Int Arch Occup Environ Health (2015) 88:943–951
DOI 10.1007/s00420-015-1023-4

ORIGINAL ARTICLE

Serum pneumoproteins in tunnel construction workers

Dag G. Ellingsen · Bente Ulvestad · Berit Bakke · Ingebjørg Seljeflot · Lars Barregard · Yngvar Thomassen

Received: 1 August 2014 / Accepted: 16 January 2015 / Published online: 30 January 2015

Abstract
Purpose The aim was to study inflammatory biomarkers in tunnel construction workers (TCW).
Methods Surfactant protein D (SP-D), Clara cell protein 16 (CC-16) and C-reactive protein (CRP) were studied in serum of 90 TCW and 50 referents before and at the end of an 11-day work period. Personal air sampling was carried out on the two consecutive days before follow-up.
Results The TCW's geometric mean exposure to particulate matter and α-quartz were 604 and 74 μg/m^3, respectively. The arithmetic mean concentration of elemental carbon was 51 μg/m^3. The arithmetic mean concentration of SP-D was reduced by 7.6 μg/L in the TCWs and 0.6 μg/L in the referents ($p = 0.04$) at the end as compared to before the work period. Subjects who had ever been TCW had lower arithmetic mean CC-16 concentrations at baseline (5.4 μg/L) than subjects who had never worked as TCW (6.4 μg/L). Years worked as TCW was significantly associated with an annual mean decline of the CC-16 concentration of 0.04 μg/L. The concentrations of the biomarker of systemic inflammation, CRP, were not affected by exposure in the TCWs. Current smoking and body mass index have a large impact on the measured biomarker concentrations.
Conclusions The results suggest that former and current TCWs have lower serum CC-16 concentrations than referents, while the concentrations of SP-D decreased during exposure. The serum biomarker of systemic inflammation, CRP, was not altered during exposure. Current smoking and BMI were related to the concentrations of all measured biomarkers.

Keywords CC-16 · SP-D · Tunnel construction · α-Quartz · Diesel exhaust

D. G. Ellingsen (✉) · B. Ulvestad · B. Bakke · Y. Thomassen
National Institute of Occupational Health, P.O. Box 8149 Dep, 0033 Oslo, Norway
e-mail: dag.ellingsen@stami.no

B. Ulvestad
Department of Occupational Medicine, Mesta AS, Moss, Norway

I. Seljeflot
Department of Cardiology, Center for Clinical Heart Research, Oslo University Hospital Ullevål, Oslo, Norway

I. Seljeflot
Faculty of Medicine, University of Oslo, Oslo, Norway

L. Barregard
Department of Occupational and Environmental Medicine, University of Gothenburg, Göteborg, Sweden

Introduction

Tunnel construction workers' exposure by inhalation is to a large extent related to the geology of the work area, type of explosives used, the use of diesel-operated engines, concrete and products used for lubricating the machinery. Thus, their occupational exposure is complex, including dust, α-quartz, emissions from diesel engines, oil mist and gases, such as nitrogen oxides and ammonia (Bakke et al. 2014).

Respiratory diseases have been found to be frequent in tunnel construction workers (Oliver et al. 2001; Arcangeli et al. 2004; Oliver and Miracle-McMahill 2006). Previous studies revealed that tunnel workers in Norway were at increased risk of long- and short-term lung function decline and chronic obstructive pulmonary disease (COPD) (Ulvestad et al. 2000; Bakke et al. 2001a; Ulvestad et al. 2001; Bakke et al. 2004). In a more recent study, we

concluded that the overall exposure levels for many of the contaminants have been reduced since then (Bakke et al. 2014).

Although tunnel construction workers are at risk of developing pulmonary diseases caused by occupational exposure, no studies of pneumoproteins have to our knowledge been conducted. Concentrations of lung-specific proteins in serum have been proposed as markers for pulmonary injury, based on the concept that these proteins move passively across the epithelial barrier into the blood in higher amounts at epithelial injury (Hermans and Bernard 1999). Clara cell protein 16 (CC-16) and surfactant protein D (SP-D) are pneumoproteins commonly used as serum biomarkers for pulmonary injury. CC-16 is a 16-kDa anti-inflammatory protein secreted predominantly from the non-ciliated bronchiolar Clara cells, but also to a lesser extent in non-pulmonary tissues (Broeckaert and Bernard 2000; Lakind et al. 2007). The highest expression of SP-D in human tissues is in the distant airways and alveoli, mainly in type 2 pneumocytes (Jaw and Sin 2012). SP-D is also expressed in pulmonary Clara cells, goblet cells and tracheo-bronchial glandular cells (Sorensen et al. 2007).

The functions of these proteins are mainly related to immune defence and to the regulation of inflammation. SP-D modulates the function of various inflammatory cells like macrophages and lymphocytes, promotes lysis of microbes and phagocytosis of pathogens and modulates cytokine and reactive oxygen networks (Jaw and Sin 2012). In an inflammatory environment, SP-D also acts as an activator of NF-κB (Jaw and Sin 2012). Transgenic mice lacking SP-D develop spontaneously emphysema (Wert et al. 2000). The concentration of SP-D in serum is increased in a number of lung diseases, such as sarcoidosis and cystic fibrosis, but also in asthma and COPD (Hartl and Griese 2006; Bowler 2012). In vitro studies indicate that by inhibiting phospholipase A2 activity, CC-16 can attenuate inflammation. Also, inhibition of monocyte and polymorphonuclear chemotaxis and phagocytosis has been shown (Broeckaert and Bernard 2000).

