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Pneumoproteins and inflammatory biomarkers in asphalt pavers

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Abstract

Pneumoproteins, biomarkers of systemic inflammation and endothelial activation were studied across a season in 72 asphalt pavers, 32 asphalt plant operators and 19 asphalt engineers. Smokers had lower concentrations of Clara cell protein (CC-16) and surfactant protein A, but higher concentrations of surfactant protein D, interleukin 6, C-reactive protein, fibrinogen and intercellular adhesion molecule (ICAM)-1 than non-smokers. Smokers reporting wheezing had lower mean CC-16 concentration than smokers not reporting wheezing (5.7 vs 8.6 μg l^{-1}; $p = 0.05$). Cholesterol, P-selectin and ICAM-1 were lower in pavers and operators at the end compared with the start of the season. This may be related to increased physical activity during the season.

Keywords: *Pneumoproteins; endothelial activation; inflammation; asphalt fumes*

Introduction

Evidence has been presented that asphalt workers have increased mortality due to pulmonary and cardiovascular diseases. A recent study reported a dose–response relationship between airborne benzo(a)pyrene and fatal ischaemic heart disease with a relative risk of 1.64 (95% confidence interval (CI) 1.13–2.38) in the highest exposed subgroup (Burstyn et al. 2005). Asphalt pavers in Norway who were employed the first time at least 25 years prior to the study had increased mortality due to circulatory (SMR = 1.52; 95% CI 1.01–2.22) and respiratory (SMR = 2.29; 95% CI 0.99–4.52) diseases when compared with national rates (Randem et al. 2003).

Asphalt pavers and asphalt plant operators can be exposed to a complex atmosphere containing oil mist and vapour, polycyclic aromatic hydrocarbons (PAHs), particles from crushed stone, road dust and vehicle exhaust including diesel particles (Elihn et al. 2008). Asphalt pavers are also exposed to ultrafine particles, and a median concentration of about 3.4×10^4 cm^{-3} was measured during paving activities (Elihn et al. 2008). Thus, asphalt pavers and asphalt plant operators can be exposed to contaminants that have the potential to cause pulmonary and, perhaps, cardiovascular health impairment.

Epidemiological studies have shown increased pulmonary and cardiovascular mortality by increasing environmental exposure to airborne pollution (Dockery et al. 1993, Nafstad et al. 2004, Pope et al. 2004a, Miller et al. 2007). Animal studies have provided evidence that exposure to particles containing SiO_2 (Nemmar et al. 2005), road dust (Radomski et al. 2005, Cascio et al. 2007, Cozzi et al. 2007) or carbon nano-tubes (Nemmar et al. 2007) can increase thrombus formation, a well-known risk factor for cardiovascular events. The latter studies also show that endothelial and platelet activation is important in the pathological process of increased thrombus formation. P-selectin has a key role in endothelial activation, and some of the studies demonstrated increased levels of P-selectin (Radomski et al. 2005, Cascio et al. 2007, Cozzi et al. 2007), while the inhibition of P-selectin reversed the pathological process in another study (Nemmar et al. 2007).

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A recent experimental study of healthy males exposed to 350 μg m^{-3} diluted diesel exhaust particles reported increased platelet activation *in vivo* and formation of thrombi *ex vivo* (Lucking et al. 2008). Increased levels of acute-phase reactants were observed in volunteers exposed to smoke generated from wood burning (Barregård et al. 2006). It has been proposed that exposure to particles may result in pulmonary inflammation, and that pulmonary macrophages produce proinflammatory cytokines (e.g. interleukin (IL)-6) inducing a low-grade systemic inflammation with increased synthesis of acute-phase reactants (e.g. C-reactive protein (CRP) or serum amyloid A (SAA)), endothelial and platelet activation and increased thrombogenicity (Scapellato & Lotti 2007). The cytokines bind to receptors on the endothelial surface, resulting in complex biological alterations in which intercellular adhesion molecule (ICAM)-1 and vascular cellular adhesion molecule (VCAM)-1 are also increasingly expressed. They mediate the adhesion of monocytes and leukocytes to the vessel wall and to already activated platelets (Huo & Ley 2001, Kuldo et al. 2005).

Studies of humans exposed to polluted ambient air have shown increased levels of CRP, IL-6, ICAM-1, fibrinogen and plasma activator inhibitor, indicating an acute-phase inflammatory response at relatively low exposure levels (Pope et al. 2004b, Dubowsky et al. 2006, Rückerl et al. 2006, 2007, Chuang et al. 2007, Shima 2007). Similar alterations were reported in subjects more highly exposed to dusts in occupational settings, although few such studies have been carried out (Hilt et al. 2002).

This study aimed to assess selected biomarkers of pulmonary epithelial alterations, proinflammatory cytokines, acute-phase reactants and endothelial activation and adhesion molecules in asphalt pavers. The associations between pulmonary function, self-reported airway symptoms, smoking habits, obesity and the determined biomarker levels were also studied. Asphalt paving is, because of long winters with snow, typical seasonal work in Norway, and the subjects experience only negligible chemical exposure outside the paving season. Thus the subjects were examined shortly before the paving season and shortly before the end of the season.

