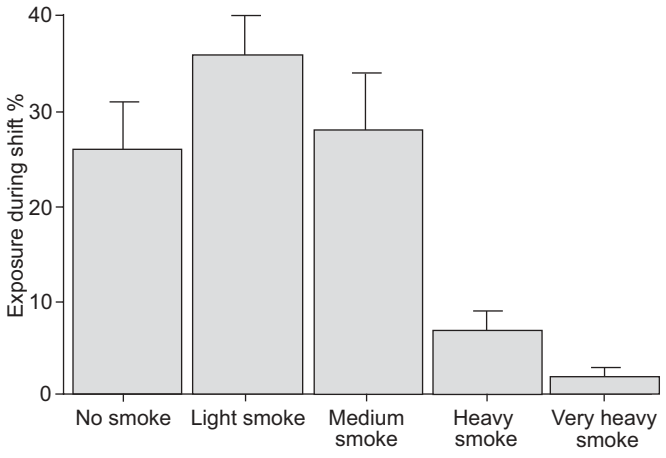

FIGURE 1. Smoke exposure during fire-fighting shifts. Smoke exposure as a percentage of fire-fighting shifts reported by 45 subjects. Firefighters were asked to score their average exposure during the work shift after which samples were colleted. Data are presented as mean±SEM.

Spirometry

Spirometry before and after fire-fighting was obtained in 23 subjects. Forced expiratory volume in one second (FEV_1; pre- and post-bronchodilation with 200 μg salbutamol) performed at baseline and after fire-fighting shifts were not different. The changes in FEV_1 following sequential inhalation of escalating concentrations of saline (3, 4 and 5%) are shown in figure 3. After inhalation of 3% hypertonic saline there was a small fractional decrease in the post-exposure group (n=12) that was not seen in pre-smoke exposure testing, but this change did not reach significance. After inhalation of 4% saline, the difference in the fractional FEV_1 change between the two groups diminished and then disappeared with inhalation of 5% saline.

Sputum analysis (lung inflammation)

Of the 34 subjects with paired sputum samples obtained at baseline and after exposure, 23 fulfilled the criteria for adequate specimens [33] in both samples. An increase in the percentage of sputum granulocytes in nonsquamous cells was

TABLE 1 Demographic characteristics of the firefighters

	All subjects			Paired sputum	Paired blood	
	Total	Baseline	Post-exposure	Baseline and post-exposure	Baseline and post-exposure	Baseline and exercise controls
Subjects n	52	43	45	23	34	9
Age yrs	30.4±1.1	32.0±1.1	30.4±1.2	28.6±1.1	32.0±1.2	30.9±2.2
Age range yrs	17–46	20–46	17–44	20–39	20–44	22–41
Female	3 (6)	3 (7)	3 (7)	3 (13)	3 (9)	0 (0)
Former smoker	14 (27)	14 (33)	12 (27)	9 (39)	12 (35)	3 (33)
Ethnicity						
Caucasian	25 (48)	25 (58)	20 (44)	19 (83)	20 (58)	9 (100)
Canadian First Nation	17 (33)	17 (40)	15 (33)	3 (13)	13 (38)	0 (0)
Other	10 (19)	1 (2)	10 (22)	1 (4)	1 (3)	0 (0)

Data are presented as n (%) or mean±SEM, unless otherwise stated.

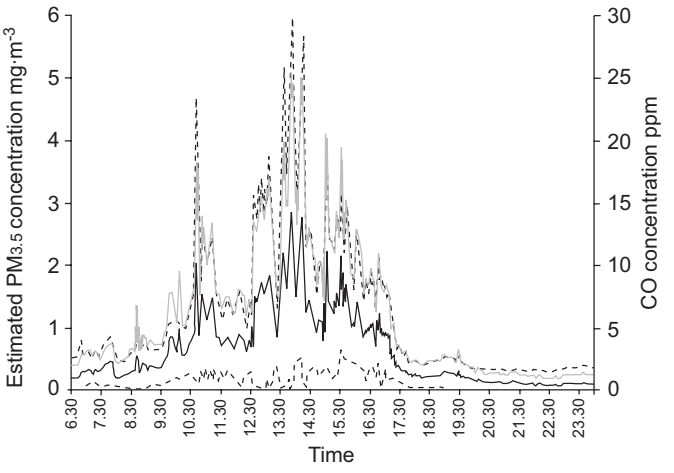

FIGURE 2. Mean measured carbon monoxide (CO) exposures (····) and mean (—) and 95% confidence intervals (- - -) of estimated respirable particulate matter ⩽3.5 μm ($PM_{3.5}$) exposures during fire-fighting shifts. A total of 17 subjects were randomly assigned to carry CO monitors during fire-fighting shifts. CO levels were recorded by the monitors at 5-min intervals. Measured CO exposures were used to estimate $PM_{3.5}$ exposures using the regression equation of REINHARDT and OTTMAR [30].

observed following fire-fighting shifts, from 6.48±1.23% at baseline to 10.87±1.36% post-exposure ($p<0.02$; fig. 4a). The total macrophage fraction in the sputum samples did not change with smoke exposure (fig. 4b); however, there was a small but statistically significant increase in inclusion-positive macrophages of 5.00±1.34 to 9.74±1.60% ($p<0.03$; fig. 4c). The fraction of inclusion-negative macrophages decreased following smoke exposure (75.65±2.31 to 65.78±2.85%; $p<0.01$) with a concurrent increase in the fraction of low positive-inclusion containing macrophages (4.83±1.29 to 9.39±1.49%; $p<0.03$; fig. 4d) with no change in the percentage of high-positive macrophages in the sputum samples (fig. 4d). Sputum lymphocyte, monocyte and bronchial epithelial cells did not show a significant change following smoke exposure (data not shown).

