Int Arch Occup Environ Health (2017) 90:309–317
DOI 10.1007/s00420-017-1199-x

ORIGINAL ARTICLE

Biomarkers of endothelial activation and thrombosis in tunnel construction workers exposed to airborne contaminants

Dag G. Ellingsen[1] · Ingebjørg Seljeflot[2,3] · Yngvar Thomassen[1] · Magny Thomassen[4] · Berit Bakke[1] · Bente Ulvestad[1,5]

Received: 30 August 2016 / Accepted: 9 January 2017 / Published online: 25 January 2017

Abstract
Objectives The aims were to study biomarkers of systemic inflammation, platelet/endothelial activation and thrombosis in tunnel construction workers (TCW).
Methods Biomarkers and blood fatty acids were measured in blood of 90 TCW and 50 referents before (baseline) and towards the end (follow-up) of a 12 days work period. They had been absent from work for 9 days at baseline. Air samples were collected by personal sampling.
Results Personal thoracic air samples showed geometric mean (GM) particulate matter and α-quartz concentrations of 604 and 74 μg/m^3, respectively. The arithmetic mean (AM) concentration of elemental carbon was 51 μg/m^3. The GM (and 95% confidence interval) concentration of the pro-inflammatory cytokine TNF-α decreased from 2.2 (2.0–2.4) at baseline to 2.0 pg/mL (1.8–2.2) ($p=0.02$) at follow-up among the TCW. Also the platelet activation biomarkers P-selectin and CD40L decreased significantly [25.4 (24.1–26.6) to 24.4 (22.9–26.0)] ng/mL, $p=0.04$ and 125 (114–137) to 105 (96–115) pg/mL, $p<0.001$, respectively. ICAM-1 concentrations increased from 249 (238–260) to 254 (243–266) ng/mL ($p=0.02$). No significant alterations were observed among the referents when assessed by paired sample *t* test. Unbeneficial alterations in blood fatty acid composition were observed between baseline and follow-up, mainly among referents.
Conclusions TCW had slightly reduced systemic inflammation and platelet activation although highly exposed to particulate matter, α-quarz and diesel exhaust, which might be due to increased physical activity during the exposure period. The slightly increased ICAM-1 may indicate monocyte recruitment to the lungs. The diet was substantially altered towards a less beneficial fatty acid profile.

Keywords P-selectin · TNF-α · Cardiovascular disease · Fatty acids

Introduction

Tunnel construction workers (TCW) are exposed to a complex mixture of airborne contaminants by inhalation, including particulate matter (PM) and gases generated through blasting and drilling and diesel engine exhaust. A considerable amount of PM is present as respirable particles, making them penetrable into the pulmonary alveolar region (Bakke et al. 2001).

Elevated levels of PM in polluted air have been associated with increased cardiovascular mortality and morbidity (Brook et al. 2010). Cardiovascular mortality has, to our knowledge, not been studied in TCW. However, there are many similarities between the work environment of TCW and miners, and increased mortality due to ischemic heart diseases has been reported in various miner populations

The study was carried out with financial support from Statoil Working Environment Fund (Norway) and The Fund for Regional Safety Representatives for Building and Construction Activities (Norway).

✉ Dag G. Ellingsen
dag.ellingsen@stami.no

[1] National Institute of Occupational Health, P.O. Box 8149 Dep, 0033 Oslo, Norway

[2] Center for Clinical Heart Research, Department of Cardiology, Oslo University Hospital, Ullevål, Norway

[3] Faculty of Medicine, University of Oslo, Oslo, Norway

[4] Norwegian University of Life Sciences, Ås, Norway

[5] Department of Occupational Medicine, Mesta AS, Lysaker, Norway

and potentially silica-exposed workers (Reid and Sluis-Cremer 1996; Weiner et al. 2007; Björ et al. 2009; Landen et al. 2011).

The biological mechanisms for the increase of cardiovascular diseases due to PM exposure are not completely understood. One potential mechanism is linked to increased systemic inflammation which is regarded as an independent risk factor for sudden cardiovascular death. Also imbalances in coagulation favoring the propagation of thrombi have been proposed.

Finally, inflammation could affect the autonomic nervous system resulting in altered heart rhytm control (Donaldson et al. 2005). Probably the most commonly proposed mechanism that is explored in this study, is the induction of a pulmonary inflammation with subsequent systemic inflammation and hypercoagulability.

Uptake of particles by alveolar macrophages is important for pulmonary particle clearance (Geiser and Kreyling 2010). Alveolar macrophages phagocytising PM express pro-inflammatory cytokines, that interact with airway epithelial cells (Hiraiwa and van Eeden 2013). This may result in systemic inflammation with induction of acute phase reactants such as C-reactive protein (CRP) (Hiraiwa and van Eeden 2013). The circulating cytokines IL-1β and TNF-α may activate a number of pro-inflammatory genes in endothelial cells, which are related to the expression of adhesion molecules, cytokines and chemokines (Kuldo et al. 2005). Pro-inflammatory cytokines are also important for tissue factor (TF) expression (Lwaleed et al. 2007). Increased expression of TF, the single most important inducer of the extrinsic pathway of the blood coagulation system, may contribute to a pro-coagulant status with increased thrombin generation which is also known to be induced during inflammation (Kuldo et al. 2005).

