Serum levels of club cell secretory protein (Clara) and short- and long-term exposure to particulate air pollution in adolescents

Eline B. Provost a,b, Agnès Chaumont c, Michal Kicinski a, Bianca Cox a, Frans Fierens d, Alfred Bernard c, Tim S. Nawrot b,c,*

a Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium
b Environmental Risk and Health, Flemish Institute for Technological Research (VITO), Mol, Belgium
c Louvain Centre for Toxicology and Applied Pharmacology, Université catholique de Louvain, Brussels, Belgium
d Belgian Interregional Environment Agency, Brussels, Belgium

Abstract

Background: Studies in populations have shown that particulate air pollution is associated with changes in lung function in adolescents.

Objective: We investigated the effect of short- and long-term exposure to particulate matter (PM10) on the pulmonary health of adolescents, using serum lung club cell secretory protein (Clara) (CC16) as a biomarker for respiratory epithelium integrity.

Methods: We measured serum CC16 in 825 adolescents (57% girls, mean age: 15 years). Short-term and long-term exposure to ambient PM10 was estimated for each participant’s home address using a kriging interpolation method. To explore the association between PM10 and serum CC16 we applied restricted cubic splines with 5 knots located at the 5th, 25th, 50th, 75th and 95th percentiles of the PM10 distribution. The explorative analyses showed a change in the slope of this association, after which a change-point analysis was performed.

Results: After adjustment for potential covariates, the analysis showed strong associations between PM10 concentrations, averaged over the week preceding the clinical examination, and serum CC16 levels. Each 5 μg/m3 increase in mean PM10 concentration in the week before the clinical examination was associated with a substantial increase of 0.52 μg/l (95% confidence interval: 0.31 to 0.73; p < 0.0001) in serum CC16 levels. The association appears nonlinear with a flattening out of the slope at mean week PM10 levels above 37 μg/m3. There was no evidence of an association between long-term exposure to PM10 and serum CC16 concentrations.

Conclusions: Our findings suggest that short-term exposure to particulate air pollution may compromise the integrity of the lung epithelium and lead to increased epithelial barrier permeability in the lungs of adolescents, even at low concentrations.

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1. Introduction

Numerous studies have demonstrated that short-term exposure to elevated levels of air pollution has detrimental effects on human health. Most of these studies detected positive associations between particulate air pollution (PM10 or PM2.5, i.e., particulate matter with an aerodynamic diameter less than 10 μm and 2.5 μm respectively) and general mortality, or the triggering of acute cardiovascular events (Nawrot et al., 2011), especially in the elderly and people with pre-existing cardiovascular and respiratory conditions (Pope, 2000; Zanobetti and Schwartz, 2005). Young people are very vulnerable to many noxious agents and their protection is an important public health challenge (Staessen et al., 2001). Recently it has been shown that, even at relatively low levels, particulate air pollution triggers mortality in susceptible newborns (Scheers et al., 2011). Lung function in children and adolescents is inversely associated with particulate air pollution (Gauderman et al., 2007; Kulklarni et al., 2006).

Club cell secretory protein (CC16), formerly known as Clara cell protein (Winckelmann and Noack, 2010), is a 16 kDa lung epithelium-specific protein. CC16 is secreted in the respiratory tract by club cells (Clara), known for their vulnerability to toxic responses. CC16 serves as an anti-oxidant and anti-inflammatory role in the lung lining fluid (Madsen et al., 2008). Human and experimental studies have however shown that CC16 can also appear in extrapulmonary fluids such as serum and urine (Broeckaert et al., 2000b; Hermans et al., 1999). Serum CC16 is therefore used as a biomarker for the integrity of the pulmonary epithelium and lung epithelial permeability. Increases in serum levels of CC16 have been observed after diverse environmental...
exposures, such as trichloroamine among regular attendees of chlorinated indoor swimming pools (Lagerkvist et al., 2004), ambient ozone (Broeckaert et al., 2000a) and particulate air pollution in elderly men (Madsen et al., 2008).