Peripheral blood analysis (systemic inflammation)

Circulating total white cell counts, differentials and band cell counts were compared before and after fire-fighting shifts. Total white blood cell counts increased following fire-fighting shifts from 5.55±017 to $7.06\pm0.18\times10^9$ cells·L^{-1} ($n=34$; $p<0.0001$; fig. 5a). Circulating PMN counts also increased after firefighting shifts (from 3.32±0.15 to $4.15\pm0.15\times10^9$ cells·L^{-1}; $p<0.001$), as did band cell counts (from 0.11±0.011 to $0.16\pm0.015\times10^9$ cells·L^{-1}; $p<0.01$; fig. 5b). Similar results were obtained in the unpaired analysis comparing all baseline samples to those collected post-exposure (data not shown). There were no significant changes in any other peripheral blood cell indices including haemoglobin, platelet counts and other white blood cell subpopulations (data not shown). Post-exposure serum IL-6 and IL-8 levels were elevated from baseline with IL-6 increasing from 0.82±0.12 to 1.89±0.43 pg·mL^{-1} ($n=34$; $p<0.02$; fig. 5c) and IL-8 increasing from 14.02±3.48 to 45.03±8.32 pg·mL^{-1} ($n=14$; $p<0.001$; fig. 5d). There were no increase in circulating granulocyte-macrophage colony-stimulating factor levels with exposure although there was a small but statistically significant increase in monocyte chemotactic protein (MCP)-1 levels (from 179±19.9 to 260±24.1 pg·mL^{-1}; $p<0.02$). C-reactive protein (CRP) did not increase with smoke exposure (data not shown). The changes in circulating band cells with smoke exposure correlate with the changes in macrophages containing particulate inclusions ($r^2=0.28$, $p<0.03$; fig. 6a) and changes in circulating IL-6 correlated with changes in the fraction of granulocytes in the sputum ($r^2=0.29$, $p<0.05$; fig. 6b).

TABLE 2 Common complaints reported in post-exposure questionnaires

Symptom	Subjects
Asymptomatic	16 (35.6)
Sputum production	13 (28.9)
Cough	11 (24.4)
Nasal congestion	9 (20.0)
Headache	6 (13.3)
Sore throat	6 (13.3)
Shortness of breath	5 (11.1)
Other	8 (17.8)
Total	45

Data are presented as n (%) or n.

To determine whether the changes seen in the circulation were due to a stress response rather than smoke exposure, nine subjects underwent sequential sampling at baseline and following shifts. This involved strenuous activity (similar to activities done during fire-fighting) in the absence of smoke exposure (exercise controls). Compared with baseline testing,

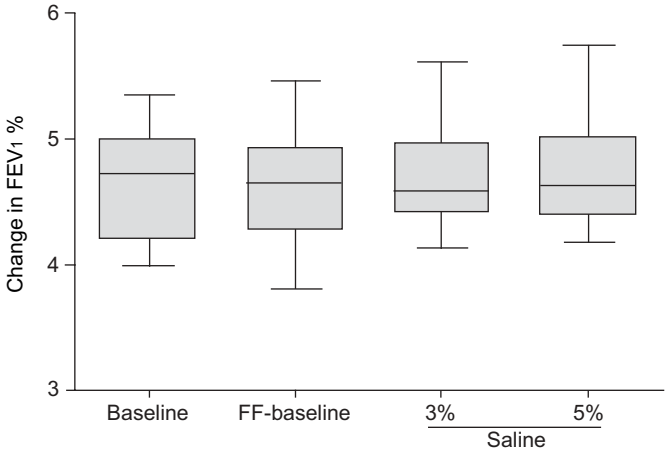

FIGURE 3. Changes in forced expiratory volume in one second (FEV_1) of firefighters at baseline (in training camp), after 1 day of wood smoke exposure (FF-baseline), and 3% (n=12) and 5% (n=3) saline inhalation. Changes in FEV_1 are expressed as a per cent change from pre-salbutamol measurements. Values are expressed as box and whisker plots with 75% of values within the box and the whiskers representing the range. There was no significant change in FEV_1 after wood smoke exposure and no increase in airway hyperresponsiveness, as measured by hypertonic saline inhalation.