Lifestyle factors, e.g., diet, are well known risk factors for cardiovascular diseases. Dietary n-6 and n-3 polyunsaturated fatty acids are involved in the pathophysiology of cardiovascular and inflammatory diseases. Improved flow-mediated dilatation after administration of n-3 fatty acids and beneficial effects on platelet aggregation and inflammation have been shown (Wang et al. 2012; Xin et al. 2012; Marangoni and Poli 2013). A number of anti-inflammatory mechanisms have been associated with marine n-3 fatty acids, including NF-κB down-regulation, decreased leukocyte chemotaxis and adhesion molecule expression and decreased leukocyte-endothelium interaction (Calder 2015).

The subjects investigated in this study were examined before returning to work from home after 9 days off and towards the end of a 12-days work period at the tunnel construction site. The TCW were exposed to a variety of airborne contaminants, including PM, α-quartz and diesel engine exhaust (Bakke et al. 2014). The aim of this study was to assess the impact of exposure to these contaminants on the concentrations of selected biomarkers of pro-coagulant activity, endothelial/platelet activation and inflammation. As the diet may differ between the work-site and at home, various fatty acids were determined to assess potential confounding. The TCW were compared to a group of referents working at the same construction sites and having the same work time schedule, but working outside the tunnels. The present work is part of a larger study of tunnel workers' health and exposure.

Materials and methods

Study design

All subjects employed at 11 different tunnel construction sites in Norway were invited to participate in this follow-up study. Altogether 92 TCW and 52 referents were available for inclusion. They were all men. One TCW and one referent refused to participate, and one TCW and one referent refused to give a blood sample. Thus, results are presented for 90 TCW and 50 referents. All participants worked for 12 consecutive days on the construction sites for approximately 10–12 h/day, before leaving home. They returned back to the work-site nine days later for their next 12 days work period.

Only currently employed subjects with at least 0.5-year of employment were included. According to the protocol subjects with known rheumatoid disease, diabetes, alcohol abuse or other chronic or acute inflammatory diseases were to be excluded from participation. However, no subjects were excluded due to these causes.

Tunnel construction workers are involved in work operations such as rock drilling, charging of explosives, blasting, loading and transport of excavated rock using dump trucks (Bakke et al. 2001). After excavation, rock is supported with steel bolts and sealing of rock by spraying wet concrete onto the surface. Mounting of ventilation ducts, installation of electrical power supply and maintenance and repair work are other common work tasks. All tunnels had forced ventilation system using fans and ventilation ducting to dilute air contaminants, while use of respirators was uncommon. The referents worked, mainly as administrative staff, at the same construction sites. However, they were not directly involved in the tunnel construction work. All participants gave an informed written consent for their voluntary participation in the study that was approved by the Norwegian Regional Ethical Committee for Medical Research (REK2) (S-09153d).

Examinations

Background data were recorded using a questionnaire including questions about respiratory symptoms, allergy, physician diagnosed asthma and self-reported symptoms of common cold at both examinations.

Blood samples were collected at baseline immediately before the subjects started their 12 days work period. The second blood samples (follow-up) were collected at the end of the work period, the day before they went home. Blood samples were collected between 4 and 6 PM both at baseline and follow-up. At follow-up the TCW had been exposed approximately the last 10 h before blood sampling.

Blood was collected from the cubital vein in vacutainer tubes without additives, EDTA tubes and tubes containing 0.129 M trisodium citrate in dilution 1:10 (BD Vacutainer, Belliver Industrial Estate, Plymouth UK) and separated by centrifugation at 2000 g for 15 min. All samples were pipetted into 4.0 mL NUNC® polypropylene cryotubes (Sigma–Aldrich, St.Louis, Missouri, US) for long-term storage at the National Institute of Occupational Health, Oslo, Norway (NIOH) at −80 °C until analysis.

Analysis of biomarkers

Serum was used for determinations of TNF-α, CRP and intercellular adhesion molecule 1 (ICAM-1), whereas EDTA-plasma was used for CD40L and citrated plasma for P-selectin, soluble TF (sTF), D-dimer and prothrombin fragment 1 + 2 (F1 + 2). All analyses were performed by ELISA; CRP with kits from DRG Instruments (Marburg/Lahn, Germany) (detection limit (DL) 0.1 mg/L), TNF-α, ICAM-1, CD40L and P-selectin (R&D Systems Europe, Abingdon, Oxon, UK), sTF (Imunobind TF, American Diagnostic Inc., Greenwich, Conn., USA), F1 + 2 (Enzygnost® F1 + 2 (monoclonal), Siemens, Marburg, Germany) and D-dimer (Asserachrom® D-dimer, Stago Diagnostica, Asniere, France). The inter-assay CVs were 8.5% (TNF-α), <5% (CRP), 4.8% (ICAM-1), 5.2% (P-selectin), 9.5% CD40L, 4.5% (F1 + 2), 7.9% (TF) and 6.6% (D-dimer). The corresponding intra-assay CVs were 6.4, 2.8, 4.0, 3.5, 6.2, 3.0, and 4.4%. Sensitivities of the assays were 0.4 pg/mL, 0.1 mg/L, 0.1 ng/mL, 0.5 ng/mL, 2.1 ng/mL, 20 pmol/L, 0.1 pg/mL and 10 ng/mL, respectively. All samples were analyzed in the same series to avoid bias due to assay variability.