Materials and methods

Study design and selection of subjects

All workers in the asphalt department of Norway's largest road construction and maintenance company were invited to a study across the asphalt paving season, including lung function tests and the assessment of biomarkers in the blood. The examinations were conducted shortly before the start of the asphalt paving season in April–May 2005 (Exam 1). The subjects were re-examined in September–October 2005 (Exam 2), shortly before the end of the season.

All 184 subjects who were invited (all males) agreed to participate in Exam 1. Some subjects ($n = 49$), mostly lorry drivers, were contract workers who left the company shortly after Exam 1. Thus, they were not considered for Exam 2. Of the remaining 135 subjects, five quit their jobs during the season. Three subjects were on vacation at Exam 2 and could not be examined. Four subjects were lost to follow-up for unknown reasons. Thus, this study comprises 123 subjects.

The study was approved by the local ethical committee for medical research. An informed written consent was obtained from the participants.

Description of the occupational groups

The subjects were categorized into three groups according to their exposure situation: asphalt pavers ($n = 72$), asphalt plant operators ($n = 32$) and asphalt engineers ($n = 19$). Asphalt pavers are directly involved in road paving. This group consists of paver operators, screed men and roller drivers. Mostly they alternate between the different work tasks. A paver operator is seated on top of the paving machine between the hopper and the screed, which discharges the hot asphalt mix onto the surface being paved. Screed men control asphalt discharge through the screed. They also fix the edges of the asphalt on the road manually, and help to spread the hot mixture discharged from the screed using a hand rake. Roller drivers drive the roller that compresses the asphalt mixture once it is applied to the surface. The roller is normally equipped with a cabin. Pavers are typically exposed to aerosols generated from the process and from the traffic passing by, such as oil mist and vapour, PAHs and nitrogen oxides (Elihn et al. 2008). During the winter season the asphalt pavers often have more sedentary work as snow plough drivers with only negligible chemical exposure.

Asphalt plant operators monitor the asphalt mixing from a control room. Asphalt mixing is a process whereby bitumen is combined with gravel. The operators must leave their cabins several times during the day for adjustments of the plant machinery. The exposure of asphalt plant operators is in many respects similar to that of the asphalt pavers. However, compounds related to road traffic passing by are generally absent from their work environment (Elihn et al. 2008). During the winter season one operator usually does maintenance work at the plant with negligible chemical exposure, while the remaining subjects have more sedentary work as snow plough drivers.

The asphalt engineers are responsible for enterprise, planning and managing the work and reporting and estimating costs. The engineers are generally only exposed

to asphalt-related products in negligible amounts. Their work is similar the whole year.

Exposure assessment

Total dust, oil mist, PAHs and gases were collected by personal samplers during the asphalt season. A random sample of workers was asked to participate in the exposure assessment. In total, 42 workers carried personal samplers, and most of the subjects (90%) were monitored on more than one occasion. The sampling duration at each occasion was 7–8 h.

The asphalt pavers had a significantly (p <0.001) higher exposure to total PAH than the plant operators, but at low levels (median 1.5 μg m^{-3}; range 0.2–6.6; n = 48 measurements vs median 0.6 μg m^{-3}; range 0.2–0.9; n = 12 measurements). The plant operators had significantly (p = 0.002) higher exposure to total dust than the pavers (median 1.1 mg m^{-3}; range 0.3–1.6; n = 9 measurements vs median 0.3 mg m^{-3}; range 0–1.7; n = 56 measurements). Exposure to nitrogen dioxide (n = 6) and oil mist (n = 16) was only measured among pavers, because it is not relevant for asphalt plant workers. Although the median exposure to nitrogen dioxide was 0.3 ppm, the pavers may have been exposed to higher levels when paving asphalt in tunnels (maximum 3.4 ppm measured). The median exposure to oil mist was 0.1 mg m^{-3}, but exposure to oil mist can also be significant on some occasions. Oil mist was measured on one occasion as 1.7 mg m^{-3} in an asphalt paving machine without a cabin. Only a few analyses of respirable dust (n = 4), volatile organic compounds (n = 4) and carbon monoxide (n =14) were done, as previous analyses showed low concentrations. Details have been published elsewhere (Ulvestad et al. 2007b).

Spirometry and questionnaire

Lung function was examined using bidirectional ultrasonic transit time measurements with a Spirare SPS310 spirometer according to recommended guidelines (American Thoracic Society 1987). The examinations were carried out between 07.00 and 10.00 AM at both examinations. The lung function variables (forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and forced expiratory flow at 50% FVC ($FEF_{50\%}$)) were expressed in absolute values and as the percentages of the predicted values according to the reference values of the European Coal and Steel Community (Quanjar et al. 1993). The subjects answered a self-administered questionnaire (a modified version of the one developed by the Medical Research Council in the UK) before Exam 1. Information on age and smoking habits was obtained from a general questionnaire.