Few studies have assessed serum pneumoproteins in workers exposed to components relevant for tunnel workers' exposure. One study reported lower CC-16 concentrations in silica-exposed quarry workers (Bernard et al. 1994). Workers diagnosed with slight silicosis and workers currently exposed to α-quartz had lower serum CC-16 concentrations (Wang et al. 2007). The workers with silicosis had also higher concentrations of SP-D in serum. Foundry workers had lower concentrations of CC-16 in serum after shift when compared to before shift (Bergamaschi et al. 2003). No statistically significant different serum CC-16 concentrations were observed when nitric acid production workers exposed to nitrogen oxides were compared to referents (Hałatek et al. 2005).

When particles are phagocytized by macrophages during pulmonary exposure, the cytokine TNF-α synthesized in the macrophages is assumed to induce the synthesis of cytokines, e.g. IL-6 in the lung epithelial cells (Martin et al. 1997). Circulating IL-6 is a powerful inducer of the synthesis of C-reactive protein (CRP) in the liver, which is considered a marker of systemic inflammation. Several studies have suggested that CRP may increase as a result of exposure to particulate matter, although a recent review concluded that the evidence is not conclusive (Li et al. 2012).

In this study, tunnel construction workers were examined before and towards the end of an 11-day work period when they were exposed to a variety of airborne contaminants. The main aim was to assess alterations in the concentrations of serum pneumoproteins and CRP during that period and to compare the alterations to referents working at the same tunnel construction sites. This study is part of a larger investigation of tunnel workers' pulmonary health and biomarkers of endothelial activation and coagulation.

Materials and methods

Study design

All tunnel construction workers ($n = 92$) employed at 11 tunnel construction sites were invited to participate in the study. One subject refused to participate, and one subject refused to give a blood sample. Referents, mainly administrative staff, were selected among subjects working at the same construction sites, but not involved directly in the tunnel construction. Fifty-two potential referents were invited, of whom one refused to participate. One referent refused to give a blood sample, but participated in other parts of the investigation. Thus, the participation rates were 98.9 and 98.0 % among the exposed subjects and referents, respectively. They were all males.

All subjects work 11 days consecutively and are then off work for 9 days. The duration of a work shift is normally 10–12 h, including two breaks of 30 min each. Blood samples were collected in all subjects before the start of the 11-day work period at around 4–6 p.m. (baseline) and at the same time of the day at the end of the work period (follow-up) together with spirometric measurements and the administration of a questionnaire.

Participation in the study was voluntary. An informed written consent was obtained from all participants prior to inclusion in the study. The study was approved by the Norwegian Regional Ethical Committee for Medical Research (REK2), according to the principles of the Helsinki declaration.

Work characteristics

Tunnel construction workers are engaged in rock drilling, charging of explosives and various support and finishing work as previously described (Bakke et al. 2001b). Briefly, after rock drilling, charging of explosives and blasting, the rock is loaded and transported out of the tunnel using dump trucks. Debris is removed using a scalar. Rock support includes fastening of unsafe rock with steel bolts and sealing of the rock by spraying wet concrete onto the excavated surface. Other work tasks include mounting of ventilation ducts, installation of electrical power supply and machine maintenance and repair. Tunnel construction workers can be grouped into drill and blast workers, support workers, mechanics, loaders, injection workers, shotcreting operators and shaft drillers. All investigated tunnels had a forced ventilation system using fans and ventilation ducting to reduce air contamination.

Clinical examinations

Background data were recorded with a questionnaire including respiratory symptoms, allergy, physician-diagnosed asthma and self-reported symptoms of cold at one of the two examinations (Randem et al. 2004). Participants were classified as never smokers, former smokers and current smokers. Former smokers were defined as those who had stopped smoking more than 12 months earlier.

Spirometry was performed according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (Miller et al. 2005). Recorded were forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and forced expiratory flow rate at 50 % (FEF_{50}) of the FVC.

Ten millilitres of whole blood was collected from the cubital vein with vacutainers without additives after cleaning of the skin with ethanol (BD Vacutainer, Belliver Industrial Estate, UK). Serum was separated by centrifugation at 2,000*g* for 15 min after a rest of 45 min and pipetted into 4.0 ml NUNC® polypropylene cryotubes (Sigma-Aldrich, St. Louis, MO, USA) for long-term storage at the National Institute of Occupational Health, Oslo, Norway (NIOH) at −70 °C until analysis.

Analysis of biomarkers

The serum concentrations of CC-16 and SP-D were measured with commercial ELISA kits (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic). The imprecision was 9 % (coefficient of variation, CV) for CC-16, and 4 % for SP-D, as calculated from duplicate samples. The methods' limits of detection (DL) were 1 µg/L for both CC-16 and SP-D. No samples contained pneumoprotein concentrations below the DL.

CRP in serum was determined by use of an enzyme-linked immunosorbent assays (DRG Instruments, Marburg/Lahn, Germany) (DL 0.1 mg/L). The inter-assay coefficient of variation was <5 %. CRP concentrations below the DL were substituted with ½ DL.

Air sampling

Details on the air sampling have been published (Bakke et al. 2014). Briefly, air samples were collected in the breathing zone of the workers and always outside personal protective respirators, if used. Personal air sampling was carried out on the two consecutive days before follow-up. No air samples were collected among the referents.