Measurements of fatty acids

Blood lipids were extracted from whole blood and methylated according to previously described methods (Bligh and Dyer 1959; Christie 1982). The extracts were analyzed by gas chromatography using a Thermo Finnigan gas chromatograph (Thermoquest, Hermel Hampstead, UK) equipped with a 100 m fused silica column (Restec RT-2560) with helium as the carrier gas. The program used was: injection at 70 °C which was kept for 2 min followed by an increase to 160 °C at the speed of 20 °C/min and then 2 °C/min to a final temperature of 230 °C which was kept for 10 min. The different methyl-esters were identified by comparison of retention times of known standards. The sum of the n-3 long-chain eicosapentaenoic (EPA;20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids (EPA + DHA) were used in the statistical analysis, to assess the impact of fatty acids on the biomarker concentrations.

Air sampling

Details on air sampling methods have been described in detail elsewhere (Bakke et al. 2014). Briefly, air samples were collected among all TCW by personal sampling during the two consecutive days before blood sampling at follow-up. The samples were collected in the workers' breathing zone, outside personal protective respirators if used. The thoracic aerosol fraction was collected on 37 mm 5.0 µm polyvinyl chloride (PVC) filters (PVC502500, Millipore Corporation, MA, USA) with BGI GK2.69 cyclones (BGI Inc., MA, USA). Elemental (EC) and organic (OC) carbon were collected on pre-cleaned quartz filters (Pallflex Tissue quartz 2500 QAT-UP, Pall Corporation, Port Washington, NY, USA). Oil mist and vapour were collected with a combined 37 mm standard three-part aerosol filter cassette (Millipore, MA, USA) equipped with a glass filter (No. 1820-037, Whatman GF, Madistone, UK) on top of a cellulose acetate filter (AAWP03700, Whatman GF, Madistone, UK) and tubes containing 150 mg charcoal (100 mg in front section) (No. 226-01, SKC, Blandford Forum, Dorset, UK).

Analysis of air samples

Details on the analysis of collected air contaminants have been published (Bakke et al. 2014). Briefly, masses of PM collected on thoracic cyclone filters were measured gravimetrically. The DL based on 8 h sampling at a flow rate of 1.6 L min was 31 µg/m^3. Oil mist was determined by Fourier transform infrared spectrophotometry while oil vapour was determined after desorption with carbon disulphide by gas chromatography, the DLs being 0.05 and 0.1 mg/m^3, respectively, based on a two hour sampling period at a flow rate of 1.4 L min. EC and OC were determined with an OCEC Dual-Optical Analyzer 5040, the DLs being 2 ng/m^3 (EC) and 2 µg/m^3 (OC) based on 8 h sampling at a flow rate of 2.0 L min. The α-quartz content in the thoracic aerosol fraction was measured by X-ray diffraction spectrometry, with a DL of 13 µg/m^3 based on 8 h sampling at a flow

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

	Exposed		Referents		*p*
	AM	Min–Max	AM	Min–Max	
Age (years)	39.3	18–59	43.8	24–61	0.03
BMI (kg/m^2)	25.7	19.9–37.0	27.2	20.3–42.7	0.02
Current smokers (%)	36.7	–	22.0	–	0.09
No. of cigarettes/day	5.4	0–40	2.4	0–30	0.02
Former smokers (%)	22.2	–	30.0	–	0.32
Use of medication (%)	10.0	–	14.0	–	0.48
Years of tunnel work	13.7	1–43	5.7	0–38	<0.001
Thoracic PM (μg/m^3)[¶]	604	110–7870	–	–	–
α-Quartz (μg/m^3)[¶]	74	<DL-1041	–	–	–
Elemental carbon (μg/m^3)	51	4–172	–	–	–
Organic carbon (μg/m^3)	175	59–608	–	–	–

AM Arithmetic mean

[¶]Geometric mean

rate of 1.6 L min. All reagents and water used for chemical analysis were of analytical quality.

Statistics

Individual exposure estimates were based on the mean concentration of the two individual days of air sampling. If only one sample was collected for an individual, this value was used. Air concentrations <DL were substituted with ½ DL for these calculations. Continuous variables were $\log_{10}$-transformed to achieve normal distribution when skewness of the distributions exceeded 2.0. For these variables geometric means (GM) are presented while arithmetic means (AM) are presented otherwise.

