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Serum pneumoproteins and biomarkers of exposure to urban air pollution: a cross-sectional comparison of policemen and foresters

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Very few biomarkers are available for the non-invasive detection of effects of urban air pollution on the respiratory tract. The objective was to evaluate whether Clara cell protein (CC16) and surfactant-associated protein-A (SP-A), two pulmonary secretory proteins, were useful in the detection of effects of urban air pollutants on the pulmonary epithelium. These proteins were determined in the serum of 53 policemen working in Brussels, Belgium, and a control group of 59 foresters working in the countryside. Except for ozone (O_3), annual concentrations of the main air pollutants (PM_{10}, NO_2, CO, SO_2 and benzene) were significantly higher in Brussels than in the country. The proportion of smokers was lower in urban policemen compared with foresters, but they smoked on average a similar number of cigarettes per day as confirmed by their urinary excretion of cotinine. Muconic acid, a marker of benzene exposure, was significantly higher in urban policemen than in foresters, in both smokers and non-smokers. Multiple regression analysis showed that the type of work, smoking habits and time spent outdoors and in a car were significant determinants of benzene uptake. Tobacco smoking impaired lung function to a similar extent in urban policemen and foresters. The serum levels of SP-A were significantly increased in smokers but were not different between policemen and foresters. Serum CC16 was significantly reduced by tobacco smoking and slightly decreased in policemen compared with foresters. Interestingly, the reduction of serum CC16 was more pronounced in the subgroup of traffic compared with survey policemen, the latter being also less exposed to benzene. The results suggest that serum pneumoproteins and especially serum CC16 could be useful in the detection of chronic effects of urban air pollutants on the respiratory epithelium of populations particularly at risk.

Keywords: lung epithelium, pneumoproteins, Clara cell protein (CC16), surfactant-associated protein (SP-A), muconic acid, policemen, foresters.

Introduction

A number of epidemiological studies have shown that exposure to urban air pollutants is associated with both an increased morbidity and mortality (Bascom *et al.* 1996, Lebowitz 1996, Samet and Cohen 1999, Thurston and Ito 1999, MacNee and Donaldson 2000, Simpson *et al.* 2000, Tellez-Rojo *et al.* 2000). Acute health effects are often mild and reversible in healthy individuals but may more seriously affect sensitive subjects such as children or patients with pre-existing lung disease. Exposure to particulate matter (PM), especially fine particles (PM_{10} and $PM_{2.5}$), has been associated with impaired lung function tests,

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increased respiratory symptoms, school absence and aggravation of cardiopulmonary diseases (Simpson *et al.* 2000). Ozone, the main oxidant of photochemical smog, can also produce a variety of adverse effects, including decreased pulmonary function, airway inflammation, airflow reduction, asthma exacerbation and increased mortality in the elderly (Kunzli *et al.* 1997, Thurston and Ito 1999). Finally, there is growing concern that indoor and outdoor air pollution may increase the risk of lung cancer in both smokers and non-smokers (Samet and Cohen 1999).

Due to the complexity of urban atmosphere, it remains difficult to identify one pollutant as being responsible for a specific adverse health effect. Although quantities of pollutants can alter the integrity of the respiratory epithelium and induce airway inflammation in the lower respiratory tract (Sato *et al.* 1995, Finlayson-Pitts and Pitts 1997, Monn and Becker 1999), the precise mechanisms underlying their pulmonary effects are not yet well understood. Depending on the pollutants chemical properties, inflammation often predominantly occurs in the distal airways, between the terminal bronchioles and alveoli (Duan *et al.* 1996), an area with specific characteristics playing an important role in the protection of the respiratory epithelium. The observed progressive loss of Clara cells with an ensuing decreased secretion of CC16 protein in this region has, for example, been suggested to weaken lung defence against oxidative stress (Johnston *et al.* 1997, Mango *et al.* 1998).