The particulate matter in the thoracic aerosol fraction was collected on 37 mm 5.0-µm-pore-size polyvinyl chloride(PVC) filters (PVC502500, Millipore Corporation, MA, USA) using BGI GK2.69 cyclones (BGI Inc., MA, USA) operated at an air flow rate of 1.6 L/min.

Elemental carbon (EC) and organic carbon (OC) were collected on pre-cleaned quartz filters (Pallflex Tissue quartz 2500QAT-UP, Pall Corporation, Port Washington, NY, USA) using a 37-mm standard three-part "total" closed-face aerosol filter cassette (Millipore, MA, USA) purchased from Sunset Laboratory Inc., (Tigard, OR, USA). An air flow rate of 2.0 L/min was used.

Oil mist and oil vapour were collected with 37 mm standard three-part "total" aerosol filter cassette (Millipore, MA, USA) equipped with a 1.6 µm-pore-size glass filter (No. 1820-037, Whatman GF, Madistone, UK) on top of a 0.8-µm-pore-size cellulose acetate filter (AAWP03700, Whatman GF, Madistone, UK) and charcoal tubes (No. 226-01, SKC, Blandford Forum, Dorset, UK) mounted in series at an air flow rate of 1.4 L/min.

Analysis of air samples

Details on the analysis of the collected air contaminants have been published (Bakke et al. 2014). Briefly, the masses of thoracic cyclone filters were measured gravimetrically using a Sartorius AG, MC 210p laboratory micro-balance (Göttingen, Germany) with a DL of 31 µg/m^3 based on 8-h sampling at a flow rate of 1.6 L/min.

Oil mist was determined with a Spectrum 100 Fourier transform infrared spectrophotometer (Perkin Elmer, Waltham, MA, USA). Oil vapour was determined using a Perkin Elmer Autosystem XL gas chromatograph and a flame ionization detector. The DLs for oil mist (0.05 mg/m^3) and oil vapour (0.1 mg/m^3) were based on a 2-h sampling period at a flow rate of 1.4 L/min. EC and OC were determined using an OCEC Dual-Optical Analyzer based on NIOSH Method No. 5040 (NIOSH 2003). The DLs of the method are approximately 2 ng/m^3 (EC) and 2 µg/m^3

Table 1 Background and exposure data among 90 tunnel construction workers and 50 referents

	Exposed		Referents		*p*
	Mean	Min–max	Mean	Min–max	
Age[a]	39.3	18–59	43.8	24–61	0.03
BMI[a]	25.7	19.9–37.0	27.2	20.3–42.7	0.02
Current smokers (%)[b]	36.7	–	22.0	–	0.09
Cigarettes/day[a]	5.4	0–40	2.4	0–30	0.02
Former smokers (%)[b]	22.2	–	30.0	–	0.32
Years of tunnel work[a]	13.7	1–43	5.7	0–38	<0.001
Thoracic dust (μg/m^3)[c,d]	604	110–7,870	–	–	–
α-Quartz (μg/m^3)[c]	74	5–1,041	–	–	–
Elemental carbon (μg/m^3)[c]	51	4–172	–	–	–
Organic carbon (μg/m^3)[c]	175	59–608	–	–	–

[a] Arithmetic mean

[b] Prevalence

[c] Geometric mean

[d] Thoracic dust and α-quartz missing for five subjects. Elemental and organic carbon missing for six subjects

(OC) based on an 8-h sampling period at a flow rate of 2.0 L/min. The α-quartz content in the thoracic aerosol fraction was determined by X-ray diffraction spectrometry using NIOSH Method No. 7500 (NIOSH 2003). The DL was 13 μg/m^3 based on an 8-h sampling period at a flow rate of 1.6 L/min. All reagents and water used for the chemical analyses were of analytical quality.

Statistics

The exposure parameters were calculated as the mean of 2 days of air sampling. The distributions of the continuous variables were assessed, and when skewness exceeded 2.0, the variable was log-transformed to achieve normal distribution. The geometric means (GM) are presented for these variables; otherwise, the arithmetic mean (AM) values are presented. Student's *t* test was applied for group comparisons of independent samples, while the paired sample *t* test was used to assess differences within the same subjects. Least square regression analysis was applied to assess univariate associations between variables, the Pearson's correlation coefficient (Pearson's *r*) being the measure of association. Multiple linear regression analysis (backwards procedure) was used to assess statistical associations between outcome variables and several independent variables simultaneously. The independent variables were currently exposed as a tunnel construction worker (1/0), age, BMI, current smoker (1/0), asthma (1/0), former smoking (1/0) and common cold (1/0). In an alternative approach, currently exposed was substituted with ever exposed as a tunnel construction worker (1/0). Associations between air exposure measures, biomarker concentrations at follow-up and the difference in biomarker concentrations between baseline and follow-up were studied among the TCWs. A two-tailed *p* value <0.05 was considered to be of statistical significance. The statistical data package SPSS 18.0 was used.

Results

The tunnel construction workers were slightly younger and had lower body mass index (BMI) as compared to the referents (Table 1). There were more current smokers among the tunnel construction workers than among the referents. Their GM exposure to particulate matter in the thoracic aerosol fraction was 604 μg/m^3. Sixteen referents were former tunnel workers.