Students *t* test was applied for comparisons of independent samples between the TCW and the referents. Paired sample *t* test was used to assess differences in biomarker concentrations and fatty acid percentages between levels measured at baseline and at follow-up within the TCW and referents separately. Univariate associations were assessed using least square regression analysis yielding Pearson's correlation coefficient as the measure of association. For the purpose of assessing biomarker concentrations with several independent variables simultaneously in one model, multiple linear regression analysis (backwards procedure) was used. The model included the independent variables exposure category (0/1), age, common cold (0/1), use of cardiovascular medication (0/1), body mass index (BMI), current tobacco smoking (0/1) and the sum of EPA + DHA percentages. For the assessment of the difference in concentrations between baseline and follow-up, common cold was substituted with common cold at either of the examinations. A two-tailed *p* value <0.05 was considered to be of statistical significance. The study had a statistical power of 0.8 to detect an effect size of 0.4 SD between the biomarker concentrations at baseline and follow-up in the 50 referents using a paired sample *t* test. The statistical data package SPSS 18.0 was used.

Results

Background data and exposure

Altogether 90 TCW and 50 referents were examined. The TCW were on average 4.5 years younger than the referents and their BMI was significantly lower (Table 1). There were more current smokers among the TCW. The GM concentration of PM and α-quartz in the thoracic aerosol fraction based on the individual average of 2 days of air sampling among all TCW were 604 and 74 μg/m^3, respectively. Correspondingly, the AM concentration of EC, a marker of diesel exhaust exposure, was 51 μg/m^3 (Table 1).

Biomarker concentrations at baseline

At baseline when the participants had been away from work for 9 days, the concentrations of the measured biomarkers were comparable among the TCW and the referents (Table 2). The concentrations remained comparable between the groups after adjusting for the differences in smoking habits, age and BMI (results not shown). In contrast, almost all fatty acid percentages in serum were statistically significantly different between the two groups at baseline, the referents having higher concentrations of long-chain n-3 fatty acids, EPA + DHA and Sum n-3 fatty acids (Table 3). The group differences remained statistically

Table 2 The geometric (GM) mean concentrations of biomarkers in 90 exposed tunnel construction workers and 50 referents at baseline

	Exposed		Referents		*p*
	GM	Min–Max	GM	Min–Max	
TNF-α (pg/mL)	2.2	1.0–17.7	2.2	1.1–5.9	0.87
CRP (mg/L)	1.4	0.03–12.3	1.6	0.2–8.4	0.36
ICAM (ng/mL)[†]	249	155–431	241	134–403	0.32
P-Selectin (ng/mL)	25.4	14.3–43.8	25.1	7.2–79.6	0.89
CD40L (pg/mL)	125	43–462	129	27–930	0.79
F 1 + 2 (pmol/L)	205	84–867	213	119–978	0.68
Tissue Factor (pg/mL)	247	151–550	243	180–484	0.67
D-dimer (ng/mL)	234[5]	124–569	266[2]	125–1636	0.19

[†]Arithmetic mean; [2,5]Number of measurements missing

Table 3 The arithmetic mean (AM) levels (in %) of fatty acids in 90 exposed tunnel construction workers and 50 referents at baseline

	Exposed		Referents		*p*
	AM	Min–Max	AM	Min–Max	
Sum n-6	23.6	12.2–34.2	25.4	18.0–32.4	0.01
Sum n-3	3.5	1.4–10.5	4.4	2.0–8.7	0.002
Ratio n-6/n-3	7.6	1.9–14.6	6.4	2.1–10.3	0.003
Ratio 20:4/20:5	9.9	1.5–29.3	8.4	2.2–16.4	0.08
Long n-3	3.3	1.1–10.1	4.2	1.8–8.3	0.001
EPA + DHA	2.5	0.8–8.5	3.3	1.4–6.9	0.001

significant after adjusting for smoking habits, age and BMI (results not shown).

Difference of biomarker concentrations between baseline and follow-up

Biomarker concentrations and fatty acids percentages at baseline and at follow-up are shown in Table 4. GM (and 95% confidence interval) concentrations of TNF-α decreased from 2.2 (2.0–2.4) at baseline to 2.0 pg/mL (1.8–2.2) ($p = 0.02$) at follow-up among the TCW. The platelet activation biomarkers P-selectin and CD40L decreased from 25.4 (24.1–26.6) to 24.4 (22.9–26.0) ng/mL ($p = 0.04$) and 125 (114–137) to 105 (96–115) pg/mL ($p < 0.001$), respectively. ICAM-1 concentrations increased among the TCW from 249 (238–260) to 254 (243–266) ng/mL ($p = 0.02$). None of these biomarker concentrations changed statistically significant across the work period among the referents. No significant changes were observed for the biomarkers of coagulation in any of the groups. There were quite substantial alterations in fatty acid percentages between baseline and follow-up, in particular among the referents. Thus, there were no significant differences in any of the fatty acid percentages between the two groups at follow-up.