Children and adolescents are considered to be more susceptible to the effects of air pollution than adults (Braga et al., 2001; Janssen et al., 2012; Scheers et al., 2011). Not only are they exposed during a critical developmental period, but children also differ from adults in physiological characteristics and exposure patterns. The airway epithelium of growing children is more permeable to air pollutants and the lung defenses against particulate and gaseous air pollution are not fully evolved (Salvi, 2007; Schwartz, 2004). It is the aim of the present study to determine whether lung permeability, as exemplified by serum CC16, is related to short-term and long-term exposure to PM10 in adolescents.

2. Material and methods

In order to investigate the association between serum levels of CC16 and exposure to particulate air pollution, a cross-sectional study design was used. Recruited adolescents were clinically examined once between March and May of 2006.

2.1. Study population

870 adolescents were recruited in three secondary schools in the southern part of Belgium, in the cities of Bastogne, Louvain-la-Neuve and Lessines. 45 participants (5%) were excluded. 14 of them had their home address in the Grand Duchy of Luxembourg, 16 were over 18 years and 15 participants had no serum CC16 measurements. The study protocol was approved by the Ethics Committee of the Faculty of Medicine of the Université catholique de Louvain (Brussels, Belgium). A questionnaire and a written agreement to participate in the study were obtained from the adolescents’ parents. The questionnaire addressed aspects related to social and medical characteristics of the adolescent and their family, and to the in-house and out-of-house environment including current smoking status of the parents. During the clinical examination, participating adolescents were inquired about their smoking habits.

2.2. Health measurements

Adolescents participating in the study were examined in their school. The clinical examination took place between 9.00 h and 16.00 h and included the drawing of a blood sample. Time of the sample collection was recorded in order to adjust for possible diurnal variations in the biomarker levels. The concentration of serum CC16 was determined by a sensitive immunoassay relying on the agglutination of latex particles coated with polyclonal antCCI6 antibodies. The agglutination is quantified by counting residual unagglutinated latex particles with a Technicon Autocounter. A detailed description of this particle counting-based latex immunoassay has been previously published in its application to urinary CC16 (Bernard et al., 1991) and the accuracy was confirmed by comparison with monoclonal anti-body based ELISA (Hermans et al., 1998a). The assay uses the rabbit antiprotein 1 antibody and standard from Dakopatts (Glostrup, Denmark). To avoid possible interferences by the complement, rheumatoid factor or chlormelonic, sera were pretreated by heating at 56 °C for 30 min and by the addition of polyethylene glycol (16%, v/v, 1:1) and trichloroacetic acid (10%, v/v, 1:40). After overnight precipitation at 4 °C, the samples were centrifuged (3000 g × 10 min) and CC16 was determined in the supernatants. The limit of detection was 0.5 μg/l and the average analytical recovery 95%. The within- and between-run coefficients of variation ranged from 5 to 10% (Hermans et al., 1998b).

2.3. Ambient air pollution exposure

Ambient air pollution concentrations were estimated for each participant’s home address using a kriging interpolation method (Jacobs et al., 2010; Janssen et al., 2008). This model provides interpolated PM10, NO2 and 8-hour maximum ozone values from the Belgian telemetric air quality network in 4 × 4 km grids. The interpolation is based on a detrended kriging interpolation model that uses land cover data obtained from satellite images in combination with monitoring stations (European Environment Agency, 2000). The ambient air pollution was estimated at lags 0 to 6 days before the clinical examination took place (short-term) as well as annual mean values for 2005 and 2006 (long-term). The Belgian Royal Meteorological Institute provided data on temperature and relative humidity, which were used to calculate apparent temperature values at lags 0 to 6 days before the clinical examination.

2.4. Statistical methods

We used SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA) for statistical analyses. Non-normally distributed variables were logtransformed. We adjusted the multiple regression models for an a priori chosen list of covariates including sex, age, apparent temperature, hour of clinical examination, smoking status, swim index (cumulative chlorinated pool attendance) and serum creatinine.