The effects of air pollutants on the integrity of the lung epithelium have classically been assessed by the analysis of the epithelial lining fluid sampled by bronchoalveolar lavage, a procedure that is too invasive to be applied to large populations. Recently, however, a new approach has been developed based on the concept that proteins secreted by the lung epithelium can be monitored in serum where they may thus serve as peripheral reporters of toxic events taking place in the respiratory tract (Hermans and Bernard 1996, Broeckaert and Bernard 2000). So far, several lung-specific proteins have been validated as non-invasive tests of lung epithelial damage among which the anti-oxidant 16-kDa Clara cell protein (CC16) mainly secreted by Clara cells in terminal bronchioles and the surfactant-associated proteins A, B and (SP-A, SP-B and SP-D), which are mainly secreted by the aveoli. Studies on human volunteers (Broeckaert *et al.* 1999, 2000, Blomberg *et al.* 2003, Nickmilder *et al.* 2003) or rodents (Arsalane *et al.* 1999, Broeckaert *et al.* 2000, 2003) exposed to O_3, on asymptomatic smokers (Bernard *et al.* 1994, Shijubo *et al.* 1997, Andersson *et al.* 2000, Dell'Omo *et al.* 2000) or on workers exposed to industrial air pollutants (Hermans and Bernard 1996, Broeckaert and Bernard 1999, Burgess *et al.* 2003) have illustrated the sensitivity and specificity of CC16 and surfactant-associated proteins as markers of lung injury.

The objective of the present study was to evaluate further pneumoproteins by comparing these biomarkers in policemen regularly exposed to urban air pollutants and foresters working in the country. Metabolites of exposure to benzene (muconic acid, MA), pyrene (hydroxypyrene) and nicotine (cotinine) were also determined to ascertain differences in inhaled air quality of these two populations.

Materials and methods

Study location and population selection

Brussels, the capital of Belgium, was selected as the polluted area (urban), and the Belgian Ardennes region as the non-polluted area (rural). Brussels is the largest city in the country with approximately 10^6 habitants and 450 000 private homes. With the exception of an incinerator, an electric power station and an airport east of the city, the essential economic activity is covered by private and public offices. These activities drain more than 250 000 vehicles each day and contribute to over 70% of the emission of air pollutants (IBGE 1996). The Ardennes area is 80–150 km south-east of Brussels and is essentially covered by forests and grasslands (150 km^2) (DGRNE 1999). No cities with more than 10 000 inhabitants are found in this area. Each region possesses its own air quality-monitoring network with approximately 40 stationary and mobile stations (IRCELINE 2000). The Belgian Interregional Cell for the Environment provided averages for PM_{10} ($\mu g\ m^{-3}$), NO_2 ($\mu g\ m^{-3}$), O_3 ($\mu g\ m^{-3}$), CO ($mg\ m^{-3}$), SO_2 ($\mu g\ m^{-3}$) and benzene ($\mu g\ m^{-3}$) and for the years 1997, 1998, 1999 and 2000. These pollutants were measured according to the European Directive 96/62/CE (JOC of 11/21/1996).

All the policemen included in the study were employed by the Brussels police department, whereas the foresters came from the Belgian federal department of water and forests and worked in different sub-areas within the country. Subjects with more than 5 years of service and who were spending the majority of their time outdoors were invited to participate by mail. Sixty-six policemen and 69 foresters accepted to take part and signed an informed consent agreement form. The examination protocol included a questionnaire, spirometry tests and the collection of a blood and urine sample. Policemen were examined in their offices (two different places) and foresters in their office or at the occupational medicine department (five different places) between January and April. In order to intimate diurnal variations in the lung function tests and the serum pneumoproteins levels (which on average show a diurnal decrease of about 10–15%), all subjects were examined in the morning. The study design was reviewed and approved by the Ethics Committee of the Catholic University of Louvain. A questionnaire available in two languages (French and Dutch), based on the European Community Respiratory Health Survey (ECRHS) (Burney *et al.* 1994), was submitted on the day of the study. Questions concerned respiratory symptoms and diseases, type and place of work, time spent in cars or in office, passive or active smoking, and home environment. Workplaces of policemen were classified based on their job description as traffic, patrol or survey.

Subjects with current respiratory infections, body mass index $>40\ kg\ m^{-2}$, incomplete questionnaires, abnormal baseline spirometry and with pack-years >40 were excluded from the final selection (three policemen and 10 foresters). Further exclusions concerned two non-smoking policemen who were found to have elevated concentrations of urinary cotinine, and all the female participants from the police corps ($n=8$) due to the absence of women foresters.

Lung function tests

Spirometric measurements were made with Vitalograph Compact II spirometers (Vitalograph Ltd, Buckingham, UK). Each apparatus was calibrated twice a day to control temperature changes with a high precision syringe of 1 litre (Vitalograph). Under the supervision of a medical doctor, each volunteer performed a minimum of three measurements and the means of the two or three best acceptable results were selected according to the American Thoracic Society criteria of reproducibility (ATS 1987). Spirometric tests included forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), and forced expiratory flows (FEF_{max}, PEF and FEF_{25-75}). All measurements were expressed as the percentage of predicted normal values using prediction equations based on race, age, sex and height.