There were no statistically significant differences in the serum concentrations of CC-16, SP-D or CRP between the tunnel construction workers and the referents at baseline (Table 2). The GM concentration of CRP was significantly lower in the tunnel construction workers at follow-up, whereas the serum concentrations of CC-16 and SP-D were comparable between the groups. However, highly statistically significantly lower concentrations of SP-D across the work period were measured among the tunnel construction workers ($p = 0.001$), while the concentrations across the work period were comparable in the referents. The difference in CC-16 across the work period was similar in the tunnel construction workers and the referents. The difference in SP-D concentrations between baseline and follow-up was statistically significantly larger in the exposed tunnel construction workers as compared to the referents (Table 2). When excluding subjects reporting a cold at one or both examinations, the mean SP-D concentration was 7.0 μg/L lower across the work period in the tunnel construction workers ($N = 78$) as compared to 1.5 μg/L higher across the work period in the referents ($N = 43$), the difference in the concentration changes being statistically significant ($p = 0.02$) (not tabulated).

The results from the multiple linear regression analysis showed that the concentrations of SP-D at baseline were not associated with being currently or ever exposed as tunnel construction worker (Table 3). In contrast, having ever been exposed, but not currently exposed, was associated with the concentrations of CC-16. The results were essentially the same when assessing the pneumoprotein concentrations measured at follow-up. The concentrations of CRP were not associated with any of the exposure category variables. The difference between the SP-D concentrations measured at follow-up and at baseline was associated with being currently exposed, while the reporting of having a

Table 2 The arithmetic mean (AM) concentrations of pneumoproteins and C-reactive protein (CRP) in serum of 90 tunnel construction workers and 50 referents at baseline, follow-up and the difference between follow-up and baseline

	Exposed		Referents		*p*
	Mean	Min–max	Mean	Min–max	
Baseline					
CC-16 (µg/L)	5.5	2.0–16.3	5.9	1.1–13.4	0.26
SP-D (µg/L)	83.8	18.0–312.7	80.1	21.3–214.1	0.65
CRP (mg/L)[a]	1.4	<DL–12.3	1.6	0.2–8.4	0.36
Follow-up[b]					
CC-16 (µg/L)	5.5	2.2–15.6	5.7	2.0–13.6	0.36
SP-D (µg/L)	76.3	18.5–231.4	79.5	13.5–208.9	0.67
CRP (mg/L)[a]	1.2	0.1–17.9	1.8	0.5–15.9	0.02
Difference					
CC-16 (µg/L)	−0.1	−3.1 to 4.9	−0.2	−3.5 to 1.6	0.76
SP-D (µg/L)	−7.6[c]	−81.3 to 93.5	−0.6	−60.1 to 51.4	0.04
CRP (mg/L)	0.1	−9.9 to 12.3	0	−5.5 to 12.4	0.81

[a] Geometric mean

[b] Samples missing for two tunnel construction workers at follow-up and for the difference

[c] $p = 0.001$ across work period

common cold infection at either of the two examinations was nearly significantly ($p = 0.051$) associated with the difference. Only age was associated with the difference for CC-16, while reporting a cold at either of the examinations accounted for the difference in CRP.

Having ever worked as tunnel construction worker was related to the concentrations of CC-16 both at baseline and at follow-up. Figure 1 shows that subjects that had never worked as tunnel construction workers had the highest concentrations of CC-16 at baseline, whereas referents who had previously been tunnel construction workers had the lowest concentrations. All subjects who had ever worked as tunnel construction worker had on average 5.4 µg/L CC-16 (95 % CI 5.0–5.8), whereas the subjects who had never worked as tunnel construction worker had 6.4 µg/L (95 % CI 5.6–7.1) ($p = 0.03$) after adjusting for age and daily current cigarette consumption. When adding years as a tunnel construction worker instead of ever exposed to the multiple regression model in addition to BMI and being a smoker, the model "$\text{CC-16}_{\text{baseline}} = 9.4$ ($p < 0.001$) − 0.11 BMI ($p = 0.03$) − 0.04 years in tunnel ($p = 0.02$) − 1.1 current smoker ($p = 0.005$) (multiple $r = 0.37$; $p < 0.001$)" was calculated. The p values of the regression coefficients

Table 3 Results from multiple linear regression analysis among all subjects (backwards procedure)

	Age	BMI	Current smoker	Cold	Currently exposed	Ever exposed	Multiple *r*
Baseline							
SP-D	1.1**** (0.5–1.8)	−2.1** (−4.1 to −0.1)	20.2*** (5.2–35.3)	ns[†]	ns	ns	0.38****
CC-16	ns	−0.13** (−0.23 to −0.03)	−1.1*** (−1.9 to −0.4)	ns	ns	−1.1** (−2.0 to −0.3)	0.37****
CRP_{lg}	0.007** (0.001–0.01)	0.04**** (0.02–0.06)	0.19** (0.05–0.33)	ns	ns	ns	0.46****
Follow-up							
SP-D	1.1**** (0.5–1.7)	−2.5*** (−4.4 to −0.7)	19*** (5–33)	−25[a,]** (−49 to −0.7)	ns	ns	0.44****
CC-16	ns	−0.16*** (−0.26 to −0.05)	−1.4*** (−2.2 to −0.6)	ns	ns	−0.9**(−1.8 to −0.1)	0.40****
CRP_{lg}	0.008*** (0.002–0.01)	0.03*** (0.007–0.04)	0.18** (0.04–0.32)	0.3[a,]** (0.07–0.55)	ns	ns	0.43****
Difference							
SP-D	ns	ns	ns	−9.6[b,]* (−19 to 0.06)	−7.3** (−13.9 to −0.7)	ns	0.24**
CC-16	−0.02** (−0.03 to −0.003)	ns	ns	ns	ns	ns	0.20**
CRP_{lg}	ns	ns	ns	1.9[b,]** (0.3–3.4)	ns	ns	0.20**