Table 4 The geometric (GM) mean concentrations of serum biomarkers and % fatty acids in 90 exposed tunnel construction workers and 50 referents at baseline and at follow-up

	Baseline		Follow-up		*p*
	GM	Min–Max	GM	Min–Max	
Exposed					
TNF-α (pg/mL)	2.2	1.0–17.7	2.0[2]	0.6–17.0	0.02
CRP (mg/L)	1.4	0.03–12.3	1.2[2]	0.1–17.9	0.47
ICAM (ng/mL)†	249	155–431	254[2]	152–436	0.02
P-Selectin (ng/mL)	25.4	14.3–43.8	24.4[2]	12.4–76.8	0.04
CD40L (pg/mL)	125	43–462	105[4]	28–446	<0.001
F 1 + 2 (pmol/L)	205	84–867	195[2]	92–1772	0.35
Tissue factor (pg/mL)	247	151–550	249[2]	158–1076	0.75
D-dimer (ng/mL)	234[5]	124–569	227[8]	81–658	0.41
Sum n-6 (%)†	23.6	12.2–34.2	24.4[3]	15.2–34.4	0.16
Sum n-3 (%)†	3.5	1.4–10.5	3.2[3]	1.1–7.7	0.09
Ratio n-6/n-3 (%)†	7.6	1.9–14.6	8.4[3]	2.0–20.0	0.07
Ratio 20:4/20:5 (%)†	9.9	1.5–29.3	11.4[3]	1.0–43.0	0.04
Long n-3 (%)†	3.3	1.1–10.1	2.9[3]	1.0–7.3	0.08
EPA + DHA (%)†	2.5	0.8–8.5	2.3[3]	0.7–4.8	0.08
Referents					
TNF-α (pg/mL)	2.2	1.1–5.9	2.0	0.8–8.4	0.12
CRP (mg/L)	1.6	0.2–8.4	1.8	0.5–15.9	0.35
ICAM (ng/mL)†	241	134–403	241[1]	142–394	0.57
P-Selectin (ng/mL)	25.1	7.2–79.6	22.5	8.4–36.7	0.11
CD40L (pg/mL)	129	27–930	122[1]	32–922	0.50
F 1 + 2 (pmol/L)	213	119–978	209[1]	96–1754	0.86
Tissue factor (pg/mL)	243	180–484	239[1]	165–434	0.44
D-dimer (ng/mL)	266[2]	125–1636	265[3]	127–1279	0.66
Sum n-6 (%)†	25.4	18.0–32.4	25.4	17.4–34.0	0.93
Sum n-3 (%)†	4.4	2.0–8.7	3.6	1.0–10.6	0.002
Ratio n-6/n-3 (%)†	6.4	2.1–10.3	7.9	2.0–19.0	<0.001
Ratio 20:4/20:5 (%)†	8.4	2.2–16.4	9.7	2.0–28.0	0.06
Long n-3 (%)†	4.2	1.8–8.3	3.4	0.8–10.1	0.001
EPA + DHA (%)†	3.3	1.4–6.9	2.6	0.7–7.4	<0.001

†Arithmetic mean; [1,2,3,4,5,8]Number of measurements missing

Regression analysis

Multiple linear regression analysis (backwards procedure) assessing biomarker concentrations measured at baseline did not show any impact of being exposed as TCW or being referent when current smoking, age, BMI, cardiovascular medication, common cold and EPA + DHA where included as potential confounders (results not shown). Concentrations of F1 + 2, D-dimer, ICAM-1 and CRP increased significantly with age. Being a current smoker was also associated with higher concentrations of ICAM-1 and CRP, while reporting an ongoing common cold was associated with higher concentrations of TNF-α and F1 + 2. BMI was associated with higher CRP concentrations. No significant associations between EPA + DHA and the concentration of any of the biomarkers at baseline were observed. Age-adjusted concentrations of TNF-α, CRP, ICAM-1, TF and P-selectin according to being a current smoker and having BMI higher or lower than 25 kg/m^2 are shown in Table 5.

Multiple linear regression analysis (backwards procedure) assessing differences in biomarker concentrations between baseline and follow-up did not show any impact of being exposed during the study period, suggesting no statistically significant differences in biomarker concentrations changes between the groups. Also no associations between any of the exposure measures and the difference in any of the biomarker concentrations between baseline and follow-up were observed. Furthermore, no associations

Table 5 The geometric (GM) mean concentrations of serum biomarkers according to body mass index (BMI) and current smoking habits in all participants at baseline adjusted for age

	BMI < 25 kg/m^2		BMI ≥ 25 kg/m^2	
	Smoke− (*N* = 37) GM (95% CI)	Smoke+ (*N* = 19) GM (95% CI)	Smoke− (*N* = 59) GM (95% CI)	Smoke+ (*N* = 25) GM (95% CI)
TNF-α (pg/mL)[b]	2.2 (1.9–2.5)	1.9 (1.5–2.2)	2.3 (2.1–2.6)	2.4 (2.0-2.8)
CRP (mg/L)[abc]	0.7 (0.6-1.0)	1.7 (1.1–2.6)	1.8 (1.4–2.3)	2.0 (1.4–2.9)
ICAM (ng/mL)[†,a]	230 (214–246)	272 (250–294)	246 (233–258)	252 (233–272)
P-Selectin (ng/mL)[a]	23 (21–25)	27 (24–31)	25 (23–27)	27 (24–30)
TF (pg/mL)[b]	248 (231–265)	277 (252–305)	238 (225–251)	238 (219–259)

[†] Arithmetic mean

[a] $p_{smoking} < 0.05$

[b] $p_{BMI} < 0.05$

[c] $p_{interaction} < 0.05$

were observed between concentrations of airborne contaminants and any of the biomarkers determined at follow-up.