Biological samples and biomarker analyses

Blood (10 ml) and urine (about 20 ml) samples were collected the day of the study and immediately stored frozen until analysis. Samples from policemen and foresters were analysed and mixed in the same series. When the concentration of a urinary metabolite was below the detection limit, it was assigned a value equal to the half of the limit of detection for the statistical analysis.

Creatinine was measured in urine by the colorimetric method of Jaffé (Taussky 1954) using a Technicon RA-1000 (Bayer Technicon, Brussels, Belgium). CC16 in serum was determined by an automated immunoassay relying on the agglutination of latex particles as described (Bernard and Lauwerys 1983, Hermans *et al.* 1998). Briefly, the assay uses rabbit anti-protein 1 antibody (Dakopatts, Glostrup, Denmark) and human CC16 purified in our laboratory as standard. To avoid possible interference by complement, rheumatoid factor or chylomicrons, serum samples (0.5 ml) were pretreated by heating at 56°C for 30 min and by the addition of polyethylene glycol (16%, 1/1 v/v) and trichloroacetic acid (10%, 1/ 40 v/v). After overnight precipitation at 4°C, the samples were centrifuged (at 2000*g* for 10 min) and CC16 was determined in the supernatants. All samples were

analysed in duplicate at two different dilutions. The assay has a detection limit of 0.5 $\mu g\,l^{-1}$, an average analytical recovery of 95% and between or within run coefficients of variation of 5–10% (Hermans *et al.* 1998). Variations due to protein degradation are unlikely as CC16 is stable in serum frozen for years and for weeks in serum kept at room at 4°C. SP-A was measured using commercially available rabbit polyclonal antibodies directed against human SP-A from bronchoalveolar lavage fluids (Chemicon International, Inc., Temecula, CA, USA). SP-A was determined by an inhibition enzyme immunosorbent assay (ELISA) similar to that developed by Doyle *et al.* (1998). In the first step, SP-A is dissociated from any associated component by the addition of 5 μl 0.1 M EDTA to 85-μl aliquots of the samples. ELISA plates (no. 3590, Costar, Rochester, NY, USA) were coated overnight at 37°C with 200 μl/well of pooled human bronchoalveolar lavage fluids (BALF) diluted 1/250 in carbonate-bicarbonate buffer (pH 9.6). Dilutions of the samples and standards were incubated in a separate ELISA plate with 50 μl/well of the primary antibody diluted 1/1000 in PBS-Tween-BSA buffer (pH 7.4). Each sample was assayed at six three-fold serial dilutions in PBS-Tween buffer containing 0.25% BSA. Standard curves comprising six three-fold serial dilutions of human SP-A were constructed. After overnight incubation, the plate coated with BALF was washed three times with PBS-Tween-BSA buffer, blocked with BSA (Sigma, St. Louis, MO, USA) (250 μl/well BSA 2% in carbonate-bicarbonate buffer), incubated 1 h at 37°C and then washed. Aliquots (75 μl) of the samples were transferred to the plate coated with BALF and incubated for an additional 120 min at 37°C. This plate was then washed and incubated for 60 min at 37°C with 75-μl aliquots of peroxidase-conjugated goat anti-rabbit polyclonal IgG (Sigma) diluted 1/1000 in PBS-Tween-BSA buffer. After washing, 190 μl acetate buffer (pH 5.5) were added to each well. The plate was then washed and developed by the addition to each well of 100 μl of the peroxidase substrate (BM blue, Boehringer Mannheim GmbH, Mannheim, Germany). After approximately 30 min, the enzymatic reaction was stopped by the addition of 100 μl/well H_2SO_4 1 mol l^{-1}. The absorbance of the substrate was measured at 450 nm by a spectrophotometer (Twinreader Titertek, Flow Laboratories, Helsinki, Finland). A standard curve was generated for each plate and concentrations of SP-A were calculated. The analytical recovery of the assay was of 89.6%. Inter- and intra-assays variation coefficients were 13.0 and 7.7%, respectively. Like serum CC16, SP-A is very stable in frozen serum. Urinary cotinine, a metabolite of nicotine, was determined as a tobacco-smoke exposure index as described (De Boeck *et al.* 2000). Briefly, 1 ml urine was treated with 250 μl sodium hydroxide 8 M, 100 μl N-ethylnorcotinine (Toronto Research Chemical, Toronto, Canada) solution (internal standard; 20 mg l^{-1} H_2O) and 15 ml chloroform. The organic phase was extracted and prepared for high-pressure liquid chromatography (HPLC) equipped with an ultraviolet light absorbance detector adjusted at 260 nm (Waters Associates, Milford, MA, USA). All solvents, including those of the mobile phase, were prepared from HPLC-grade and Milli-Q® water (Millipore, Brussels, Belgium). The limit of detection was determined as 0.05 mg l^{-1}. The within- and between-day variations ($n=10$) were 4.3 and 4.7%, respectively. The assay was calibrated with standard of cotinine (Sigma Aldrich, Bornem, Belgium) diluted in urine samples. *Trans-trans* MA was measured by HPLC by using a slightly modified method of Ducos *et al.* (1992). Briefly, 200 μl urinary samples were purified on a anion-exchange column (SB 100 Chromabond, no. 730079, Macherey Nagel, Düren, Germany) conditioned with methanol (Janssens Chemica, Beerse, Belgium). Elution was realized by the addition of 1 ml acetic acid/H_2O (1/99, v/v). Analysis of MA was performed on a 1100 automatic HPLC analyser (Agilent, Wilmington, DA, USA). Calibration was made by using synthesized MA (98% purity, Sigma Aldrich M-9000–3). The lowest detection limit was 20 mg l^{-1}. Analytical recovery and reproducibility generally exceeded 90%. Urinary hydroxypyrene (HP), a metabolite of pyrene, constituent of the polycyclic aromatic hydrocarbons (PAH) family was measured using the method of Buchet *et al.* (1995). All solvents and water used were of HPLC grade. Analysis was based on enzymatic hydrolysis followed by a concentration step and the determination of the metabolite with HPLC equipped with a fluorescence spectrophotometer. The calibration was made with a control urine sample spiked with synthesized HP (Janssens Chemica). The detection limit was 0.1 $\mu g\,l^{-1}$.