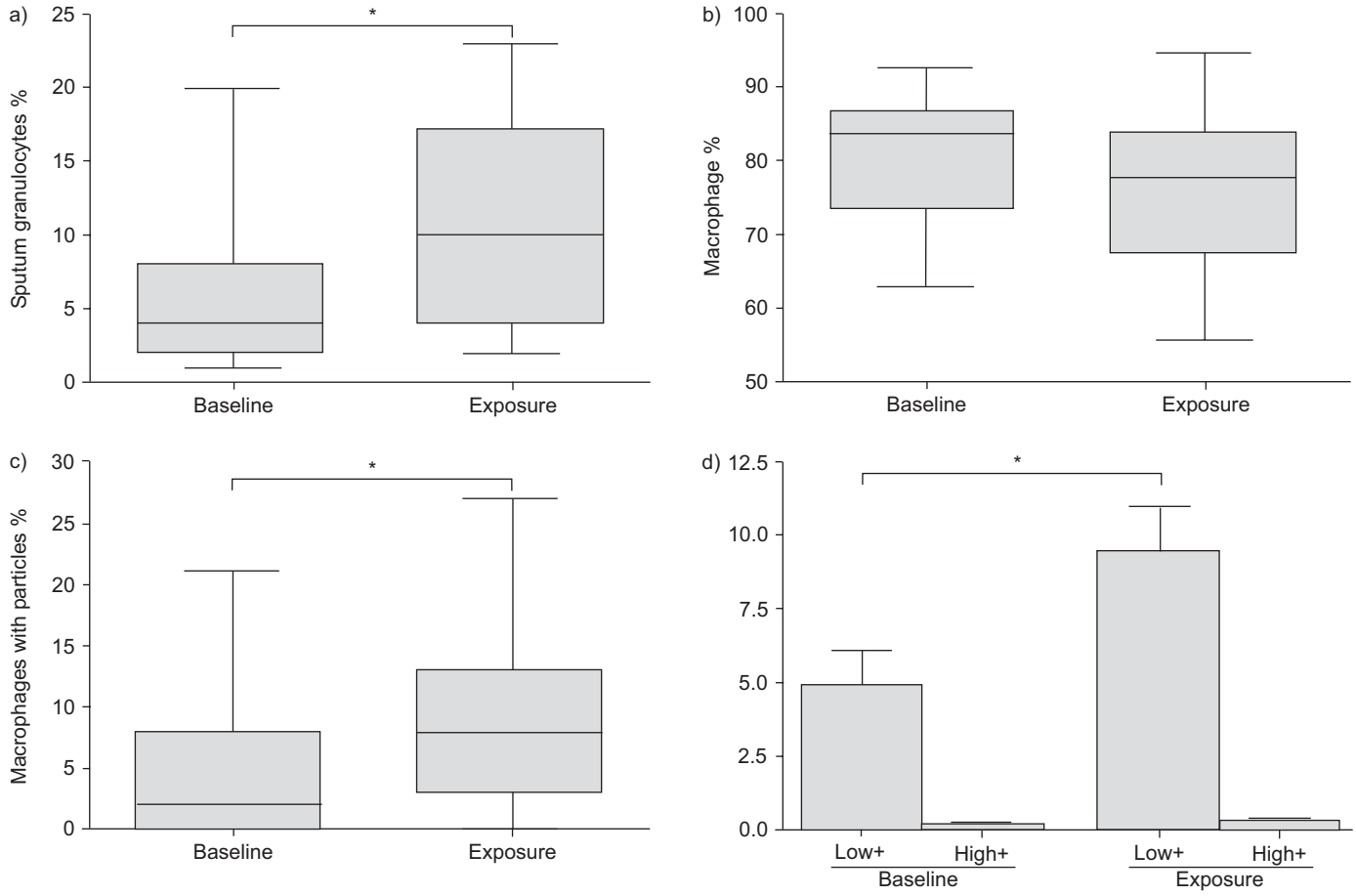

FIGURE 4. Pre- and post-exposure induced sputum cell counts and macrophage particle inclusions. Cellular composition of the sputum expressed as a percentage of total nonsquamous cells from 23 firefighters with paired samples both at baseline and post-exposure: a) mean sputum granulocyte fraction; b) total macrophage counts; c) total inclusion positive macrophage counts; and d) low- and high-positive inclusion macrophages. Inclusion-negative macrophages were defined as those lacking visible particulate inclusions by light microscopy at 630× magnification while low-positive and high-positive macrophages were defined as containing particulate inclusion that comprised less than or greater than 5% of their cytoplasm, respectively. Whiskers represent SEM. *: $p<0.05$.

exercise increased circulating white blood cell counts (from 5.60 ± 0.33 to $7.23\pm0.33\times10^9$ cells·L^{-1}; $p<0.001$; fig. 7a) and PMN counts (3.48 ± 0.28 to $4.89\pm0.37\times10^9$cells·L^{-1}; $p<0.02$). However, neither band cell counts nor IL-6 or IL-8 levels changed significantly following strenuous exercise compared with baseline testing (fig. 7b–d).

DISCUSSION

The majority of epidemiological and biochemical studies linking air pollution to respiratory and cardiovascular disease have focused on urban sources [1–5]. Exposure to urban air pollution is associated with an inflammatory response in the lung that is thought to be responsible for the adverse respiratory health effects, while the associated systemic inflammatory is implicated in adverse cardiovascular effects [35–39]. Although exposure to air pollution from biomass burning remains a significant problem in both developed countries (exposure during wildland fire-fighting, in communities in proximity to wildland fires and from residential wood smoke) and developing nations (exposure from indoor cooking and agricultural burning) [21], the mechanisms of the adverse heath effects of biomass smoke inhalation have not been as rigorously studied to date. The present study has demonstrated that inhalation of smoke from forest fires by young healthy firefighters induces a measurable inflammatory response in the lung characterised by an increase in sputum granulocytes. These changes were associated with symptoms of both upper and lower respiratory tract irritation. Additionally, systemic inflammation, characterised by stimulation of the bone marrow (increased circulating band cells) and increased circulating cytokine levels, was found. Such local and systemic inflammatory responses may contribute to the observed adverse health effects reported in epidemiological studies of those exposed to smoke from forest fires [21, 23, 24].

Carbon monoxide monitoring was used in the present study in order to confirm the presence of ambient smoke on the day when post-shift samples were collected (fig. 2). Carbon monoxide has been previously shown to correlate with major constituents of biomass smoke such as acrolein, benzene, formaldehyde and respirable particulate matter, and is therefore a quantitative indicator of smoke exposure [30, 40]. Based on those previous studies and the measured carbon monoxide exposures in the present study, high exposures to particulate