Discussion

This is to our knowledge the first study of biomarkers of endothelial/platelet activation, inflammation and coagulation in TCW. The TCW were exposed to a GM concentration of 604 μg/m^3 of PM, which includes α-quartz and EC from diesel exhaust emissions. The study shows that the concentrations of the biomarkers of platelet activation CD40L and P-selectin and the pro-inflammatory cytokine TNF-α were slightly lower in TCW after 11 days of exposure. The study further shows that the concentration of ICAM-1, a marker of endothelial activation, was slightly higher at follow-up. The significant alterations in blood fatty acid composition was in particular observed among the referents.

Exposure

The TCW were exposed to the GM concentrations of 604 μg/m^3 of PM and 74 μg/m^3 of α-quartz in the thoracic aerosol fraction. The AM concentration of EC, a marker of diesel exhaust exposure, was 51 μg/m^3. Epidemiological studies have shown increased mortality due to ischemic heart disease in miners, who have many similar work conditions as TCW (Reid and SluisCremer 1996; Weiner et al. 2007). The increased risk in those studies was attributed to α-quartz exposure. Increased mortality due to ischemic heart diseases was also shown in coal and iron miners (Björ et al. 2009; Landen et al. 2011). The increased ischemic heart disease mortality among operators of heavy equipment was attributed to diesel exhaust exposure (Finkelstein et al. 2004; Laden et al. 2007; Torén et al. 2007). Thus, exposure to airborne contaminants that are present in the work environment of TCW have been associated with increased cardiovascular mortality.

Platelet activation

In this study TNF-α, CD40L and P-selectin levels were slightly reduced among the TCW after 11 days of exposure. In contrast, results from experimental studies of humans exposed to diesel exhaust particle mass concentrations ranging from 300 to 350 μg/m^3, showed increased concentrations of TNF-α, P-Selectin and CD40L (Törnqvist et al. 2007; Lucking et al. 2008). Also increased pro-coagulant activity was reported in humans exposed to diesel exhaust particulates (Mills et al. 2007; Lucking et al. 2008). None of the coagulation biomarker concentrations increased in the present study. However, diesel exhaust exposure among the TCW was lower compared to the concentrations used in the experimental studies. It has been proposed that increased thrombogenicity and platelet activation occur within the first hours after exposure (Langrish et al. 2012). The TCW had been continuously exposed the last 10 h before blood sampling and were thus at risk of such effects.

Studies assessing biomarkers of inflammation, coagulation and endothelial activation have been carried out in populations typically exposed to ambient air pollution. Increased levels of CRP, ICAM-1, F 1 + 2, IL-6, fibrinogen and plasminogen activator inhibitor type-1 (PAI-1) have been reported, although the concentrations of EC and PM are substantially lower in urban air than during tunnel construction (Dubowsky et al. 2006; Rűckerl et al. 2006, 2007; Chuang et al. 2007; Shima 2007). Animal studies have shown that pro-inflammatory reactions after exposure to α-quartz occur after a latency period, and it has been speculated that α-quartz particles mainly are phagocytized by M-2 polarized macrophages, that have a down-regulating

effect of the classical pro-inflammatory M-1 polarized macrophages (Kawasaki 2015). A recent in vitro study showed the predominant uptake of silica nanoparticles by M-2 polarized macrophages (Hoppstädter et al. 2015). Whether α-quartz exposure may down-regulate pro-inflammatory effects induced by, e.g., diesel exhaust exposure among TCW remains to be elucidated.

Physical activity

Tunnel construction workers carry out physically demanding work tasks, and it is reasonable to assume that their physical activity is substantially larger during work than when they are at home. This was in contrast to the more sedentary work of the referents. Several studies have shown that physical activity can reduce systemic inflammation, but whether this effect is related to the activity as such or to weight reduction associated with physical activity is not completely elucidated (Beavers et al. 2010). A recent review concluded that there was strong evidence that physical activity decreases TNF-α in cardiovascular risk patients (Palmefors et al. 2014). That review also suggested reduced ICAM-1 during physical exercise, while the evidence of a reduction in P-selectin was inconclusive. We have previously shown a reduction of P-selectin among asphalt pavers (Ellingsen et al. 2010). Asphalt pavers in Norway have a physically active work during the paving season and more sedentary work outside the season.