Statistics

For statistical analysis, NCSS software was used. Results are expressed mean±SE. Variables not normally distributed were normalized by a logarithmic transformation. Differences between policemen and foresters or between smokers and non-smokers were assessed by an unpaired Student's *t*-test. One-way analysis of variance (ANOVA) and the Student–Newman–Keuls post-test were used to compare policemen categorized by type of work (traffic, patrol and survey). A two-way ANOVA was also used to detect possible interactions between the type of work and the smoking habits (smokers or non-smokers) for CC16, SP-A, MA, HP, cotinine and lung function tests. Multiple linear regression tests were used to assess the association of biomarkers (CC16, SP-A, MA, HP, cotinine) and lung function tests with possible predictors. The level of statistical significance was set at $p<0.05$ or $p<0.01$ where specified (two-tailed).

Results

Air pollution data and characteristics of policemen and foresters

Table 1 provides the air pollution data for Brussels and the country (Ardennes areas) for 1997–2000. Except for O_3 levels, the mean annual and upper range values for PM_{10}, CO, SO_2, NO_2 and benzene were higher in Brussels than in the Ardennes areas for the four years considered. The characteristics of policemen and foresters are presented in table 2 for all subjects and separately for non-smokers and smokers. There were no significant differences between both groups with respect to age, BMI and duration of employment. The proportion of smokers was however significantly higher among foresters than among policemen (41 versus 28%; $p < 0.05$) but the average pack-years and numbers of cigarettes consumed per day were rather similar between smokers of both groups. On average, working policemen spent more time in vehicles and less time outdoors than foresters.

Biomarkers of exposure to environmental pollutants

Concentrations of metabolites of benzene (MA), PAHs (hydroxypyrene, HP) and nicotine (cotinine) are shown in table 3. The urinary excretion of MA was overall greater in policemen than in foresters, significantly so when comparing smoking policemen to smoking foresters ($p < 0.1$) and were also increased in smokers compared with non-smokers of the same cohorts. Two-way ANOVA revealed a significant interaction between the type of work and smoking habits on MA excretion ($p < 0.01$). Smokers also had a higher urinary excretion of HP compared with non-smokers, both in policemen and foresters ($p < 0.05$ for policemen), but unexpectedly policemen had lower urinary levels of HP compared with foresters. Cotinine levels in the urine of smokers were similar between policemen and foresters, and were as expected significantly higher in smokers compared with non-smokers.