The subjects were classified as never smokers, former smokers and current smokers. Former smokers had stopped smoking more than 12 months earlier. The subjects were asked about job tasks they had had during the season at Exam 2, and were categorized into the occupational groups according to these tasks.

Blood sampling and processing

The participants were asked to abstain from smoking, eating and drinking after midnight on the day of the examinations. Blood samples were collected by venipuncture between 07.00 and 10.00 AM at both examinations. Whole blood was collected in 7 ml vacutainers with EDTA, 5 ml tubes with heparin, 5 ml tubes with citrate and in 10 ml tubes without additives (BD Diagnostics, Plymouth, UK).

The EDTA-, citrate- and heparin-containing blood samples were kept on ice for a maximum of 60 min before centrifugation at 2000 *g* for 15 min. The samples without additives were kept at room temperature for coagulation from 60–120 min before centrifugation at 1300 *g* for 15 min. Plasma/serum was pipetted off and transferred into NUNC® cryotubes (NUNC, Roskilde, Denmark), which were put on dry ice and transported to the laboratory for storage at -70°C.

Analytical methods

Samples collected before the asphalt paving season and before the end of season were analysed in one run at laboratories without knowledge of exposure status and sampling time. IL-6 was analysed in EDTA plasma with the commercial enzyme-linked immunosorbent assay (ELISA) kit Quantikine HS (R&D Systems, Abingdon, UK). Fibrinogen was measured in citrate plasma, using reagents from bioMerieux (Durham, NC, USA) as adapted to their Stago analyser. High-sensitivity micro-CRP was determined by an immunoturbidometric method (Roche, Basel, Switzerland) using a Hitachi 917 analyser. Details have been published elsewhere (Ulvestad et al. 2007b).

Commercial ELISA kits were used for the determination of ICAM-1, VCAM-1, P-selectin (R&D systems), CC-16, surfactant protein D (SP-D) (BioVendor Laboratory Medicine Inc., Brno, Czech Republic) and amyloid A (Anogen, Mississauga, Ontario, Canada) in serum. Surfactant protein A (SP-A) in serum was measured using a homemade sandwich ELISA using two different antibodies to human SP-A, one polyclonal and one monoclonal. Pooled serum from 16 of the asphalt workers was used as the standard, and the concentration of SP-A in this internal standard was set at 1033 mg l^{-1}. The concentration of the standard was calculated by comparing the absorbance of the standard with the absorbance from a sample of native SP-A with a known concentration of 105 mg l^{-1}, kindly provided by Ida Tornoe and her colleagues at the Department of Immunology and

Microbiology, Institute of Medical Biology, University of Southern Denmark, Odense, Denmark. The concentration of the native SP-A was estimated by absorbance of unmodified protein at optical density at 280 nm (OD280). The comparison was made with triplet samples on two different plates and the median of the six values was used as the standard concentration. Immunoassay plates (Medisorp 96-well; NUNC) were coated and incubated for 2 h at 37°C with a polyclonal goat antibody for human SP-A (AB3422; Millipore, Billerica, MA, USA, and Canada) diluted in phosphate-buffered saline (PBS) (10 µl antibody in 11 ml PBS). The plates were then washed three times with PBS/0.05% Tween-20, blocked using gelatin in PBS (PBS/5% gelatin) and incubated for 2 h at 37°C. Samples and standards were immediately diluted in PBS. Thereafter the plates were washed three times with PBS/0.05% Tween-20, and the samples and standards were added to the wells. The plates were incubated at 4°C overnight. On the following day the plates were washed three times, and the biotin-labelled monoclonal antibody for human SP-A (HYB 238-04; Antibody Shop, Gentofte, Denmark) was added to each well, 10 µl antibody in 11 ml PBS). After incubation for 2 h at 37°C, the wells were washed three times with PBS/0.05% Tween-20 and incubated with diluted streptavidin–HRP conjugate (Sigma-Aldrich, St Louis, MO, USA) (10 µl in 11 ml PBS) for 2 h at 37°C. The wells were again washed three times with PBS/0.05% Tween-20 before adding OPD/H_2O_2 (Dako, Glostrup, Denmark) for colour development for about 7 min after which the development was stopped with 0.6 M H_2SO_4. The optical density was read at 450 and 490 nm using a microtitre plate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA).

Statistics

Distributions of continuous variables with skewness exceeding 2.0 were log-transformed to achieve normal distributions. Thus, the concentrations of IL-6, SAA, CRP, SP-A and SP-D were log-transformed, and the geometric means (GM) are presented. Otherwise the arithmetic mean (AM) values are given. Analysis of variance (ANOVA) was applied for group comparisons of continuous variables, the least square difference (LSD) being calculated when more than two groups were compared. When concentrations were compared in the same individuals before season and before the end of season, the paired samples *t*-test was used. Univariate associations were based on least square regression, calculating the Pearson's correlation coefficient (Pearson's *r*). Multiple linear regression analysis (stepwise backwards procedure) was used to assess associations between biomarkers and several independent variables simultaneously. The level of significance was set at 0.05 (two-tailed). The statistics were calculated with the data package SPSS 15.0 on a PC.