Presented are β-coefficients and the 95 % CI (in parentheses)

[†] Not significant; * $p < 0.10$; ** $p < 0.05$; *** $p < 0.01$; **** $p < 0.001$

[a] Cold reported at follow-up

[b] Cold reported at baseline, follow-up or both

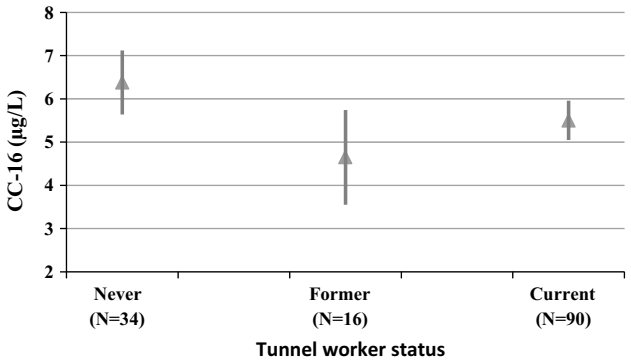

Fig. 1 The arithmetic mean (and 95 % CI) concentrations of CC-16 at baseline adjusted for age, BMI and amount of smoking (cig./day) according to work history as tunnel worker. *p* values: never versus former = 0.036, never versus current = 0.028

Table 4 The concentrations of pneumoproteins and C-reactive protein (CRP) in serum of tunnel construction workers and referents measured at baseline according to current smoking habits

	Current smokers ($N = 44$)		Current non-smokers ($N = 96$)		*p*
	AM	95 % CI	AM	95 % CI	
CC-16 (µg/L)	4.8	4.3–5.3	6.0	5.5–6.5	0.003
CC-16 (µg/L)$_{Adj.}$	4.7	4.1–5.4	6.0	5.6–6.5	0.002
SP-D (µg/L)	97.1	80.3–114.0	75.7	68.2–83.3	0.02
SP-D (µg/L)$_{Adj.}$	96.3	83.9–108.8	76.1	67.7–84.5	0.009
CRP (mg/L)[a]	1.90	1.48–2.43	1.27	1.02–1.57	0.03
CRP (mg/L)$^{a}_{Adj.}$	1.93	1.48–2.53	1.26	1.05–1.51	0.01

AM arithmetic mean, *Adj.* adjusted for age and BMI

[a] Geometric mean

are shown in parentheses. The same independent variables were statistically significantly included in the model when the CC-16 concentrations measured at follow-up were assessed, but the *p* value for "years in tunnel" was stronger ($p = 0.002$). No statistically significant associations were observed between any of the air exposure measures and the biomarkers.

Smoking habits had a substantial impact on the determined biomarker concentrations (Table 4). The impact was comparable at baseline and at follow-up. The concentrations of CC-16 were around 20 % lower in smokers as compared to non-smokers, while the concentrations of SP-D and CRP were substantially higher. The differences in the concentrations between never smokers ($N = 61$) and former smokers ($N = 35$) were far from attaining statistical significance for any of the three biomarkers.

Slight associations between CC-16 and FEV_1 at baseline (Pearson's $r = 0.23$; $p = 0.006$) and at follow-up (Pearson's $r = 0.22$; $p = 0.008$) were observed. Participants reporting wheezing had 4.8 µg/L (95 % CI 4.0–5.6) of CC-16 in serum at baseline as compared to 5.7 µg/L (95 % CI 5.3–6.2) among participants not reporting wheezing after adjusting for current smoking and BMI ($p = 0.053$). Other reported pulmonary symptoms were not associated with the measured pneumoprotein concentrations (results not shown).

Discussion

This is to our knowledge the first study of serum pneumoproteins in tunnel construction workers. The main result is that the serum concentrations of SP-D in currently exposed tunnel construction workers are lower at the end of a work period of 11 days. No alterations were observed in the concentrations of CC-16 related to current exposure. The results indicate, however, that subjects having ever worked as a tunnel construction worker had lower CC-16 concentrations. Also, number of years employed as a tunnel construction worker was significantly associated with lower serum concentrations of CC-16, the point estimate of the reduction being around −0.04 µg/L/year. The concentrations of the serum biomarker of systemic inflammation, CRP, were not altered during the work period. All biomarkers were associated with current smoking, the concentrations of CRP and SP-D being higher and CC-16 lower in current smokers. The serum concentrations of CC-16 were slightly associated with FEV_1. A possible association with reporting of wheezing and CC-16 was also observed.