To explore the association between serum CC16 and PM10, and to allow nonlinearity, we applied restricted cubic splines with 5 knots located at the 5th, 25th, 50th, 75th and 95th percentiles of the PM10 distribution (Harrell, 2001). These explorative analyses showed a change in the slope of the association between serum CC16 and PM10. Therefore, nonlinear associations were modeled with a change-point analysis, where it is assumed that the slope might change at a certain concentration (Steenland and Deddens, 2004). The associations were fitted using the nonlinear regression PROC NLIN procedure in SAS and the change-point was estimated by the model (Pope et al., 2011). The effect on serum CC16 levels of a 5 μg/m3 increase in ambient PM10 concentrations at the day of the clinical examination (lag 0), the day before the clinical examination (lag 1) and 6 days previous to the clinical examination (mean lag 0–6) was estimated. Similarly, we investigated the association between serum CC16 and ambient NO2. We estimated the effect on serum CC16 levels of 5 μg/m3 increases of ambient NO2 at the day of the clinical examination (lag 0), the day before the clinical examination (lag 1) and 6 days previous to the clinical examination (mean lag 0–6).

3. Results

The study population included 825 adolescents (56.61% girls) aged 13–18 years of whom the characteristics are listed in Table 1. Serum CC16 averaged (SD) 9.20 μg/l (3.70). Serum CC16 was positively associated with age (r = 0.12, p = 0.0005), serum creatinine (r = 0.16, p < 0.0001), apparent temperature (r = 0.08, p = 0.0182) and inversely associated with the swim index (r = −0.11, p = 0.0015). Additional to these significant predictors, sex, hour of clinical examination and smoking status were forced in future adjusted analyses for serum CC16.

The analysis showed that the effect on serum CC16 levels of an increase in average PM10 concentrations during the week preceding the clinical examination (mean lag 0–6) is most pronounced for relatively low PM10 concentrations (Fig. 1 and 2). Each 5 μg/m3 increase in mean PM10 concentration in the week before clinical examination was associated with a substantial increase of 0.52 μg/l (95% CI: 0.31 to 0.73) in serum CC16 levels (Table 2). The association appears nonlinear, with a significant change in slope (p = 0.0012) above a mean week PM10 concentration of 37.0 μg/m3, after which the association reaches a plateau. A similar pattern was observed for PM10 concentrations the day before the clinical examination (lag 1). Serum CC16 levels were significantly and
positively associated with PM10 levels the day before the clinical examination, where a plateau effect is reached at PM10 levels above 50.5 μg/m³ (p-value for slope change = 0.0047).

The shape of the association, as seen with PM10, e.g. steep rise at low concentration and plateau effect at higher concentrations, was different for ambient NO2 (Fig. 3). The average NO2 concentration in the week preceding the clinical examination (mean lag 0–6) was significantly associated with serum CC16 levels, but NO2 concentrations higher than 39.59 μg/m³ resulted in a more substantial increase in serum CC16 levels (Table 3). In multiple exposure–response models, we adjusted the effect of PM10 concentrations for ambient NO2 levels and vice versa. The multiple exposure models showed similarly significant effects on serum CC16 levels for PM10 while the association between serum CC16 and NO2 did not reach statistical significance (data not shown).

In further analyses, we added long-term exposure to PM10 along with the same covariates mentioned previously and the mean week averaged PM10 before clinical examination. These analyses showed no significant associations between serum CC16 and long-term PM exposure [−0.16 μg/l per 5 μg/m³ increase in PM10 (95% CI − 0.50 to 0.18, \(p = 0.35\)]. Long-term exposure to NO2 was also not significantly associated with serum CC16 levels [0.11 μg/l per 5 μg/m³ increase in NO2 (95% CI − 0.05 to 0.32)]. We observed no significant associations between serum CC16 levels and 8-hour maximum ozone concentrations, either on the day of the clinical examination (lag 0; \(p = 0.64\)) or averaged over the week before (mean lag 0–6; \(p = 0.42\)). Corresponding effect sizes for a 5 μg/m³ increase in 8-hour maximum ozone concentration were 0.02 μg/l (95% CI − 0.05 to 0.09) and 0.09 μg/l (95% CI − 0.13 to 0.32), respectively.