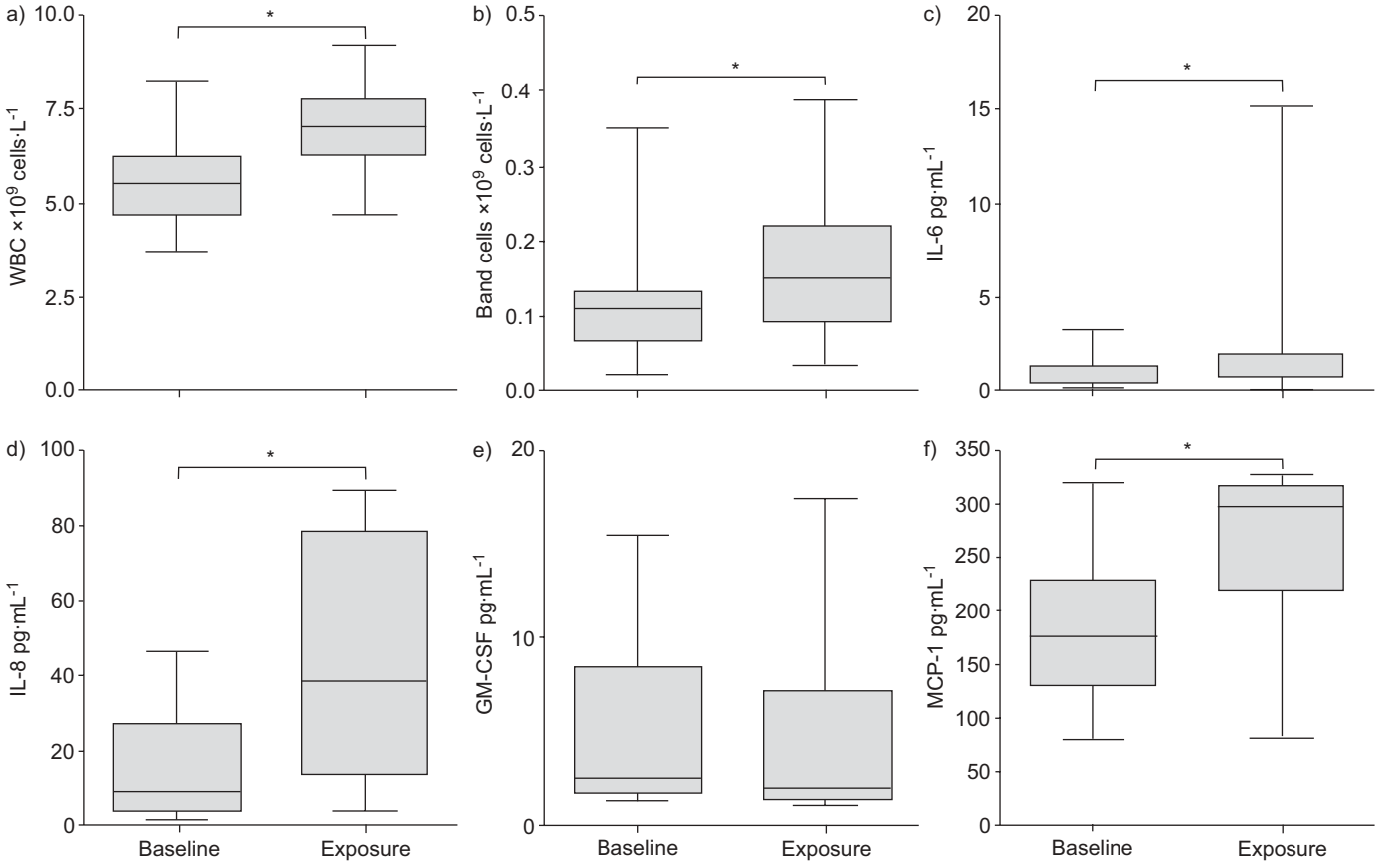

FIGURE 5. Mean circulating a) white blood cell (WBC; n=34; p<0.0001 between baseline and exposure), b) band cell (n=34; p<0.02), c) interleukin (IL)-6 (n=34; p<0.001), d) IL-8 (n=14; p<0.01), e) granulocyte-macrophage colony-stimulating factor (GM-CSF; n=14; p=0.47) and f) monocyte chemotactic protein (MCP)-1 (n=14; p<0.04) changes induced by fire-fighting, presented as mean at baseline and after exposure. Whiskers represent SEM. *: p<0.05.

matter during fire-fighting were estimated. The estimated exposure to combustion-source respirable particles, in the 1–2 mg·m^{-3} range, are similar to those experienced by females and children in developing countries who cook with biomass over open fires, and more than one order of magnitude above concentrations of ambient fine particles found in highly polluted cites or in communities impacted by forest fire smoke [21]. While the observed effects in the present study may be attributed to

