Respiratory Effects Associated With Wood Fuel Use: A Cross-Sectional Biomarker Study Among Adolescents

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Summary. The use of wood as heating and cooking fuel can result in elevated levels of indoor air pollution, but to what extent this is related to respiratory diseases and allergies is still inconclusive. Here, we report a cross-sectional study among 744 school adolescents (median age 15 years) using as main outcomes respiratory symptoms and diseases, exhaled nitric oxide, total and aeroallergen-specific IgE in serum, and two epithelial biomarkers in nasal lavage fluid (NALF) or serum, that is, Clara cell protein (CC16) and surfactant-associated protein D (SPD). Information about the wood fuel use and potential confounders was collected via a personal interview of the adolescent and a questionnaire filled out by the parents. Two approaches were used to limit the possible influence of confounders, that is, multivariate analysis using the complete study population or pairwise analysis of matched sub-populations obtained using an automated procedure. Wood fuel use was associated with a decrease of CC16 and an increase of SPD in serum, which resulted in a decreased serum CC16/SPD ratio (median −9%, $P = 0.001$). No consistent differences were observed for the biomarkers measured in exhaled breath or NALF. Wood fuel use was also associated with increased odds for asthma [odds ratio (OR) 2.2, 95% CI: 1.1–4.4, $P = 0.02$], hay fever (OR = 2.4, 95% CI: 1.4–4.3, $P = 0.002$), and sensitization against pollen allergens (OR = 2.1, 95% CI: 1.3–3.4, $P = 0.002$). The risks of respiratory tract infections, self-reported symptoms, and sensitization against house-dust mite were not increased by wood fuel use. The increased risks of asthma, hay fever and aeroallergen sensitization, and the changes of lung-specific biomarkers consistently pointed towards respiratory effects associated with the use of wood fuel. **Pediatr Pulmonol.**

Key words: indoor pollution; lung biomarkers; respiratory disease; sensitization; wood fuel use; wood smoke.

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INTRODUCTION

Evidence is increasing that indoor pollution plays an important role in the development of respiratory diseases, especially during childhood.[1] A number of risk factors have already been identified, such as volatile organic compounds,[2] ultra-fine particles,[3] house dust allergens,[4] environmental tobacco smoke,[5] biomass burning smoke,[6] and chlorination products in swimming pools.[7] The use of wood as heating and cooking fuel can result in elevated levels of indoor air pollution and is considered to be an important public health problem in the developing world.[8] Illustrative for the currently increased attention to wood smoke are the recent classification by the International Agency for Research on Cancer of indoor emissions from biomass fuel (primarily wood) as a probable human carcinogen (group 2A) and the recent report by Hosgood et al. confirming the positive association between in-home wood use and the lung cancer risk.[9,10] Epton et al.[11] did not detect a significant effect of ambient wood smoke particulate air pollution on lung function of healthy school-aged students but they observed associations with respiratory symptoms. In a recent review, Po et al.[12] reported significant associations between solid biomass fuel exposure and the risk of acute respiratory infection in children [odds ratio (OR) 3.53, 95% CI: 1.94–6.43] but these authors found no association with asthma. Barry et al.[13] reported that cooking indoors with wood and coal for more than 6 months significantly increased the

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odds of current asthma (OR = 2.3, 95% CI: 1.1–5.0), whereas no effect was seen with history of heating indoors with wood and coal (OR = 0.8, 95% CI: 0.4–1.8). In another recent study, Nguyen et al.[14] reported a statistically significant negative association between asthma and the use of a wood-burning stove. Maier et al.[15] did not observe an association between the use of a wood stove and asthma or wheezing. On the other hand, in the experimental study by Samuelsen et al.,[16] wood smoke exhibited an adjuvant-like activity in allergic sensitization.

In recent years, there have been significant advances in the development of non- or less-invasive tools to monitor airways damage or inflammation. The amount of data supporting nitric oxide (NO) in exhaled air as a non-invasive marker of airways inflammation in asthma and allergic diseases has been steadily growing.[17] Also, Clara cell-specific protein (CC16) and surfactant protein D (SPD), which is mainly produced by the type II pneumocytes, have been shown to be sensitive biomarkers of epithelial damage in lower airways.[18] The measurement of biomarkers in nasal lavage fluid (NALF) is another non-invasive approach, which is receiving increased interest because it allows to detect nasal epithelial changes relevant to the deeper lung.[19] Barregard et al.[20] were among the first to use such biomarkers to assess the respiratory effects of wood smoke. In a controlled human exposure study, these authors showed that a single exposure to wood smoke causes increases of NO in exhaled air and of CC16 in serum. The increased level of NO is indicative of an inflammation at the distal part of the airways, while the increased CC16 concentration in serum is the reflection of an increased permeability of the air–blood barrier.