Determinants of urinary MA were assessed by multiple regression analysis testing as independent variables the type of work, the cotinine excretion, the time spent outdoors at work and the time spent in car at work. This analysis revealed that the type of work (partial $r^2 = 0.094$; $p = 0.0363$), the excretion of

Table 1. Levels of selected air pollutants in Brussels and the Ardennes areas for 1997–2000.

	Brussels		Ardennes areas	
	Annual means (range)	Minimum–maximum	Annual means (range)	Minimum–maximum
PM_{10} ($\mu g\ m^{-3}$)	27–40	1–178	12–14*	2–81
NO_2 ($\mu g\ m^{-3}$)	42–46	2–167	12–15	3–59
O_3 ($\mu g\ m^{-3}$)	36–40	2–227	52–61	2–184
CO ($mg\ m^{-3}$)	72–81	12–370	3–4	1–17
Benzene ($\mu g\ m^{-3}$)	17–26	1–103	3–3	1–11
SO_2 ($\mu g\ m^{-3}$)	10–16	1–94	7–7	4–60

Values are arithmetic means; minimum – maximum are the minimum and maximum of the daily averages recorded during the four years. All data were given by the respective regional annual reports except for rural PM_{10} values, which came from the *Flemish region.

Table 2. Characteristics of Brussels policemen and Ardennes foresters included in the study.

	Policemen			Foresters		
	All ($n=53$)	Non-smokers ($n=38$)	Smokers ($n=15$)	All ($n=59$)	Non-smokers ($n=35$)	Smokers ($n=24$)
Age (years)	41.7±9.5	42.3±9.4	40.2±10.1	44.5±9.8	46.4±9.8	41.8±9.3
BMI (kg m^{-2})	26.6±2.9	26.2±3.1	27.5±2.6	25.8±3.7	27.2±3.5	23.8±2.9
Pack years	–	–	17.8±11.5	–	–	19.4±9.1
Cigarettes/day	–	–	17.6±6.9	–	–	16.8±8.2
Duration of employment (years)	17.8±8.7	17.9±9.1	17.5±8.1	21.3±9.5	22.6±9.7	19.3±8.9
Time spent outdoors at work (h day^{-1})	5.2±1.8*	5.0±1.7*	5.7±2.0*	7.4±1.1	7.4±1.2	7.5±0.9
Time spent in car (h day^{-1})	3.3±2.4*	3.3±2.3*	3.1±2.5*	1.1±0.9	1.3±0.9	0.9±1.0

Values are mean±SE; BMI: body mass index; *: $p<0.05$, significantly different from foresters.

cotinine (partial $r^2=0.238$; $p<0.0001$) and the time spent in a vehicle at work (partial $r^2=0.0525$; $p=0.0467$) significantly fitted the model. When introduced into the model, these independent variables for urinary MA had a determination coefficient (r^2) of 0.4024 ($p=0.0014$). A significant correlation was found between MA and cotinine in urine for smoking policemen and foresters (log MA = 0.71 log cotinine + 0.67, $r^2=0.156$, $p=0.004$). As shown in figure 1, when policemen were classified according to their workplaces (traffic, patrol and survey), subjects working in traffic showed significantly higher MA excretion than surveying policemen (0.25±0.07 versus 0.14±0.03 mg g^{-1} creatinine respectively, $p<0.05$).

Multiple regression analysis showed that the only significant determinant for HP excretion was tobacco smoking (cotinine excretion, $p=0.0134$), explaining 20.7% of the variance of HP in urine. As expected, urinary excretion of cotinine (mg g^{-1} creatinine) was significantly higher in smokers compared with non-smokers ($p<0.0001$) although detectable concentrations of this metabolite were also found in non-smoking policemen and foresters (table 3).