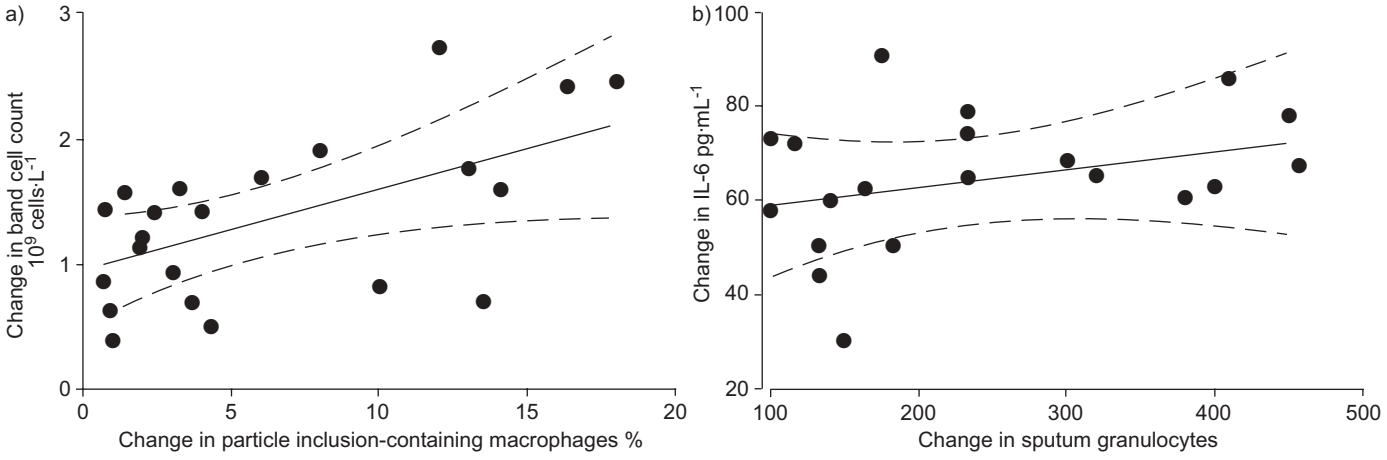

FIGURE 6. Correlations between a) the change in band cell counts and the number of macrophages containing particulate inclusions (r^2=0.28, p<0.03) and b) the change in interleukin (IL)-6 and sputum granulocyte counts (r^2=0.29, p<0.05) after smoke exposure. Spearman rank correlation was used to explore these relationships in 23 firefighters with paired sputum samples and blood cell counts. – – – –: 95% confidence intervals.

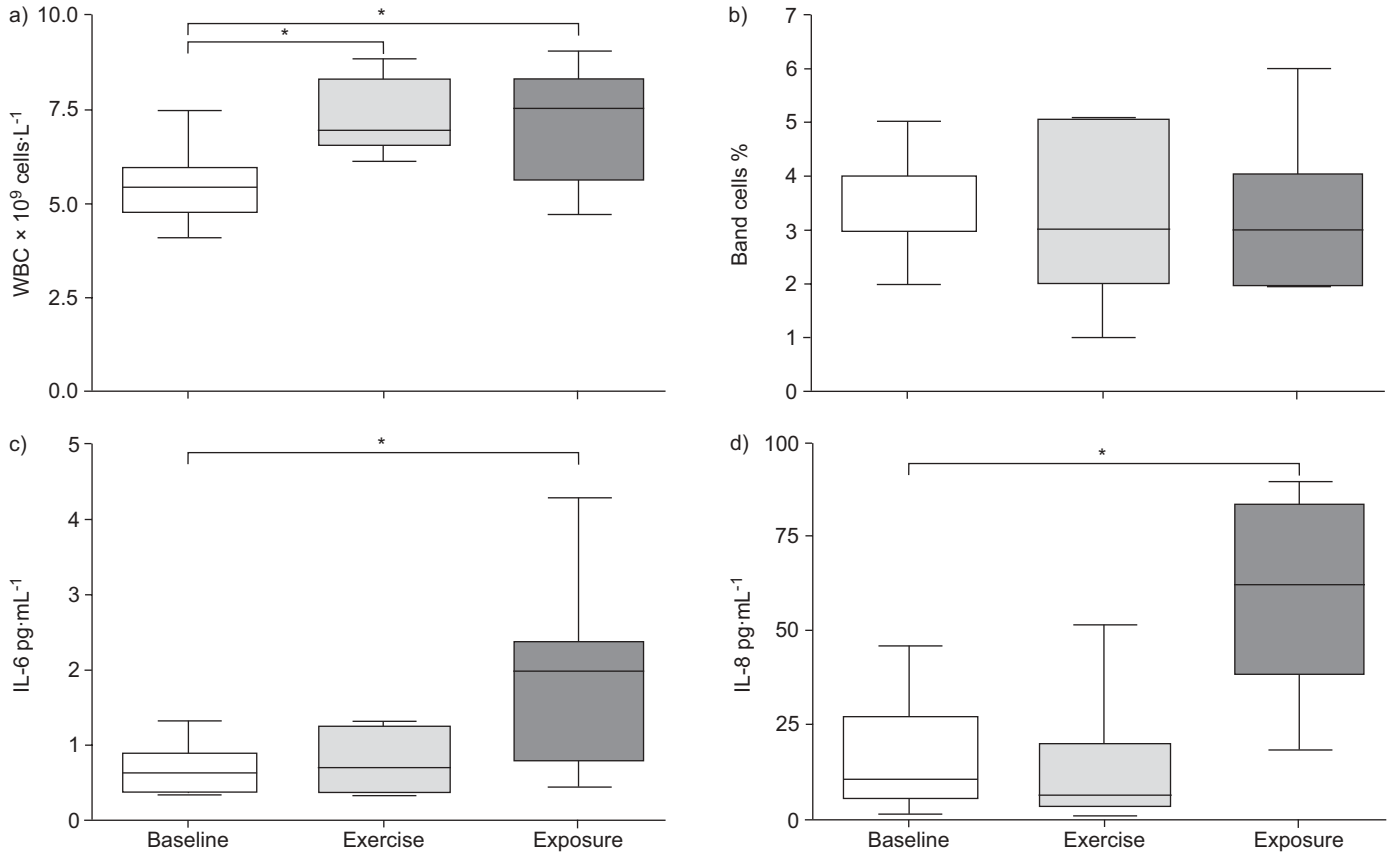

FIGURE 7. Mean circulating a) white blood cell (WBC), b) band cell, c) interleukin (IL)-6 and d) IL-8 level changes at baseline and with exercise. Data are expressed as mean from nine firefighters with paired sampling at baseline (□) and after shifts involving strenuous activity but no smoke exposure (▒) and with smoke exposure (■). Whiskers represent SEM. *: $p<0.05$.

exposures other than to particulate matter, the increase in sputum macrophages with carbon inclusions confirms increased inhalation of airborne particulate matter in the exposure group compared with baseline sampling. The largest increase was seen in the macrophages with few particle inclusions, suggesting mild to moderate exposure. Alternatively, the low particle load of macrophages could also be due to impaired macrophage phagocytic ability following exposure to airborne particulate matter, as previously reported by RUDELL *et al.* [11].