ICAM-1

There is evidence that also the concentration of ICAM-1, which is regarded as a marker of endothelial activation in atherosclerosis, is reduced due to physical activity (Palmefors et al. 2014). However, the concentrations increased slightly in the TCW, while no changes from baseline to follow-up were observed among the referents. Studies of wild-type mice have shown substantial amounts of ICAM-1 in bronchoalveolar lavage fluid and that type I alveolar epithelial cells are the source of origin, but pro-inflammatory cytokines had no or only slight effect on the concentrations (Mendez et al. 2006). In contrast, pro-inflammatory cytokines have strong effect on the expression of ICAM-1 in endothelial cells in vitro (Tsakadze et al. 2004). Mice exposed to hyperoxia had increased levels of ICAM-1 in the BAL fluid (Mendez et al. 2006). It has also been shown that children exposed to environmental tobacco smoke had substantially higher concentrations of ICAM in the BAL fluid than non-exposed children (Grigg et al. 1999). ICAM-1 is highly expressed in lung tissue, endothelial cells and other tissues. We have previously reported that the examined TCW had pulmonary alterations as evidenced by lower pulmonary function and alterations in the serum concentrations of surfactant protein D (Ellingsen et al. 2015; Ulvestad et al. 2015). Thus, it is tempting to speculate that the increase in ICAM-1 is a pulmonary endothelial response as part of the process of attracting monocytes to the lung for the handling of their pulmonary inflammation.

Fatty acids

The referents had a more favourable blood fatty acid composition than the exposed subjects at baseline, in particular substantially higher levels of EPA + DHA. These fatty acids are well known to have anti-inflammatory properties, e.g., by down-regulating the inflammatory transcription factor NF-κB or activating peroxisome proliferator-activated receptor (PPAR) (Adkins and Kelley 2010; Calder 2015). However, there were no significant associations between EPA + DHA and the concentrations of any of the determined biomarkers at baseline.

None of the fatty acid percentages differed statistically significant between the groups at follow-up. This was mostly due to the substantial alterations in the fatty acid composition among the referents between baseline and follow-up. The difference in dietary habits between the groups and the lower prevalence of smokers among the referents suggest a healthier life-style among the referents than among the TCW.

Strengths and limitations

The high participation rates of this follow-up study indicate that selection bias may be of minor importance. There was also no loss to follow-up. The exposed subjects and referents were employed in the same companies suggesting similar recruitment to work. However, the prevalence of current smokers was higher among the TCW who also had less beneficial blood fatty acid composition than the referents at baseline, indicating slight differences in sociodemographic background and dietary habits between the groups. These differences between the groups did not impact the overall results of the study. Only presumably healthy men were examined, thus one should be cautious to extrapolate the results to female populations or, e.g., to male populations with pre-existing cardiovascular diseases. A low occasional airborne exposure to PM among the referents cannot be excluded, but no alterations in their biomarker concentrations were observed. Also, such exposure would not confound the observations of altered biomarker concentrations among the TCW.

In summary, biomarker concentrations of platelet activation and inflammation decreased slightly in TCW during 11 days of exposure to gases and PM, including α-quartz and diesel exhaust. The ICAM-1 levels increased slightly, perhaps as a sign of monocyte recruitment to the pulmonary

endothelial layer. Whether the alterations may be of clinical significance in the long run remains to be elucidated.

Acknowledgements We thank medical technologist Vibeke Bratseth for excellent laboratory work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

References

Adkins Y, Kelley DS (2010) Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J Nutr Biochem 21:781–792

Bakke B, Stewart P, Ulvestad B, Eduard W (2001) Dust and gas exposure in tunnel construction work. AIHAJ 62(4):457–465

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 58(7):818–829

Beavers KM, Brinkley TE, Nicklas BJ (2010) Effects of exercise training on chronic inflammation. Clin Chim Acta 411:785–793

Björ B, Burström L, Jonsson H, Nathanaelsson L, Damber L, Nilsson T (2009) Fifty-year follow-up of mortality among a cohort of iron-ore miners in Sweden, with specific reference to myocardial infarction mortality. Occup Environ Med 66:264–268

Bligh EG, Dyer WI (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV et al (2010) Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation 121:2331–2378

Calder PC (2015) Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta 1851(4):469–484

Christie WW (1982) A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. J Lipid Res 23:1072–1075

Chuang K-J, Chan C-C, Su T-C, Lee C-T, Tang C-S (2007) The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. Am J Resp Crit Care Med 176:370–376

Donaldson K, Mills N, MacNee W, Robinson S, Newby D (2005) Role of inflammation in cardiopulmonary health effects of PM. Toxicol Appl Pharmacol 207(2 Suppl):S483–S488

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(6):992–998

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

Ellingsen DG, Ulvestad B, Bakke B, Seljeflot I, Barregard L, Thomassen Y (2015) Serum pneumoproteins in tunnel construction workers. Int Arch Occup Environ Health 88(7):943–951

Finkelstein MM, Verma DK, Sahai D, Stefov E (2004) Ischemic heart disease mortality among heavy equipment operators. Am J Ind Med 46:16–22