Increased leakage of pneumoproteins into the vascular compartment after inhalation of various contaminants inducing injury to the alveolar–endothelial barrier has been proposed as a mechanism for increased blood levels of pneumoproteins (Broeckaert and Bernard 2000; Sorensen et al. 2007). In the present study, the concentrations of SP-D decreased across the work period, while the CC-16 concentrations remained unaltered. This could suggest that the barrier between blood and lung is not the target for the air contaminants assessed in this study or that the exposure has been too low to compromise the barrier. There are few studies available on the concentration of SP-D in serum as a biomarker of toxic injury to the lung and even fewer assessing exposure of relevance to tunnel construction workers. However, one study reported that silica-exposed pyrite mine workers without radiologically detectable silicosis had SP-D concentrations comparable to the referents (Wang et al. 2007). That study also reported that pyrite miners with radiographic-determined silicosis had significantly higher concentrations of SP-D in serum. None of the participants in the present study had known silicosis, and their lung function measurements did not show a restrictive pattern indicating fibrosis. Further, their serum SP-D

concentrations were comparable with the referents as in the silica-exposed pyrite miners. The decline in the SP-D concentrations across the work period has never been shown previously, but could reflect acute effects induced by one of the exposure parameters, including α-quartz, which was assessed in this study. This acute effect of exposure is most likely reversible in the short term, as the SP-D concentrations were comparable to that of the referents at baseline. It has been pointed out that the role of a relative SP-D deficiency in the pathogenesis of pulmonary diseases needs to be further investigated (Orgeig et al. 2010). Given the important roles of SP-D in the pulmonary physiology such as modulating functions of macrophages and neurophils, binding various microorganisms or under certain circumstances activating NF-κB, it is important to know the consequences, if any, of altering the SP-D homeostasis induced by environmental exposures (Jaw and Sin 2012).

The referents and the tunnel construction workers had comparable concentrations of CC-16 in serum at baseline. However, the reference group also comprised subjects who were former tunnel construction workers. These subjects had significantly lower CC-16 concentration than the other referents and concentrations comparable to current tunnel construction workers. The subjects who had never worked as tunnel construction worker had significantly higher CC-16 concentrations in serum than those who had ever worked as such. These results are in agreement with the aforementioned study of silica-exposed pyrite miners (Wang et al. 2007). That study reported lower concentrations of CC-16 in the silica-exposed subjects and no differences in the CC-16 concentrations in miners without silicosis, with silicosis or with suspected silicosis. Similar results were reported in quarry workers who had been exposed to silica-rich dust for on average 15.2 months (Bernard et al. 1994).

Hyperplasia of Clara cells has been reported in rats exposed to α-quartz, but not after exposure to titanium dioxide (Albrecht et al. 2001). Potential pathological consequences of this hyperplasia remain to be elucidated. It is, however, in this context of interest that one important function of Clara cells is to control the extent of inflammation (Reynolds and Malkinson 2010). Based on studies in mice, it was suggested that Clara cells inhibit pulmonary inflammation induced by crystalline silica by inhibiting gene expression of matrix metalloproteinases in vivo (Yatera et al. 2001). These components are involved in the degradation of extracellular matrix. It appears that Clara cells may be involved in the pathogenesis of silica-induced pulmonary fibrosis. We have, however, not been able to identify experimental studies assessing serum or bronchio-alveolar fluid concentrations of CC-16 after α-quartz exposure.

The tunnel construction workers are exposed to components that are also *typically present in* ambient air pollution, such as nitrous oxides and other combustion products from diesel engines, although their exposure is substantially higher. Several epidemiological studies of subjects exposed to ambient air contaminants have shown increased concentrations of acute phase reactants such as CRP or fibrinogen in serum (Pope et al. 2004; Dubowsky et al. 2006; Rückerl et al. 2006; Shima 2007; Chuang et al. 2007; Li et al. 2012). Generally, the exposure characterization has not been extensive in these studies, and a comparison with the present study is difficult. From the results of this study, it appears that exposure to diesel exhaust and α-quartz are not causing increased CRP, at least not at the air concentrations determined in this study.

There is a quite substantial impact of current smoking on the measured serum pneumoprotein concentrations, SP-D concentrations being around 30 % higher and CC-16 being around 20 % lower in current smokers as compared to non-smokers. Higher concentrations of SP-D and lower concentrations of CC-16 in serum in current smokers is in accordance with previous reports (Robin et al. 2002; Berthoin et al. 2004; Mutti et al. 2006; Sorensen et al. 2006a; Madsen et al. 2008; Ellingsen et al. 2010). Several studies have shown that the concentrations of CC-16 (Bernard et al. 1992; Lesur et al. 1996; Shijubo et al. 1998) and SP-D (Honda et al. 1996; Betsuyaku et al. 2004) are lower in BAL fluids in smokers as compared to non-smokers. Increased pulmonary SP-D mRNA and protein expression were shown in cigarette smoke-exposed mice (Hirama et al. 2007). This could suggest that the decreased content of SP-D in BAL fluid is not related to decreased pulmonary synthesis, but to increased clearance, perhaps through the alveolar–capillary membrane. The proportion of Clara cells in the terminal and respiratory bronchioles was significantly reduced in Wistar rats exposed to cigarette smoke, and also, CC-16 mRNA was substantially, although not significantly, lower (Liao et al. 2010). These latter animal studies could explain the rather contradictory results of the present study of increased serum SP-D and reduced CC-16 concentrations in smokers. However, it also shows the importance of collecting reliable smoking data, because smoking can operate as a confounder in studies of pneumoproteins.

BMI had a substantial impact on the measured pneumoprotein concentrations, higher BMI resulting in lower serum concentrations of SP-D and CC-16 and higher concentrations of CRP. A negative impact on the serum concentrations of SP-D related to BMI has been reported in humans (Sorensen et al. 2006b; Zhao et al. 2007; Ellingsen et al. 2010). SP-D-deficient mice had significantly higher weight gain than wild-type mice, and pulmonary lipid accumulation occurs in SP-D-deficient mice phenotypes (Sorensen et al. 2006b). However, the role of SP-D in obesity is not known. The association between obesity

and increased concentrations of CRP is well known (Choi et al. 2013).