Results

Background data, lung function parameters and the concentrations of the measured biomarkers at Exam 1 are presented in Table 1. Thirty subjects reported being current smokers, while 48 subjects reported never having been active smokers. There was a wide range in the body mass index (BMI) of the participants. The pulmonary functional measures FVC% and FEV_1% were on average in the range of the expected values. Eleven subjects had the ratio FEV/FVC below 0.70 (without testing with bronchodilatator).

The Pearson's correlation coefficients between the biomarker concentrations measured at Exam 1 and Exam 2 were all high and highly statistically significant (p <0.001) (not tabulated) ($r_{CC\text{-}16}=0.85$; $r_{SP\text{-}A}=0.99$; $r_{SP\text{-}D}=0.95$; $r_{ICAM\text{-}1}=0.91$; $r_{VCAM\text{-}1}=0.92$; $r_{P\text{-}selectin}=0.92$; $r_{cholesterol}=0.81$; $r_{fibrinogen}=0.65$; $r_{CRP}=0.71$; $r_{IL\text{-}6}=0.62$; $r_{SAA}=0.46$).

Smoking habits had a substantial impact on the determined biomarker levels. Because background data were collected at Exam 1, the concentrations of the biomarkers determined at Exam 1 are presented according to self-reported smoking habits (Table 2). The concentrations of IL-6, CRP and SP-D were statistically significantly higher in the current smokers compared with the never-smokers and the former smokers. Current smokers had

Table 1. Background data of all participants (n=123) and the concentrations of biomarkers collected at Exam 1.

	Mean	Range
Age (years)	46.6	19–61
FVC (% of expected)	97.9	62–122
FEV_1 (% of expected)	94.0	63–126
FEV_1/FVC	0.78	0.47–0.89
BMI (kg m^{-2})	27.3	18.4–42.0
Current smokers (%)	24.4	–
Ex-smokers (%)	36.6	–
Never-smokers (%)	39.0	–
Asthma (%)	10.6	–
IL-6[a] (mg l^{-1})	1.6	0.3–15.0
CRP[ab] (mg l^{-1})	1.2	0.01–15.0
SAA[a] (mg l^{-1})	2.5	0.4–33.7
Fibrinogen (g l^{-1})	3.5	2.2–7.1
Cholesterol (mmol l^{-1})	5.8	3.6–8.5
CC-16 (µg l^{-1})	8.7	0.9–20.1
SP-A[ab] (mg l^{-1})	81	1.3–35548
SP-D[ac] (µg l^{-1})	110	24–404
P-Selectin[b] (µg l^{-1})	176	63–470
ICAM-1[b] (µg l^{-1})	280	136–483
VCAM-1[b] (µg l^{-1})	617	284–1767

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; BMI, body mass index; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; CC-16, Clara cell protein; SP, surfactant protein; ICAM, intercellular adhesion molecule; VCAM, vascular cellular adhesion molecule.

[a]Geometric mean; [b]two subjects missing; [c]seven subjects missing.

significantly lower concentrations of CC-16 and SP-A compared with the never-smokers. Also former smokers had significantly lower levels of SP-A than never-smokers. Four subjects had, for unknown reasons, very high SP-A concentrations (>10 000 mg l^{-1}) at both sampling occasions. The differences between the never-smokers and the former and current smokers, respectively, remained after excluding these subjects. When the results of the biomarkers presented in Table 2 were statistically adjusted according to BMI and age, the results remained similar. However, currents smokers had significantly higher levels of SAA than never-smokers (3.2 vs 2.2 mg l^{-1}; $p = 0.049$).

Multiple linear regression analysis was used to study further the quantitative impact of self-reported smoking habits on the determined biomarkers that were significantly different in the smokers and non-smokers, taking into consideration the BMI and age (Table 3). Current smoking (no. of cigarettes/day) was negatively associated with CC-16 and positively associated with IL-6, CRP, SP-D, ICAM-1 and fibrinogen. SP-A was not associated with current smoking, but negatively with previous smoking as assessed by pack-years. BMI was associated positively with IL-6 and the acute-phase reactants CRP and fibrinogen (and with SAA, but result not shown). A negative association between SP-D and BMI was also found.