Geiser M, Kreyling WG (2010) Deposition and biokinetics of inhaled nanoparticles. Part Fibre Toxicol 7:2

Grigg J, Riedler J, Robertson CF (1999) Soluble intercellular adhesion molecule-1 in the bronchoalveolar lavage fluid of normal children exposed to parental cigarette smoke. Eur Respir J 13:810–813

Hiraiwa K, van Eeden SF (2013) Contribution of lung macrophages to the inflammatory responses induced by exposure to air pollutants. Mediators Inflamm. doi:10.1155/2013/619523

Hoppstädter J, Seif M, Dembek A, Cavelius C, Huwer H, Kraegeloh A, et al (2015) M2 polarization enhances silica nanoparticle uptake by macrophages. Front Pharmacol 6:55. doi:10.3389/fphar.2015.00055

Kawasaki H (2015) A mechanistic review of silica-induced inhalation toxicity. Inhal Toxicol 27(8):363–377

Kuldo JM, Ogawara KI, Werner N, Asgeirsdottir SA, Kamps JAAM, Kok RJ et al (2005) Differential effects of NF-κB and p38 MAPK inhibitors and combinations thereof on TNF-α and IL-1β-induced proinflammatory status of endothelial cells in vitro. Am J Physiol Cell Physiol 289:C1229–C1239

Laden F, Hart JE, Smith TJ, Davis ME, Garshick E (2007) Cause-specific mortality in the unionized U.S. trucking industry. Environ Health Perspect 115:1192–1196

Landen DD, Wassell JT, McWilliams L, Patel A (2011) Coal dust exposure and mortality from ischemic heart disease among a cohort of U.S. coal miners. Am J Ind Med 54:727–733

Langrish JP, Bosson J, Unosson J, Muala A, Newby DE, Mills NL et al (2012) Cardiovascular effects of particulate air pollution exposure: time course and underlying mechanisms. J Intern Med 272:224–239

Lucking AJ, Lundback M, Mills NL, Faratian D, Barath SL, Pourazar J et al (2008) Diesel exhaust inhalation increases thrombus formation in man. Eur Heart J 29:3043–3051

Lwaleed BA, Cooper AJ, Voegeli D, Getliffe K (2007) Tissue factor: A critical role in inflammation and cancer. Biol Res Nurse 9:97–107

Marangoni F, Poli A (2013) Clinical pharmacology of n-3 polyunsaturated fatty acids: non-lipidic metabolic and hemodynamic effects in human patients. Atherosclerosis 14:230–236

Mendez MP, Morris SB, Wilcoxen S, Greeson E, Moore B, Paine R 3rd (2006) Shedding of soluble ICAM-1 into the alveolar space in murine models of acute lung injury. Am J Physiol Lung Cell Mol Physiol 290:L962–L970

Mills NL, Törnqvist H, Gonzales MC, Vink E, Robinson SD, Söderberg S et al (2007) Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. New Eng. J Med 357:1075–1082

Palmefors H, DuttaRoy S, Rundqvist B, Börjesson M (2014) The effect of physical activity or exercise on key biomarkers in atherosclerosis—a systematic review. Atherosclerosis 235:150–161

Reid PJ, SluisCremer GK (1996) Mortality of white South African gold miners. Occup Environ Med 53:11–16

Rűckerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J et al (2006) Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. Am J Respir Crit Care Med 173:432–441

Rűckerl R, Greven S, Ljungman P, Aalto P, Antoniades C, Bellander T et al (2007) Air pollution and inflammation (Interleukin-6, C-Reactive Protein, Fibrinogen) in myocardial infarction survivors. Environ Health Perspect 115:1072–1080

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

Torén K, Bergdahl IA, Nilsson T, Järvholm B (2007) Occupational exposure to particulate air pollution and mortality due to ischaemic heart disease and cerbrovascular disease. Occup Environ Med 64:515–519

Törnqvist H, Mills NL Gonzales MC, Miller MR, Robinson SD, Megson IL (2007) Persistent endothelial dysfunction in humans after diesel exhaust inhalation. Am J Respir Crit Care Med 176:395–400

Tsakadze NL, Sen U, Zhao Z, Sithu SD, English WR, D'Souza SE (2004) Signals mediating the cleavage of intercellular adhesion molecule-1. Am J Physiol Cell Physiol 287:C55–C63

Ulvestad B, Lund MB, Bakke B, Thomassen Y, Ellingsen DG (2015) Short-term lung function decline in tunnel construction workers. Occup Environ Med 72:108–113

Wang Q, Liang X, Wang L, Lu X, Huang J, Cao J et al (2012) Effects of omega-3 fatty acids supplementation on endothelial function: A meta-analysis of randomized controlled trials. Atherosclerosis 221(2):536–543

Weiner J, Barlow L, Sjögren B (2007) Ischemic heart disease mortality among miners and other potentially silica-exposed workers. Am J Ind Med 50:403–408

Xin W, Wei W, Li X (2012) Effect of fish oil supplementation on fasting vascular endothelial function in humans: A meta-analysis of randomized controlled trials. PLoS One 7(9):e46028