Clara cell protein (CC16) and surfactant protein-A (SP-A) in serum

Table 3 shows the concentrations of CC16 and SP-A in the serum of policemen and foresters. No statistically significant difference was found between policemen and foresters for both serum biomarkers. The results confirmed that tobacco smoking indeed decreases serum CC16 levels and increases the concentrations of SP-A in smokers compared with non-smoking subjects. Multiple regression analysis concerning serum CC16 showed it was indeed significantly negatively correlated to the number of pack-years (partial $r^2=0.0837$, $p=0.0022$), whilst type of work, time spent inside a vehicle or outdoors did not significantly affect serum CC16. When policemen were classified according to their workplaces (traffic, patrol and survey), it appeared that policemen working in traffic showed lower CC16 levels (figure 1) than policemen doing other activities although this difference did not reach the level of statistical significance.

Table 3. Biomarkers of exposure, serum pneumoproteins and lung function tests in policemen and foresters.

	Policemen			Foresters		
	All (n = 53)	Non-smokers (n = 38)	Smokers (n = 15)	All (n = 59)	Non-smokers (n = 35)	Smokers (n = 24)
Muconic acid in urine (mg g^{-1} creatinine)	0.16 ± 0.04	0.13 ± 0.04	0.24 ± 0.07*†	0.10 ± 0.03	0.08 ± 0.02	0.14 ± 0.02†
Hydroxypyrene in urine (μg g^{-1} creatinine)	0.18 ± 0.05	0.13 ± 0.04	0.31 ± 0.09†	0.32 ± 0.10	0.26 ± 0.10	0.41 ± 0.10
Cotinine in urine (mg g^{-1} creatinine)	0.94 ± 0.20	0.04 ± 0.02	3.20 ± 0.6†	1.16 ± 0.24	0.02 ± 0.01	3.10 ± 0.5†
Clara cell protein in serum (μg l^{-1})	11.1 ± 0.9	11.8 ± 0.8	9.1 ± 0.9‡	11.9 ± 0.8	12.4 ± 0.8	10.9 ± 0.7‡
Surfactant-associated protein A in serum (μg l^{-1})	71.1 ± 6.9	64.2 ± 7.8	90.3 ± 13.9‡	71.4 ± 6.4	61.5 ± 6.5	84.1 ± 11.8‡
FVC (% predicted value)	97.6 ± 4.7	98.0 ± 6.1	96.5 ± 4.9	94.9 ± 4.4	95.6 ± 7.7	93.6 ± 6.1
FEV_1 (% predicted value)	98.1 ± 6.6	99.4 ± 3.9	94.7 ± 4.6	93.6 ± 3.5	96.3 ± 5.7	89.9 ± 5.3
PEF (% predicted value)	96.2 ± 3.5	98.7 ± 3.2	89.7 ± 3.7	94.2 ± 4.5	97.0 ± 3.6	92.3 ± 8.3
FEF_{25-75} (% predicted value)	106.5 ± 8.6	115.9 ± 12.0[3]	82.7 ± 4.9‡	118.7 ± 9.6	131.2 ± 8.9[3]	102.5 ± 12.8‡

Values are mean ± SE.

FVC, forced vital capacity; FEV_1, forced expiratory volume at 1 s of VC; PEF, peak expiratory flow; FEF_{25-75}, maximum expiratory flows between 25 and 75% of the forced vital capacity.

*$p < 0.1$ compared with foresters; †$p < 0.05$ compared with non-smokers; ‡$p < 0.1$ compared with non-smokers.

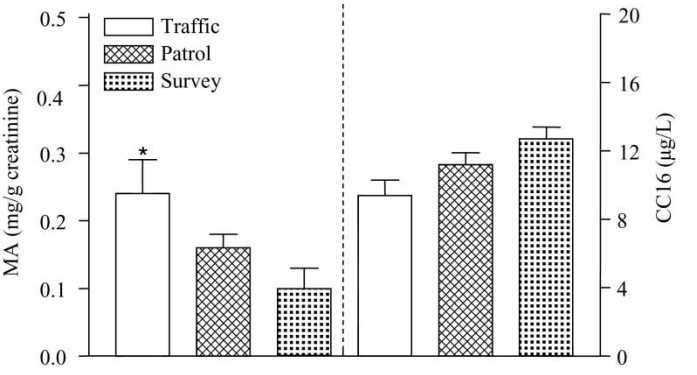

Figure 1. Concentrations of urinary muconic acid (MA) and serum Clara cell protein in policemen according to the type of work. Values are mean ± SE; *significantly different from the survey group, $p < 0.05$.

The number of pack-years significantly influenced SP-A levels in serum (partial $r^2 = 0.0542$, $p = 0.0024$) and the correlation was positive, by contrast to that with serum CC16.