4. Discussion

The key finding of our study is that serum CC16, a marker of lung permeability, is positively associated with short-term exposure to PM10 among adolescents aged 13–18 years, even at low concentrations of ambient PM10. This association could not be explained by other potential covariates or confounding factors such as age, smoking, apparent temperature or trichloroamine exposure from swimming pools, as represented by the swim index.

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Table 1
Description of the study population.

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>825</td>
</tr>
<tr>
<td>Anthropometrics</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>15.43 ± 0.77</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>467 (56.61)</td>
</tr>
<tr>
<td>Lifestyle</td>
<td></td>
</tr>
<tr>
<td>Current smoking participants</td>
<td>48 (5.87)</td>
</tr>
<tr>
<td>Environmental tobacco smoke exposure</td>
<td>274 (33.33)</td>
</tr>
<tr>
<td>Swim index (cumulative chlorinated</td>
<td>389 (229–811)</td>
</tr>
<tr>
<td>attendance in hours</td>
<td></td>
</tr>
<tr>
<td>Biologic markers</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.90 ± 0.15</td>
</tr>
<tr>
<td>Serum club cell secretory protein (Clara) 16 (μg/l)</td>
<td>9.20 ± 3.70</td>
</tr>
<tr>
<td>Ambient pollutants</td>
<td></td>
</tr>
<tr>
<td>PM10 (μg/m³)</td>
<td></td>
</tr>
<tr>
<td>Short term (lag 0)</td>
<td>30.62 ± 17.04</td>
</tr>
<tr>
<td>Short term (mean lag 0–6)</td>
<td>32.61 ± 13.35</td>
</tr>
<tr>
<td>Long term (annual mean 2005–2006)</td>
<td>27.52 ± 5.55</td>
</tr>
<tr>
<td>Ozone (μg/m³)</td>
<td></td>
</tr>
<tr>
<td>Short term (lag 0)</td>
<td>82.08 ± 21.82</td>
</tr>
<tr>
<td>Short term (mean lag 0–6)</td>
<td>82.47 ± 18.25</td>
</tr>
<tr>
<td>NO2 (μg/m³)</td>
<td></td>
</tr>
<tr>
<td>Short term (lag 0)</td>
<td>21.25 ± 10.74</td>
</tr>
<tr>
<td>Short term (mean lag 0–6)</td>
<td>21.34 ± 7.84</td>
</tr>
</tbody>
</table>

Values are number (%) or mean ± SD except for swim index, which was not normally distributed, for which arithmetic mean (25%–75% quartiles) is given.
Interestingly, the association between serum CC16 and PM10 appears to increase steeply at low concentrations, but reaches a plateau (no further increases) at higher PM10 concentrations. This plateau effect is independent of NO2, probably due to the fact that the used interpolation model provides exposure data in 4 by 4 km grids and therefore small-scale traffic impacts affecting NO2 concentration may not be reflected accurately. Research has shown that relatively low levels of fine particulate exposure, from either air pollution or secondhand cigarette smoke, are sufficient to induce adverse biologic responses increasing the risk of cardiovascular disease mortality and that the exposure–response relationship is relatively steep at low levels of exposure and flattens out at higher exposures (Pope et al., 2009). Numerous theories have been postulated regarding the underlying mechanism leading to the nonlinear shape including exposure measurement error at higher exposure levels and biologic saturation (Lewtas et al., 1997; Vineis et al., 2000). Within the ULTRA-study, Timonen et al. (2004) found a short-term effect on urinary CC16 levels in the study population of Helsinki (Finland), where the exposure to particulate air pollution is low compared to the study populations of Erfurt (Germany) or Amsterdam (The Netherlands), where they did not find an association with urinary CC16. These observations strengthen our findings that there is a strong short-term effect on serum CC16 at low concentrations of ambient PM10, whereas the effect diminishes at higher levels of particulate air pollution.