Having a ratio FEV_1/FVC below 0.70 did not contribute to the statistical models. The concentrations of the pneumoproteins determined at baseline were not associated with lung function parameters as assessed by spirometry. However, the self-reporting of subjective symptoms related to lung function appears to have an impact on some biomarkers, in particular the reporting of wheezing. The mean concentrations of IL-6 (GM 2.1 vs 1.4 ng l^{-1}; $p = 0.005$), fibrinogen (3.7 vs 3.4 g l^{-1}; $p = 0.009$) and ICAM-1 (297 vs 272 μg l^{-1}; $p = 0.03$) were statistically significantly higher in 41 subjects reporting wheezing compared with subjects not reporting wheezing, while the higher concentrations of the acute-phase reactants CRP (1.6 vs 1.1 mg l^{-1}; $p = 0.07$) and SAA (3.0 vs 2.2 mg l^{-1}; $p = 0.07$) were of borderline significance. Subjects reporting wheezing had also nearly statistically significantly lower concentrations of SP-A (51 vs 102 mg l^{-1}; $p = 0.08$) and CC-16 (7.8 vs 9.2 μg l^{-1}; $p = 0.08$). The 22 subjects reporting morning cough had higher concentrations of SP-D (141 vs 104 μg l^{-1}; $p = 0.02$) and ICAM-1 (316 vs 272 μg l^{-1}; $p = 0.03$) and lower concentrations of SP-A (34 vs 98 mg l^{-1}; $p = 0.03$) than subjects not reporting this symptom. For the other subjective symptoms only small differences in the biomarker levels between reporting or not reporting the symptom were observed.

Because the biomarker concentrations measured at Exam 1 were apparently related both to smoking and the reporting of wheezing, the subjects were stratified into four groups according to being a smoker or not, and reporting wheezing or not. Figure 1 shows the CC-16 concentrations measured at Exam 1 adjusted for age. When considering the current smokers only, reporting wheezing in addition to being a current smoker reduced the mean level of CC-16 substantially compared with smokers not reporting wheezing, the *p*-value for the difference being 0.05. Pack-years or amount of current smoking did not contribute to the difference, but the point estimate of

Table 2. The arithmetic mean concentrations and ranges of biomarkers measured in serum collected at Exam 1 according to smoking habits.

	Smoking status					
	Never smoker ($n = 48$)		Former smoker ($n = 45$)		Current smoker ($n = 30$)	
	Mean	Range	Mean	Range	Mean	Range
Age** (years)	43.6	19.0–60.0	49.5	24.0–61.0	47.1	31.0–61.0
BMI (kg m^{-2})	28.3	22.0–42.0	26.7	18.4–35.4	26.6	22.0–37.5
Smoking (g daily)	0	–	0	–	13.9	3.0–20.0
Smoking (pack-years)	0	–	15.1	1.8–60.0	19.2	1.7–35.0
IL-6[a]* (ng l^{-1})	1.4	0.3–9.1	1.5	0.6–12.4	2.3	0.6–15.0
CRP[a]* (mg l^{-1})	1.2	0.2–12.8	1.0	0.1–15.0	1.8[d]	0.01–12.5
SAA[a] (mg l^{-1})	2.3	0.4–10.8	2.3	0.4–30.1	3.1	0.7–33.7
Fibrinogen* (g l^{-1})	3.3	2.2–5.7	3.5	2.2–7.1	3.8	2.8–5.5
Cholesterol (mmol l^{-1})	5.6	3.8–7.7	6.0	3.7–8.0	5.8	3.6–8.5
CC-16* (μg l^{-1})	9.6	2.6–19.1	8.8	0.9–20.1	7.2	2.4–18.8
SP-A[a]*,** (mg l^{-1})	222[c]	1.8–26944	47[c]	1.3–35548	37	2.3–2672
SP-A[a,b]*,** (mg l^{-1})	162[c]	1.8–4463	41[c]	1.3–720	37	2.3–2672
SP-D*,*** (μg l^{-1})	90	29.9–241	111[e]	24.1–389	157[e]	46.8–404
P-selectin (μg l^{-1})	166[c]	63–299	180[c]	95–470	184	90–288
ICAM-1*,*** (μg l^{-1})	267[c]	192–399	269[c]	136–392	318	223–483
VCAM-1 (μg l^{-1})	621[c]	375–1012	633[c]	284–1767	589	393–1237

BMI, body mass index; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; CC-16, Clara cell protein; SP, surfactant protein; ICAM, intercellular adhesion molecule; VCAM, vascular cellular adhesion molecule.

[a]Geometric mean; [b]four subjects excluded; [c]one subject missing; [d]two subjects missing; [e]three subjects missing; *$p < 0.05$ between never-smokers and current smokers; **$p < 0.05$ between never-smokers and former smokers; ***$p < 0.05$ between current smokers and former smokers.

Table 3. Results from multiple linear regression analysis (backwards procedure) assessing relationships between biomarkers collected at Exam 1 and age, body mass index, current (g daily) and previous (pack-years) smoking habits as independent variables.

				Smoking		
		Age	BMI	Current	Previous	Mult. *r*
IL-6[a] (ng l^{-1})	–0.88***	0.01**	0.02**	0.01**	–	0.44***
CRP[a] (mg l^{-1})	–0.89**	–	0.03**	0.02**	–	0.39***
CC-16 (µg l^{-1})	9.2***	–	–	–0.13*	–	0.21*
SP-A[a] (Units)	2.18***	–	–	–	–0.03***	0.35***
SP-D[a] (µg l^{-1})	2.33***	0.004*	–0.019***	0.014***	–	0.54***
ICAM-1 (µg l^{-1})	268***	–	–	3.7***	–	0.42***
Fibrinogen (g l^{-1})	1.42*	0.02*	0.04**	0.02*	–	0.35**
SAA[a] (mg l^{-1})	–0.26^{ns}	–	0.024**	–	–	0.26**

IL, interleukin; CRP, C-reactive protein; CC-16, Clara cell protein; SP, surfactant protein; ICAM, intercellular adhesion molecule; SAA, serum amyloid A.