Lung function tests

As expected, all lung function parameters were reduced in smokers compared with non-smokers, both in policemen and foresters, but only FEF_{25-75} decreased significantly (table 3). Lung function tests did not vary significantly in policemen classified according to their type of work. These results were confirmed by multiple regression analysis (data not shown).

Discussion

With the exception of ozone, which on average was slightly higher in the countryside than in Brussels, all other pollutants showed annual concentrations that were much higher in Brussels than in the control area. The greatest difference was observed in the mean concentrations of benzene in Brussels which were five to 10 times higher than in rural areas of Belgium. Mean benzene levels in Brussels usually range between 15 and 20 $\mu g\ m^{-3}$ but concentrations of 40–200 $\mu g\ m^{-3}$ may be found along main streets or tunnels (IBGE 1998). These values are actually more elevated than in typical European cities, where annual means of benzene in air usually range between 2.0 and 15.5 $\mu g\ m^{-3}$ for urban areas with respectively low and high traffic density, with rural background concentrations of 0.5–2 $\mu g\ m^{-3}$ (EBS 1996). This higher environmental pollution by benzene was reflected by higher levels of urinary MA in policemen than in foresters, significantly so in smokers. Some traffic policemen had urinary levels of MA that were between those typically found in bus drivers and those characterizing exposure in petrochemical industry workers (Dor *et al.* 1999). Interestingly, MA excretion was shown by multiple regression analysis to depend on the number of hours spent in cars. This association may reflect exposure to both in-vehicle environmental tobacco smoke

(Yu and Weisel 1996) and benzene, the levels of which may reach values five to 38 times higher than outdoors (Leung and Harrison 1998, Rushton and Cameron 1999). By contrast, exposure to pyrene, a constituent of PAHs, was higher in foresters than in policemen judging by the urinary excretion of 1-hydroxypyrene. This unexpected observation could be explained by differences in heating systems since 60% of foresters reported wood burning as primary or secondary heating system compared with only 5% in policemen (Dor *et al.* 1999). However, multiple regression analysis did not permit to identify determinants of 1-hydroxypyren excretion other than tobacco smoking (Siwinska *et al.* 1999).

As expected, tobacco smoking emerged as the main determinant of lung epithelium integrity. Smokers had significantly decreased serum CC16 levels compared with non-smokers, mirroring a progressive destruction of Clara cells due to tobacco smoking with an ensuing reduced secretion of the protein in bronchoalveolar lavage fluid (BALF) (Bernard *et al.* 1992, Hermans and Bernard 1996, Shijubo *et al.* 1997, Andersson *et al.* 2000). By contrast, serum levels of SP-A increased in smokers reflecting most likely an increased permeability of the lung epithelium as reported in previous studies (Jones *et al.* 1980, Robin *et al.* 2002). These effects of tobacco smoke were found both in foresters and policemen. Although the numbers of subjects examined were too low to reach statistical significance, the effects tended to be more pronounced in policemen than in foresters, especially in policemen who despite similar cigarette consumption showed on average the lowest values of serum CC16 and the highest values of SP-A (table 3). The possibility of an exacerbated effect of smoking on the distal airways in case of simultaneous traffic air pollution exposure is supported by the FEF_{25-75} results, which were significantly lower in smoking policemen compared with smoking foresters. Non-smoking policemen also showed a trend towards having lower serum CC16 values with increasing exposure to traffic air pollutants, which is consistent with the hypothesis of traffic-related distal airway damage additive to that of tobacco smoke. However, whether these changes can be extrapolated to the general population, which is not necessarily occupationally exposed to air pollutants remains uncertain. Recently, indeed, we examined the lung parameters of schoolchildren living in Brussels by applying the same protocol as in the present study (Bernard *et al.* 2003) and compared them with those of children living in the Ardennes rural areas. We found no statistically significant differences in the urban and rural schoolchildren's lung function, Clara cell protein and surfactant-associated proteins A and B levels. Extending these studies to a European level, we again found no significant difference in lung parameters of children living in areas with different levels of pollution by ambient ozone (North of Sweden, Brussels and Northern Italy) (European HELIOS project, to be published).

In conclusion, although observed differences in biomarkers did not always reach statistical significance because of the low numbers of subjects examined, the overall results of the present study confirm the potential of biomarkers to assess exposure to urban air pollutants and to detect their possible impact on the respiratory epithelium of subjects like policemen who are particularly at risk.

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