The majority of symptoms reported by the firefighters in the present study were relatively mild complaints related to irritation of the respiratory tract. These findings are consistent with previous studies that have shown an increase in respiratory symptoms, including nasal irritation, cough, sputum production and wheezing [24, 41]. The clinical importance of this respiratory irritation may be minor in the present study population, which was comprised of health subjects with no underlying respiratory conditions. However, in ''at-risk'' subjects with conditions such as asthma and chronic obstructive pulmonary disease, this upper and lower respiratory tract irritation may be sufficient to exacerbate the lung condition. This is supported by the significant increase in outpatient visits and hospitalisations for respiratory illnesses in children, during the forest fires of Southern California, USA [24], and in both Singapore [25] and Indonesia [26] in 1997.

In the present study, a decline in FEV1 with exposure to forest fire smoke was not demonstrated. Additionally, a significant degree of airway hyperreactivity induced by hypertonic saline inhalation in post-exposure testing was not detected. This differs from the findings of other studies; several have shown a small decline in pulmonary function (FEV1 and forced vital capacity) associated with exposure to wildfire smoke [40, 41]. BETCHLEY *et al.* [40] have also reported a small but persistent post-season decrement in FEV1 and mean forced expiratory flow between 25 and 75% of forced vital capacity, 2.5 months after cessation of fire-fighting activity; this suggests that the respiratory effects of forest fire smoke inhalation last long after exposure is resolved. The discrepancy between the present findings and those of previous studies may also be related to the relatively low degree of accumulated exposure in the present study. Furthermore, a larger study would be necessary in order to establish whether acute exposure of healthy nonasthmatic subjects (such as those in the present study) to wild fire smoke induces airway reactivity.

Analysis of induced sputum samples showed an increase in the fraction of granulocytes (mostly neutrophils) in sputum samples following smoke exposure. This suggests recruitment of granulocytes into the airway and is consistent with numerous previous studies in both animals [42–46] and humans [7, 9–11], which have shown increased granulocyte

counts, most commonly neutrophilia, in bronchoalveolar lavage and induced sputum samples following exposure to particulate matter. An increase in the number of macrophages in induced sputum was not found in the present study, which suggests that the predominant inflammatory response in the lung with acute wood smoke exposure is neutrophilic in nature.

Airspace macrophages that phagocytose airborne particles are not only capable of eliciting a local inflammatory response in the lung, but also contribute to the initiation of a systemic inflammatory response [17, 18, 20]. In the present study, a significant increase in circulating white blood cells, PMN and band cell counts following fire-fighting shifts has been demonstrated. Band cells are immature PMN produced in the bone marrow; an increase in band cell count in the peripheral circulation is an indication that the marrow has been stimulated to increase the release of granulocytes [47]. This bone marrow stimulation, induced by forest fire smoke exposure, is consistent with animal studies that have shown an increase in circulating band cell counts following exposure to ambient air pollution particles [17–19]. In addition, these same animal studies provided further evidence for stimulation of the bone marrow by demonstrating a decrease in the transit time of PMN cells and monocytes through the bone marrow after exposure to ambient particles [17–19]. Bone marrow stimulation induced by exposure to air pollutants is also supported by the study of TAN *et al.* [47], which showed an increase in circulating band cell counts in healthy army recruits exposed to smoke haze during the 1997 Southeast Asian forest fires.

In order to further support the idea that biomass smoke exposure induces a systemic inflammatory response, the present study showed elevated levels of circulating cytokines (IL-6, IL-8 and MCP-1) in firefighters following smoke exposure (fig. 5). IL-6 is considered an important multifunctional cytokine involved in the regulation of a variety of cellular responses, including the induction of acute-phase protein synthesis, lymphocyte activation and haematopoiesis [48]. It has been shown previously that IL-6 is a potent stimulator of the bone marrow that increases the transit of PMN and monocytes through the marrow and their release into the circulation [49]. IL-6 also promotes the sequestration of PMN in the lung [50] in a similar manner to that of IL-8 [51]. IL-6 has also been shown to play an important role in the pathogenesis of atherosclerosis either directly *via* the release of platelets from the marrow, or indirectly by increasing acute phase proteins such as CRP and fibrinogen [52, 53]. In the present study, CRP was not elevated following exposure, suggesting that IL-6 alone is insufficient to increase circulating CRP levels following wood smoke exposure. Changes in the fraction of macrophages with particle inclusions correlated with changes in band cell counts (fig. 6a) and circulating IL-6 correlates with the increase in airspace granulocytes (fig. 6b) following exposure. This suggests that lung inflammation impacts systemic inflammation. Furthermore, the similarity of mediators produced by macrophages exposed to particles [12, 17, 18, 20] and increased circulating mediators suggests that these cells are critically important in generating the systemic inflammatory response induced by exposure to wood smoke.

It is known that vigorous exercise is associated with an increase in circulating white blood cells and PMN counts, largely due to mobilisation of these cells from the intravascular marginated pool by catecholamines [54]. In a subgroup of nine firefighters, an increase in circulating white blood cell and PMN counts, equivalent to those seen following smoke exposure, was induced by strenuous exercise without smoke exposure. Therefore, the increase in white blood cell and PMN counts appears to be part of a nonspecific stress response and not a feature unique to smoke inhalation. The absence of an increase in band cell counts, IL-6 and IL-8 in the exercise control samples suggests that the increase in band cells post-exposure is not a stress response due to the physical exertion of fire-fighting, but rather a unique response to forest fire smoke inhalation. Collectively, the increase in peripheral band cell counts, as well as serum IL-6 and IL-8 levels, following exposure to forest fire smoke provides evidence for stimulation of the bone marrow and the initiation of a systemic inflammatory response as a consequence of smoke inhalation.