The concentration of serum CC16 is a well-validated marker of the lung epithelium barrier integrity, reflecting either the number of club cells (Clara) lining the terminal airways or the permeability of the alveolar–capillary barrier (Hermans and Bernard, 1999, 1999). Exposure to tobacco smoke (Bernard et al., 1994; Robin et al., 2002), indoor wood fuel use (Barregard et al., 2008; Van Miert et al., 2012) and chronic exposure to particulate air pollution in policemen (Berthoin et al., 2004) are associated with decreases in CC16. In line with previous evidence in elderly (Madsen et al., 2008), we did not find an effect of chronic exposure to ambient air pollution but observed an increase in serum CC16 in association with short-term exposure to particulate air pollution. The magnitude of change in our study for a short-term increase in PM10 was comparable to estimates of serum CC16 in elderly (Madsen et al., 2008) and with changes in urinary concentration in one of the three studied populations (including persons with respiratory ailments) of the ULTRA-study (Timonen et al., 2004). Whereas a decrease of serum CC16 could reflect a decreased production of the club cells (Clara) due to irritation/cytotoxicity, the driving mechanism in the case of short-term exposures is most likely lung hyperpermeability and not a reduced production of CC16 (Van Miert et al., 2012).

In cyclists, increased serum levels of CC16 have been reported in association with 2 h of exercise during an ozone episode (Broeckaert et al., 2000). However, in our study, 8-hour maximum ozone concentrations appeared not to be significant, which can be explained by the fact that our study was not performed during summer and ozone levels were relatively low. Exposure to trichloroamine via chlorinated indoor swimming pools is also known to be associated with serum CC16 levels (Carboneille et al., 2002; Font-Ribera et al., 2010; Lagerkvist et al., 2004). We adjusted our models accordingly via the swim index, which represents the cumulative hours of chlorinated pool attendance. Weather-related differences over the seasons and over days may both be associated with air pollution and serum concentrations of CC16. Therefore, we adjusted our models for apparent temperature. In order to account for possible diurnal variations in serum CC16 levels, models were adjusted for hour of the clinical examination.

A limitation of this cross-sectional study is that it can only suggest an association between serum concentrations of CC16 and PM10 exposure since observational studies do not prove causality. Due to lack of personal exposure measurements, we used interpolated particulate air pollution data. Nevertheless, our previous studies have validated the use of these exposure estimates (Jacobs et al., 2010; Janssen et al., 2008).

![Fig. 3](image-url) Association between serum club cell secretory protein (Clara) (CC16) and (A) NO2 moving averages at lags 0 to 6 and (B) NO2 at lags 0 to 6. The associations were modeled with restricted cubic splines with 5 knots located at the 5th, 25th, 50th, 75th and 95th percentiles.
Table 3

<table>
<thead>
<tr>
<th>Day of clinical examination (lag 0)</th>
<th>Change in serum CC16 (µg/l) before change-point</th>
<th>Day before clinical examination (lag 1)</th>
<th>Change-point NO2 concentration (µg/m³)</th>
<th>Week before clinical examination (mean 0-6)</th>
<th>Change in serum CC16 (µg/l) after change-point</th>
<th>p-Value for change in slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>(0.06 to 0.39)</td>
<td>0.39</td>
<td>(0.17 to 0.62)*</td>
<td>0.56</td>
<td>(0.29 to 0.82)*</td>
<td>0.0393</td>
</tr>
<tr>
<td>35.24</td>
<td>(26.60 to 48.88)</td>
<td>33.20</td>
<td>(13.00 to 53.40)</td>
<td>39.59</td>
<td>(25.48 to 53.70)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Estimates are given for a 5 µg/m³ increase in NO2 concentration with 95% confidence intervals. The analyses were adjusted for age, sex, apparent temperature, hour of clinical examination, smoking status, swim index (cumulative chlorinated pool attendance in hours) and serum creatinine.

5. Conclusions

The present study supports the evidence that short-term exposure to particulate air pollution may compromise the integrity of the lung epithelium and lead to increased epithelial barrier permeability in the lungs of adolescents, even at low concentrations.

Acknowledgments

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