[a]Geometric mean; $^{*}p$ <0.05; $^{**}p$ <0.01; $^{***}p$ <0.001; ^{ns}not significant.

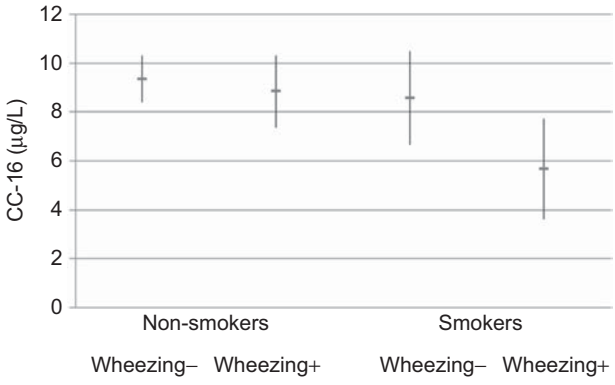

Figure 1. The arithmetic mean concentrations (and 95% confidence interval) of CC-16 determined at Exam 1 according to current smoking habits and the reporting of wheezing (adjusted for age).

the difference between the two groups increased from 2.8 to 3.5 ($p = 0.02$) when the difference in age was adjusted for. The results were similar when the CC-16 concentrations measured at Exam 2 were considered. The difference between smokers reporting wheezing or not did not attain statistical significance for the other biomarkers referred to above.

The biomarker concentrations measured at Exam 1 and Exam 2 according to occupation is shown in Table 4. CC-16 increased statistically significantly in all three occupational groups, but the highest increase being measured in the pavers. However, similar alterations across season were not observed for the other investigated pneumoproteins SP-A and SP-D.

The concentrations of the determined acute-phase reactants were comparable in all groups. The levels of the biomarkers indicative of endothelial activation (ICAM-1, VCAM-1 and P-selectin) decreased across the season in the plant operators and the pavers. This was also the case for the total cholesterol concentrations. When considering current non-smokers only, the same pattern of reduced ICAM-1 and P-selectin during the paving season emerged in the plant operators and the pavers (results not shown). However, CC-16 increased only slightly, and non-significantly, in the non-smoking engineers.

Discussion

This first study of biomarkers of endothelial/platelet activation and pneumoprotein levels in asphalt workers shows that self-reported smoking habits have a substantial impact on the determined concentrations of the pneumoproteins and biomarkers of inflammation. The reporting of subjective pulmonary symptoms, in particular wheezing, may also be an important determinant for some of the determined biomarkers. Lower concentrations of the biomarkers of endothelial/platelet activation in the asphalt pavers and plant operators before the end of season compared with before the season were observed. As previously reported, CC-16 increased in the pavers and plant operators across the season (Ulvestad et al. 2007a). A similar increase was not observed for the pneumoproteins SP-A and SP-D. A previous report from this study reported increased IL-6 in asphalt pavers (Ulvestad et al. 2007b). Although pavers who had done substantial asphalt stripping were not included in the present study, non-smoking asphalt pavers without a history of asthma had significantly increased IL-6 across the season (GM 1.3 vs 1.6 ng l^{-1}; $p = 0.02$).

Current smokers had lower CC-16 concentrations than current non-smokers as reported in other studies (Broeckart & Bernard 2000, Robin et al. 2002, Berthoin et al. 2004, Madsen et al. 2008). The impact of smoking habits on the serum concentrations of SP-A and SP-D has been less studied. Our study shows that smokers and ex-smokers have lower SP-A concentrations than never-smokers, and regression analysis indicates pack-years to be the more important determinant compared with the amount of current smoking. This is, however, in contrast to studies in humans that have shown similar (Robin et al. 2002, Mutti et al. 2006, Widmeier et al. 2007)

Table 4. The concentrations of the measured biomarkers before (Exam 1) and before the end of season (Exam 2) in all subjects according to occupation.