In summary, former and current tunnel construction workers have lower serum CC-16 concentrations than the referents. The concentration of SP-D is decreased during exposure. Both current smoking and BMI are related to the concentrations of the measured biomarkers and must be appropriately accounted for in epidemiological studies. The serum concentrations of a biomarker of systemic inflammation, CRP, were not altered during exposure to particulate matter and diesel engine emissions.

Acknowledgments The study was carried out with financial support from Statoil Work Environment Fund (Norway) and the Fund for Regional Delegate for the Construction Industry (Norway).

Conflict of interest Bente Ulvestad has a part-time position as occupational physician in one of the companies where the study was carried out. The remaining authors declare no conflict of interests.

References

Albrecht C, Adolf B, Weishaupt C, Höhr D, Zeitträger I, Friemann J, Born PJ (2001) Clara-cell hyperplasia after quartz and coal-dust instillation in rat lung. Inhal Toxicol 13:191–205

Arcangeli G, Cupelli V, Montalti M, Pristera M, Baldasseroni A, Giuliano G (2004) Respiratory risks in tunnel construction workers. Int J Immunopathol Pharmacol 17:91–96

Bakke B, Ulvestad B, Stewart P, Lund MB, Eduard W (2001a) Effects of blasting fumes on exposure and short-term lung function changes in tunnel construction workers. Scand J Work Environ Health 27:250–257

Bakke B, Stewart P, Ulvestad B, Eduard W (2001b) Dust and gas exposure in tunnel construction work. Am Ind Hyg Assoc J 62(4):457–465

Bakke B, Ulvestad B, Stewart P, Eduard W (2004) Cumulative exposure to dust and gases as determinants of lung function decline in tunnel construction workers. Occup Environ Med 61:262–269

Bakke B, Ulvestad B, Thomassen Y, Woldbæk T, Ellingsen DG (2014) Characterization of occupational exposure to air contaminants in modern tunneling operations. Ann Occup Hyg. doi:10.1093/annhyg/meu034

Bergamaschi E, Apostoli P, Catalani S, Festa D, Folesani G, Andreoli R, Manini P, Schenato S, DePalma G, Franchini I, Bernard A, Mutti A (2003) Indicators of pulmonary epithelial damage among workers at a foundry exposed to airborne pollutants. G Ital Med Lav Ergon 25(3):104–106

Bernard A, Marchandise FX, Depelchin S, Lauwerys R, Sibille Y (1992) Clara cell protein in serum and bronchoalveolar lavage. Eur Resp J 5:1231–1238

Bernard AM, Gonzalez-Lorenzo JM, Siles E, Trujillano G, Lauwerys R (1994) Early decrease of serum Clara cell protein in silica-exposed workers. Eur Resp J 7:1932–1937

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

Betsuyaku T, Kuroki Y, Nagai K, Nasuhara Y, Nishimura M (2004) Effects of ageing and smoking on SP-A and SP-D levels in bronchoalveolar lavage fluid. Eur Resp J 24:964–970

Bowler RP (2012) Surfactant protein as a biomarker for chronic obstructive pulmonary disease. COPD 9:651–653

Broeckaert F, Bernard A (2000) Clara cell secretory protein (CC16): characteristics and perspectives as lung peripheral biomarker. Clin Exp Allergy 30:469–475

Choi J, Joseph L, Pilote L (2013) Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. Obes Rev 14(3):232–244

Chuang KJ, Chan CC, Su TC, Lee CT, Tang CS (2007) The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. Am J Respir Crit Care Med 176:370–376

Dubowsky SD, Suh H, Schwartz J, Coull BA, Gold DR (2006) Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. Environ Health Perspect 114:992–998

Ellingsen DG, Ulvestad B, Andersson L, Barregard L (2010) Pneumoproteins and inflammatory biomarkers in asphalt pavers. Biomarkers 15(6):498–507

Hałatek T, Gromadzińska J, Wasowicz W, Rydzyński K (2005) Serum clara-cell protein and beta2-microglobulin as early markers of occupational exposure to nitric oxides. Inhal Toxicol 17(2):87–97

Hartl D, Griese M (2006) Surfactant protein D in human lung diseases. Eur J Clin Invest 36:423–435

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

Hirama N, Shibata Y, Otake K, Machiya J, Wada T, Inoue S, Abe S, Takabatake N, Sata M, Kubota I (2007) Increased surfactant protein-D and foamy macrophages in smoking-induced mouse emphysema. Respirology 12:191–201

Honda Y, Takahashi H, Kuroki Y, Akino T, Abe S (1996) Decreased contents of surfactant proteins A and D in BAL fluids of healthy smokers. Chest 109:1006–1009

Jaw JE, Sin DD (2012) Unifying thoracic biomarkers: surfactant protein-D and beyond. Expert Rev Respir Med 6:147–154

Lakind JS, Holgate ST, Ownby DR, Mansur AH, Helms PJ, Pyatt D, Hays SM (2007) A critical review of the use of Clara cell secretory protein (CC16) as a biomarker of acute or chronic pulmonary effects. Biomarkers 12:445–467

Lesur O, Bernard AM, Begin RO (1996) Clara cell protein (CC16) and surfactant-associated protein A (SP-A) in asbestos-exposed workers. Chest 109:467–474

Li Y, Rittenhouse-Olson K, Scheider WL, Mu L (2012) Effect of particulate matter air pollution on C-reactive protein: a review of epidemiologic studies. Rev Environ Health 27:133–149