In summary, the present study has shown that healthy seasonal forest firefighters exposed to biomass smoke mount both a pulmonary and systemic inflammatory response. Examination of forest firefighters exposed to wildfire smoke provides a unique opportunity to specifically assess the acute effects of biomass air pollution as well as furthering the understanding of the toxicity of particulate matter air pollution in humans in general. The present results provide a plausible mechanism for the increased cardiopulmonary morbidity and mortality that epidemiological studies have associated with air pollution from biomass smoke inhalation. The findings also support a growing body of research showing that inhalation of particulate matter from a variety of sources generates a local inflammatory response within the lungs, which subsequently initiates a systemic response resulting in the adverse health consequences associated with air pollution exposure. These adverse health effects may be of little clinical consequence for young healthy firefighters; however in susceptible subjects exposed to wildfire smoke they could trigger exacerbations of asthma and chronic obstructive pulmonary disease, predispose to lung infection or precipitate acute cardiac events [53, 55]. The long-term consequences of smoke exposure for seasonal firefighters is less well studied but there is clearly a need for methods to reduce it, in order to attenuate both the local (lung) and systemic effects of exposure to wildfire smoke.

ACKNOWLEDGEMENTS

The present authors would like to thank British Columbia Forest Services for their cooperation and generous support to make this study possible.

REFERENCES

1 Goldberg MS, Burnett RT, Bailar JC 3rd, *et al*. The association between daily mortality and ambient air particle pollution in Montreal, Quebec. 1. Nonaccidental mortality. *Environ Res* 2001; 86: 12–25.

2 Goldberg MS, Burnett RT, Bailar JC 3rd, *et al*. The association between daily mortality and ambient air particle pollution in Montreal, Quebec. 2. Cause-specific mortality. *Environ Res* 2001; 86: 26–36.

3 Dockery DW, Pope CA 3rd, Xu X, *et al*. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993; 329: 1753–1759.
4 Samet JM, Dominici F, Curriero FC, Coursac I, Zeger SL. Fine particulate air pollution and mortality in 20 U.S. cities, 1987–1994. *N Engl J Med* 2000; 343: 1742–1749.
5 Filleul L, Le Tertre A, Baldi I, Tessier JF. Difference in the relation between daily mortality and air pollution among elderly and all-ages populations in southwestern France. *Environ Res* 2004; 94: 249–253.
6 Seaton A, Macnee W, Donaldson K, Godden D. Particulate air pollution and acute health effects. *Lancet* 1995; 345: 176–178.
7 Salvi S, Blomberg A, Rudell B, *et al.* 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* 1999; 159: 702–709.
8 Mukae H, Vincent R, Quinlan K, *et al.* The effect of repeated exposure to particulate air pollution (PM10) on the bone marrow. *Am J Respir Crit Care Med* 2001; 163: 201–209.
9 Ghio AJ, Devlin RB. Inflammatory lung injury after bronchial instillation of air pollution particles. *Am J Respir Crit Care Med* 2001; 164: 704–708.
10 Nordenhäll C, Pourazar J, Blomberg A, Levin JO, Sandström T, Adelroth E. Airway inflammation following exposure to diesel exhaust: a study of time kinetics using induced sputum. *Eur Respir J* 2000; 15: 1046–1051.
11 Rudell B, Blomberg A, Helleday R, *et al.* Bronchoalveolar inflammation after exposure to diesel exhaust: comparison between unfiltered and particle trap filtered exhaust. *Occup Environ Med* 1999; 56: 527–534.
12 Becker S, Soukup JM, Sioutas C, Cassee FR. Response of human alveolar macrophages to ultrafine, fine, and coarse urban air pollution particles. *Exp Lung Res* 2003; 29: 29–44.
13 Fujii T, Hayashi S, Hogg JC, Vincent R, van Eeden SF. Particulate matter induces cytokine expression in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2001; 25: 265–271.
14 Ishii H, Fujii T, Hogg JC, *et al.* Contribution of IL-1β and TNF-α to the initiation of the peripheral lung response to atmospheric particulates (PM10). *Am J Physiol Lung Cell Mol Physiol* 2004; 287: L176–L183.
15 Suwa T, Hogg JC, Vincent R, Mukae H, Fujii T, van Eeden SF. Ambient air particulates stimulate alveolar macrophages of smokers to promote differentiation of myeloid precursor cells. *Exp Lung Res* 2002; 28: 1–18.
16 van Eeden SF, Tan WC, Suwa T, *et al.* Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM10). *Am J Respir Crit Care Med* 2001; 164: 826–830.
17 Mukae H, Hogg JC, English D, Vincent R, van Eeden SF. Phagocytosis of particulate air pollutants by human alveolar macrophages stimulates the bone marrow. *Am J Physiol Lung Cell Mol Physiol* 2000; 279: L924–L931.
18 Goto Y, Ishii H, Hogg JC, *et al.* Particulate matter air pollution stimulates monocyte release from the bone marrow. *Am J Respir Crit Care Med* 2004; 170: 891–897.
19 Fujii T, Hayashi S, Hogg JC, *et al.* Interaction of alveolar macrophages and airway epithelial cells following exposure to particulate matter produces mediators that stimulate the bone marrow. *Am J Respir Cell Mol Biol* 2002; 27: 34–41.
20 Terashima T, Wiggs B, English D, Hogg JC, van Eeden SF. Phagocytosis of small carbon particles (PM10) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow. *Am J Respir Crit Care Med* 1997; 155: 1441–1447.
21 Naeher LP, Brauer M, Lipsett M, *et al.* Woodsmoke health effects: a review. *Inhal Toxicol* 2007; 19: 67–106.
22 Hays MD, Geron CD, Linna KJ, Smith ND, Schauer JJ. Specifications of gas-phase and fine particle emissions from burning of foliar fuels. *Environ Sci Technol* 2002; 36: 2281–2295.
23 Vedal S, Dutton SJ. Wildfire air pollution and daily mortality in a large urban area. *Environ Res* 2006; 102: 29–35.
24 Künzli N, Avol E, Wu J, *et al.* Health effects of the 2003 Southern California wildfires on children. *Am J Respir Crit Care Med* 2006; 174: 1221–1228.
25 Emmanuel SC. Impact to lung health of haze from forest fires: the Singapore experience. *Respirology* 2000; 5: 175–182.
26 Swinbanks D. Forest fires cause pollution crisis in Asia. *Nature* 1997; 389: 321.
27 Sastry N. Forest fires, air pollution, and mortality in southeast Asia. *Demography* 2002; 39: 1–23.
28 Reinhardt TE, Ottmar RD. Smoke exposure at Western wildfires. US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Research Paper PNW-RP-525, 2000.
29 Swiston J, Davidson W, Attridge S, Li G, Brauer M, van Eeden SF. Acute pulmonary and vascular effects of wildfire smoke inhalation. *Proc Am Thorac Soc* 2006; 3: A804.
30 Reinhardt TE, Ottmar RD. Baseline measurements of smoke exposure among wildland firefighters. *J Occup Environ Hyg* 2004; 1: 593–606.
31 Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995; 152: 1107–1136.
32 Paggiaro PL, Chanez P, Holz O, *et al.* Sputum induction. *Eur Respir J* 2002; 20: Suppl. 37, 3S–8S.
33 Efthimiadis A, Spanevello A, Hamid Q, *et al.* Methods of sputum processing for cell counts, immunocytochemistry and *in situ* hybridisation. *Eur Respir J* 2002; 20: Suppl. 37, 19S–23S.
34 Kelly MM, Hargreave FE, Cox G. A method to preserve sputum for delayed examination. *Eur Respir J* 2003; 22: 996–1000.
35 Nel A. Atmosphere. Air pollution-related illness: effects of particles. *Science* 2005; 308: 804–806.
36 Utell MJ, Frampton MW, Zareba W, Devlin RB, Cascio WE. Cardiovascular effects associated with air pollution: potential mechanisms and methods of testing. *Inhal Toxicol* 2002; 14: 1231–1247.
37 Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society. Health effects of outdoor air pollution. *Am J Respir Crit Care Med* 1996; 153: 3–50.
38 Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society. Health effects of outdoor air pollution. Part 2. *Am J Respir Crit Care Med* 1996; 153: 477–98.