	Engineers ($n=19$)		Plant operators ($n=32$)		Pavers ($n=72$)	
	Exam 1	Exam 2	Exam 1	Exam 2	Exam 1	Exam 2
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
IL-6[a] (ng l^{-1})	1.6 (1.2–2.1)	1.5 (1.1–1.9)	1.8 (1.1–2.5)	1.7 (1.3–2.2)	1.6 (1.3–1.8)[b]	1.7 (1.4–2.0)
CRP[a] (mg l^{-1})	1.2 (0.7–2.0)[b]	1.1 (0.7–1.8)	1.6 (1.1–2.3)[b]	1.2* (0.9–1.7)	1.1 (0.9–1.4)[b]	1.1 (0.9–1.3)
SAA[a] (mg l^{-1})	3.2 (2.1–4.8)	2.3 (1.7–3.1)	2.5 (1.9–3.2)	2.3 (1.7–3.3)	2.3 (1.9–2.8)	2.4 (2.0–2.9)
Fibrinogen (g l^{-1})	3.6 (3.1–4.1)	3.4 (3.1–3.7)	3.5 (3.2–3.7)	3.5 (3.2–3.9)	3.5 (3.4–3.7)	3.4 (3.3–3.6)
Cholesterol (mmol l^{-1})	5.7 (5.1–6.2)	5.6 (5.1–6.1)	6.0 (5.6–6.3)	5.6** (5.3–6.0)	5.8 (5.6–6.0)	5.6** (5.3–5.8)
CC-16 (μg l^{-1})	8.7 (7.3–10.2)	10.1** (8.5–11.7)	9.1 (7.5–10.7)	11.4*** (9.9–13.0)	8.5 (7.6–9.4)	11.3*** (10.2–12.4)
SP-A† (mg l^{-1})	44 (20–101)	45 (20–102)	47 (20–108)	43 (18–103)	123 (76–197)[c]	122 (76–196)
SP-D (μg l^{-1})	120 (94–155)	123 (93–162)	107 (89–129)[b]	105 (85–129)	108 (94–123)[d]	104 (90–120)
P-Selectin (μg l^{-1})	185 (168–202)	185 (165–206)	182 (162–203)	167** (147–186)	170 (156–185)[c]	163** (149–178)
ICAM-1 (μg l^{-1})	283 (254–312)	274 (247–301)	277 (255–298)	265* (240–291)	281 (268–295)[c]	273** (260–286)
VCAM-1 (μg l^{-1})	601 (491–710)	579 (491–666)	654 (561–748)	615* (529–702)	605 (561–649)[c]	599 (553–644)

IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; CC-16, Clara cell protein; SP, surfactant protein; ICAM, intercellular adhesion molecule; VCAM, vascular cellular adhesion molecule.

[a]Geometric mean; [b]one pair missing; [c]two pairs missing; [d]seven pairs missing;*$p<0.05$; **$p<0.01$; ***$p<0.001$;

or higher SP-A concentrations (Berthoin et al. 2004, Kida et al. 1997, Kobayashi et al. 2008) in smokers compared with non-smokers. In contrast, the levels of serum SP-D were higher in current smokers in our study, which has been reported previously (Mutti et al. 2006, Sorensen et al. 2006a). The determined pneumoprotein levels collected at Exam 1 were used for these statistical calculations. However, using the concentrations measured at Exam 2 did not alter the overall results with respect to smoking habits.

The levels of CC-16 (Bernard et al. 1992, Lesur et al. 1996, Shijubo et al. 1998), SP-D (Honda et al. 1996, Betsuyaku et al. 2004) and SP-A (Lesur et al. 1996, Shijubo et al. 1998, Honda et al. 1996, Betsuyaku et al. 2004) in bronchoalveolar lavage (BAL) fluids are lower in smokers than in non-smokers. Also Sprague-Dawley rats exposed to cigarette smoke had lower concentrations of SP-A in BAL fluid, while the number of cells positive for SP-A was lower in Wistar rats (Subramaniam et al. 1996, Hu et al. 2008). This could indicate that smoking primarily causes decreased pulmonary synthesis or increased pulmonary degradation of the pneumoproteins.

Increased leakage of pneumoproteins into the vascular compartment upon injury to the alveolar–endothelial barrier has been proposed as a mechanism for increased blood levels of pneumoproteins after inhalation of various contaminants (Sorensen et al. 2007). However, we measured increased serum levels of SP-D and lower levels of CC-16 in smokers. This may point to more complex biological mechanisms than can be explained by increased leakage alone. SP-D has for instance been viewed as a biomarker superior to SP-A because of its higher hydrophilicity (Sorensen et al. 2007). CC-16 levels in serum and BAL fluid are lower in smokers because of damage to the Clara cells (Broeckart & Bernard 2000, Robin et al. 2002, Mutti et al. 2006). However, our data indicate this damage at least in part to be reversible because former smokers have CC-16 levels comparable to those of never-smokers. This is in agreement with findings by Backé et al (2004).

A substantial negative impact from BMI on the determined levels of SP-D was observed, which was reported in humans recently (Sorensen et al. 2006b, Zhao et al. 2007). One of the studies also reported that SP-D-deficient mice had significantly higher weight gain than wild-type mice, in particular when given a high-fat diet. Slightly increased serum SP-D levels were observed in wastewater workers. That study also reported a negative impact of BMI on the serum SP-D levels of the examined workers (Daneshzadeh et al. 2010). The role of SP-D in obesity is not known, but pulmonary lipid accumulation occurs in SP-D-deficient mice phenotypes (Sorensen et al. 2006b).