Liao J-P, Chi C-H, Li H-C, Tang X-Y (2010) Effects of N-acetylcysteine on Clara cells in rats with cigarette smoke exposure. Chin Med J 123(4):412–417

Madsen C, Durand KL, Nafstad P, Schwarze PE, Rønningen KS, Håheim LL (2008) Associations between environmental exposures and serum concentrations of Clara cell protein among elderly men in Oslo, Norway. Environ Res 108:354–360

Martin LD, Rochelle LG, Fischer BM, Krunkosky TM, Adler KB (1997) Airway epithelium as an effector of inflammation: molecular regulation of secondary mediators. Eur Resp J 10:2139–4216

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, ATS/ERS Task Force (2005) Standardisation of spirometry. Eur Respir J 26:319–338

Mutti A, Corradi M, Goldoni M, Vittori MV, Bernard A, Apostoli P (2006) Exhaled metallic elements and serum pneumoproteins in asymptomatic smokers and patients with COPD or asthma. Chest 129:1288–1297

NIOSH (2003) Manual of analytical methods (NMAM) (Suppl 3). In:NIOSH, Schlect PC, O'Connor PF (eds) 4th edn. National Institute forOccupational Safety and Health DHHS (NIOSH), Cincinnati, pub. no. 5040

Oliver LC, Miracle-McMahill H (2006) Airway disease in highway and tunnel construction workers exposed to silica. Am J Ind Med 49:983–996

Oliver LC, Miracle-McMahill H, Littman AB, Oakes JM, Gaita RR Jr (2001) Respiratory symptoms and lung function in workers in heavy and highway construction: a cross-sectional study. Am J Ind Med 40:73–86

Orgeig S, Hiemstra PS, Veldhuizen EJA, Casals C, Clark HW, Haczku A, Knudsen L, Possmayer F (2010) Recent advances in alveolar biology: evolution and function of alveolar proteins. Respir Physiol Neurobiol 173S:43–54

Pope CA III, Hansen ML, Long RW, Nielsen KR, Eatough NL, Wilson WE, Eatough DJ (2004) Ambient particulate air pollution, heart rate variability, and blood markers of inflammation in a panel of elderly subjects. Environ Health Perspect 112:339–345

Randem BG, Ulvestad B, Burstyn I, Kongerud J (2004) Respiratory symptoms and airflow limitation in asphalt workers. Occup Environ Med 61:367–369

Reynolds SD, Malkinson AM (2010) Clara cell: progenitor for the bronchiolar epithelium. Int J Biochem Cell Biol 42(1):1–4

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 Resp J 20:1152–1161

Rückerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J, Heinrich J, Marder V, Frampton M, Wichmann HE, Peters A (2006) Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. Am J Respir Crit Care Med 173:432–441

Shijubo N, Honda Y, Itoh Y, Yamaguchi T, Kuroki Y, Akino T, Kawai T, Abe S (1998) BAL surfactant protein A and Clara cell 10-kDa protein levels in healthy subjects. Lung 176:257–265

Shima M (2007) Air pollution and serum C-reactive protein concentration in children. J Epidemiol 17(5):169–176

Sorensen GL, Hjelmborg JV, Kyvik KO, Fenger M, Hoj A, Bendixen C, Sørensen TI, Holmskov U (2006a) Genetic and environmental influences of surfactant protein D serum levels. Am J Physiol Lung Cell Mol Physiol 290(5):1010–1017

Sorensen GL, Hjelmborg JV, Leth-Larsen R, Schmidt V, Fenger M, Poulain F, Hawgood S, Sørensen TI, Kyvik KO, Holmskov U (2006b) Surfactant protein D of the innate immune defence is inversely associated with human obesity and SP-D deficiency infers increased body weight in mice. Scand J Immunol 64:633–638

Sorensen GL, Husby S, Holmskov U (2007) Surfactant protein A and surfactant protein D variation in pulmonary disease. Immunobiology 212:381–416

Ulvestad B, Bakke B, Melbostad E, Fuglerud P, Kongerud J, Lund MB (2000) Increased risk of obstructive pulmonary disease in tunnel workers. Thorax 55:277–282

Ulvestad B, Bakke B, Eduard W, Kongerud J, Lund MB (2001) Cumulative exposure to dust causes accelerated decline in lung function in tunnel workers. Occup Environ Med 58:663–669

Wang S-X, Liu P, Wei M-T, Chen L, Guo Y, Wang R-Y, Tu Z-G, Liang X-C (2007) Roles of serum Clara cell protein 16 and surfactant protein-D in early diagnosis and progression of silicosis. J Occup Environ Med 49:834–839

Wert SE, Yoshida M, LeVine AM, Ikegami M, Jones T, Ross GF, Fisher JH, Korfhagen TR, Whitsett JA (2000) Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. Proc Natl Acad Sci USA 97(11):5972–5977

Yatera K, Morimoto Y, Kim HN, Yamato H, Tanaka I, Kido M (2001) Increased expression of matrix metalloproteinase in Clara cell-ablated mice inhaling crystalline silica. Environ Health Perspect 109:795–799

Zhao XM, Wu YP, Wei R, Cai HX, Tornoe I, Han JJ, Wang Y, de Groot PG, Holmskov U, Xia ZL, Sorensen GL (2007) Plasma surfactant protein D levels and the relation to body mass index in a Chinese population. Scand J Immunol 66(1):71–76