Using the large database which has been generated by our laboratory during a cross-sectional survey of more than 800 adolescents, we investigated whether wood fuel use was associated with respiratory diseases, sensitization to aeroallergens, self-reported symptoms, or changes of epithelial biomarkers in serum, exhaled breath and NALF.

ABBREVIATIONS:

CC16	Clara cell protein CC16
CI	confidence interval
FEV_1	forced expiratory volume in 1 sec
FVC	forced vital capacity
IgE	immunoglobulin E
NALF	nasal lavage fluid
NO	nitric oxide
OR	odds ratio
SPD	surfactant protein D

METHODS

Study Population

The database on adolescents was generated according to a study protocol which was approved by the Ethics committee of the Faculty of Medicine of the Catholic University of Louvain and which complied with all applicable requirements of international regulations. The adolescents attended school in the cities of Louvain-la-Neuve, Bastogne or Lessines, 3 small-size (<30,000 inhabitants) cities in the French speaking part of Belgium. The first 2 cities are located in rural regions, whereas the third one is part of a larger urban region. One school per city was selected, and of the 1,200 students (3rd or 4th grade of secondary school) who were contacted, 1,137 (94.8%) returned the questionnaire. Among them, 847 had written the agreement from their parents to participate in the study, resulting in an overall participation rate of 71.4%. As active smoking could act as a major confounder, all students who had declared during the personal interview to be active smokers were excluded from the study population. When excluding the active smokers (n = 84) and the adolescents lacking a properly filled-out questionnaire (n = 19), a total of 744 adolescents were retained for further analysis.

Questionnaire

The adolescents' parents were asked to complete a questionnaire about the personal or familial characteristics, the in-house and out-house environment, medical characteristics and antecedents, and self-reported symptoms. The information about the personal and familial characteristics comprised gender, ethnicity (not included in further analyses because only 3% were of non-Caucasian origin), age, birth weight, smoking status of mother during pregnancy, a parent with allergy or asthma, having older siblings, day care attendance, and having a parent with bachelor level education or higher (as a marker for social status). Investigated characteristics of the out-house environment where living in a rural area and the cumulated attendance to chlorinated pools. The in-house environment was characterized by living in a house built after 1950, having a furred pet, low ventilation of the bedroom (one or less times per week), presence of molds on walls, the use of bleach as cleaning agent, smoking parents (i.e., being exposed to environmental tobacco smoke), use of air fresheners, and having a heating system with wood (e.g., fireplace, stove). Medical characteristics were doctor-diagnosed asthma, hay fever (seasonal allergic rhinitis), and allergic rhinitis (non-seasonal allergic rhinitis). Information about medical antecedents concerned the past occurrence of lower respiratory tract

infections (pneumonia, bronchitis, bronchiolitis) and frequent colds (>4×/year).

Examination of Students

The adolescents participating in the study were examined in their school between March 7 and May 15, 2006. Examinations took place between 09:00 and 15:00. For each test or sample collection, the time was recorded to adjust for the possible diurnal variations in the biomarker levels. The examination started with an interview inquiring about the respiratory symptoms during the past 12 months (wheezing, cough crisis, chest tightness, and shortness of breath), the smoking habits, and the medication consumption. Additionally, the answers to the main questions of the questionnaire, such as those relating to the characteristics of the home environment, were reviewed with the study participant. In case of contradictions, the answer obtained during the interview outweighed the answer from the questionnaire. Next, the adolescents underwent a series of tests including the measurement of height and weight, NO in exhaled breath and lung function, and the collection of blood and NALF samples. The forced expiratory volume in 1 sec (FEV_1), and the forced vital capacity (FVC) were measured with a Medikro spirometer (Spiro2000 v1.7.3, Medikro OY, Finland) according to the American Thoracic Society Standards.[21] The concentration of NO in exhaled air was determined with the NIOX^{TM} analyzer (Aerocrine AB, Solna, Sweden) according to the guidelines of the American Thoracic Society.[22] The sample of venous blood was collected on a dry tube, allowed to clot overnight at 4°C, and then centrifuged at 2,000*g* for 10 min. Serum was decanted and stored at −18°C until biomarker analysis. NALF samples were collected from both nostrils. Participants were asked to sit down, bend forward, and put their heads down. Two and a half milliliters of sterile physiological saline at 37°C were instilled into each nostril by a disposable tip connected to a peristaltic pump. After 10 sec, students were asked to lift their head and the lavage fluid was collected using a small funnel. The NALF samples were stored at −20°C until evaluation.