The reporting of subjective symptoms, especially wheezing, appeared to add to the effect of smoking on some of the biomarker concentrations. This was most pronounced for the pneumoproteins, and smokers reporting wheezing had significantly lower CC-16 compared with smokers not reporting wheezing. The latter group had only slightly lower concentrations of CC-16 than non-smokers. This has previously, to our knowledge, not been reported, and could point to a more extensive damage of Clara cells in smokers reporting wheezing than in smokers not reporting wheezing. Several studies have indicated that self-reported wheezing is an important marker of chronic obstructive pulmonary disease (COPD), independent of for instance age and smoking (Freeman et al. 2005, Vandevoorde et al. 2007, Medbø & Melbye 2008).

We have previously reported that CC-16 in serum increased in the pavers during their period of work when

they were exposed to airborne contaminants from paving and passing traffic (Ulvestad et al. 2007a). Thus, it is of interest that a simultaneous increase in the serum levels of SP-A and SP-D did not occur, indicating different mechanisms for increasing the blood level of different pneumoproteins. The passage across the bronchoalveolar–blood barrier may be easier for CC16 with a small molecular radius than that of the surfactant proteins, which mainly occur in oligomers (Hermans & Bernard 1998, Hermans et al. 1999). A main focus of the study was to address the possibility that pulmonary inflammation induced by work-related exposure by inhalation could eventually result in alterations of biomarkers of endothelial activation, such as P-selectin. This was, however, not the case.

When lungs are exposed to particulate matters a number of molecular and cellular alterations occur, e.g. increased pulmonary expression of cytokines such as IL-6 (Martin et al. 1997). It is well known that the circulating cytokines IL-1, IL-6 and tumour necrosis factor-α are powerful inducers of acute-phase reactants, and that they also can activate endothelial cells. Current smokers in this study had higher concentrations of IL-6, CRP, fibrinogen and ICAM-1, and, after adjusting for BMI and age, also SAA. This suggests that smoking can induce the effects that are likely to be expected upon exposure to environmental factors. However, none of these effects could be observed in relation to occupational exposure when all subjects were considered. Asphalt pavers are exposed to asphalt fumes, but because they in general work where there is road traffic, they are also exposed to emissions from vehicles, such as diesel fume particles and nitrous oxides. The exposure assessment showed GM concentrations of total dust ranging from 0.3 to 0.4 mg m^{-3} for different work operations among the asphalt pavers and 0.9 mg m^{-3} among the plant operators (Ulvestad et al. 2007b). These exposure levels are substantially higher than normally reported from environmental studies.

Thus, our results are in contrast to environmental studies of subjects exposed to polluted ambient air. Studies have reported increased concentrations of serum CRP (Pope et al. 2004b, Dubowsky et al. 2006, Rückerl et al. 2006, Chuang et al. 2007, Shima 2007) or plasma fibrinogen (Chuang et al. 2007, Rückerl et al. 2007). Also increased concentrations of ICAM-1 and IL-6 (Rückerl et al. 2006, 2007) have been reported. Volunteers exposed to smoke generated from the burning of wood had increased levels of SAA at a $PM_{2.5}$ exposure level of around 250 μg m^{-3} (Barregård et al. 2006).

Actually, the asphalt pavers and plant operators had lower concentrations of ICAM-1 and P-selectin at Exam 2 compared with Exam 1. This result is in contrast to our a priori hypothesis, and may indicate a reduction in their level of endothelial activation. It is not likely that asphalt fumes per se have the ability to reduce the level of these biomarkers. However, the workers also had a concomitant reduction of serum cholesterol. The pavers have definitively increased their level of physical activity. During the winter they have sedentary work as lorry drivers removing snow, while paving also implies hard manual work. Also the plant workers are mostly engaged during the winter as drivers removing snow, and only a small minority is not engaged in this sedentary work during winter. The engineers maintain the same level of job-related physical activity all year around.

Thus, the lower total cholesterol level at Exam 2 compared with Exam 1 for the pavers and the plant operators could be an effect of increased level of job-related physical activity. Lower cholesterol and improved brachial artery flow-mediated dilatation, indicative of improved endothelial function were found after a 3-month treadmill exercise programme of a sedentary workforce of office and laboratory workers (Lippincott et al. 2008). Diabetes type 2 patients administered a moderate training programme for 6 months experienced a significant decrease in the concentrations of P-selectin and ICAM-1, while chronic heart failure patients had a significantly decrease in P-selectin after 20 weeks of moderate training (Zoppini et al. 2006, Bjørnstad et al. 2008). Rabbits fed high-cholesterol diets had lower P-selectin when exercising compared with non-exercising rabbits (Yang & Chen 2003). It has been suggested that moderate exercise has a beneficial effect on platelet function by reducing platelet activation, while strenuous exercise increases platelet activation (Wang 2006).

In conclusion, increased systemic inflammation and endothelial activation could not be detected in the asphalt workers across the season. The study shows that several potential confounders, such as smoking habits, BMI and possibly the level of physical activity need to be accounted for when performing such studies.

Declaration of interest

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