39 Sharkey B, ed. Health Hazards of Smoke: Recommendations of the April 1997 Consensus Conference. Missoula, United States Department of Agriculture, Forest Service, Technology and Development Program, 1997.

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

41 Rothman N, Ford DP, Baser ME, *et al.* Pulmonary function and respiratory symptoms in wildland firefighters. *J Occup Med* 1991; 33: 1163–1167.

42 Dick CA, Singh P, Daniels M, Evansky P, Becker S, Gilmour MI. Murine pulmonary inflammatory responses following instillation of size-fractionated ambient particulate matter. *J Toxicol Environ Health A* 2003; 66: 2193–2207.

43 Nemmar A, Hoylaerts MF, Hoet PH, Vermylen J, Nemery B. Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis. *Toxicol Appl Pharmacol* 2003; 186: 38–45.

44 Renwick LC, Brown D, Clouter A, Donaldson K. Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. *Occup Environ Med* 2004; 61: 442–447.

45 Rhoden CR, Lawrence J, Godleski JJ, González-Flecha B. *N*-Acetylcysteine prevents lung inflammation after short-term inhalation exposure to concentrated ambient particles. *Toxicol Sci* 2004; 79: 296–303.

46 Roberts ES, Richards JH, Jaskot R, Dreher KL. Oxidative stress mediates air pollution particle-induced acute lung injury and molecular pathology. *Inhal Toxicol* 2003; 15: 1327–1346.

47 Tan WC, Qiu D, Liam BL, *et al.* The human bone marrow response to acute air pollution caused by forest fires. *Am J Respir Crit Care Med* 2000; 161: 1213–1217.

48 Kamimura D, Ishihara K, Hirano T. IL-6 signal transduction and its physiological roles: the signal orchestration model. *Rev Physiol Biochem Pharmacol* 2003; 149: 1–38.

49 Suwa T, Hogg JC, English D, van Eeden SF. Interleukin-6 induces demargination of intravascular neutrophils and shortens their transit in marrow. *Am J Physiol Heart Circ Physiol* 2000; 279: H2954–H2960.

50 Suwa T, Hogg JC, Klut ME, Hards J, van Eeden SF. Interleukin-6 changes deformability of neutrophils and induces their sequestration in the lung. *Am J Respir Crit Care Med* 2001; 163: 970–976.

51 Terashima T, English D, Hogg JC, van Eeden SF. Release of polymorphonuclear leukocytes from the bone marrow by interleukin-8. *Blood* 1998; 92: 1062–1069.

52 Rattazzi M, Puato M, Faggin E, Bertipaglia B, Zambon A, Pauletto P. C-reactive protein and interleukin-6 in vascular disease: culprits or passive bystanders? *J Hypertens* 2003; 21: 1787–1803.

53 Magliano DJ, Liew D, Ashton EL, Sundararajan V, McNeil JJ. Novel biomedical risk markers for cardiovascular disease. *J Cardiovasc Risk* 2003; 10: 41–55.

54 Foster NK, Martyn JB, Rangno RE, Hogg JC, Pardy RL. Leukocytosis of exercise: role of cardiac output and catecholamines. *J Appl Physiol* 1986; 61: 2218–2223.

55 Bai N, Khazaei M, van Eeden SF, Laher I. The pharmacology of particulate matter air pollution-induced cardiovascular dysfunction. *Pharmacol Ther* 2007; 113: 16–29.