Analyses

Total and aeroallergen-specific IgE concentrations in serum were determined using the Immulite® IgE kit (Diagnostic Products Company, Los Angeles, CA). The presence of IgE against the following allergens was verified: house-dust mite, cat epithelium, dog dander, molds, tree pollen, grass pollen, and herbaceous pollen mixture (weeds). Sensitization against specific aeroallergens was defined as a serum concentration of specific IgE >0.35 kIU/L. The CC16 concentration in serum and NALF was determined by latex immunoassay using a rabbit anti-CC16 antibody (Dakopatts, Glostrup, Denmark) and CC16 standards purified in our laboratory.[23] The serum concentration of SPD was measured using the commercially available SPD EIA kit (Code No YSE-7744; Yamasa Corporation, Chiba, Japan). Results from the NALF samples were available for a total of 419 adolescents. Care was taken to include only the results from adolescents in the study who did not had a cold 2 weeks prior to the sample collection as this could possibly bias the interpretation. Concentrations of biomarkers in NALF were adjusted for the variable dilution of the recovered epithelial lining fluid either by calculating the absolute amount of recovered protein or by adjusting the concentration in NALF with the plasma/NALF concentration ratio of urea.[24,25] These adjustments were made for each nostril separately and then the mean value was calculated and used for the statistical analyses.

Statistical Analyses

Bivariate and multivariate analyses were performed using R.[26–28] Graphs and statistics on adjusted concentrations were generated using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, www.graphpad.com). Statistical procedures on continuous variables were performed after log-transformation. The threshold for statistical significance was set at $P < 0.05$. Comparisons of continuous variables between the non-exposed and exposed adolescents were performed using the *t*-test. To eliminate the influence of confounding factors, two approaches were used, that is, matching prior to statistical testing and multivariate analyses. In the first approach, pairwise statistical tests were performed after matching of adolescents exposed to wood (smoke, n = 226) to a comparable population of non-exposed adolescents (n = 226) by means of the automated matching procedure "optmatch[29]" using the characteristics: "gender," "age," "parental allergy or asthma," "parental higher education," "living in a rural area," and "living in a house built after 1950." Multivariate analyses were performed on the total study population (744 adolescents). Adjusted OR were obtained by means of the generalized linear modeling using the binomial distribution, whereas multivariate models for biomarkers in NALF, blood and exhaled air were built via the same approach but using the Gaussian distribution. Initial models were defined associating each biomarker with wood fuel use and with age, gender, body mass index (BMI), birth weight, total serum IgE concentration (except for sensitization outcomes), time of sample collection (biomarkers only), the social and medical characteristics, and the variables reflecting the in- and out-house environment quality.

For serum markers, also the serum creatinine concentration was included in the initial model to account for the renal function. The obtained models were optimized using a backward stepwise algorithm using the Akaike information criterion (AIC, R-function: ''step'').

RESULTS

The characteristics of the sub-populations with or without matching are shown in Table 1. When comparing the unmatched sub-populations, adolescents living in a house heated with wood did not differ from their peers with respect to gender, age, older siblings, parental history of asthma, and allergy and chlorinated pool attendance. The group had a lower socio-economic status as reflected by the parental education and fewer had attended a day care center during infancy. Not surprisingly, houses heated with wood were more often old houses localized in a rural area. When comparing the matched sub-populations, none of the above-mentioned significant differences was present anymore.

Table 2 displays the respiratory health characteristics of adolescents with or without wood fuel use. The self-reported symptoms, allergic rhinitis, and the respiratory tract infections did not differ meaningfully between the two groups, irrespective whether the sub-populations were matched or not. Multivariate analysis confirmed the above-mentioned absence of findings. Wood fuel use was not retained in any of the optimized models with a P-value <0.05. On the other hand, the wood fuel use group showed increased prevalence of asthma and hay fever. Atopic asthma, that is, doctor-diagnosed asthma with a concurrent aeroallergen sensitization, was consistently associated with wood fuel use. The OR for non-atopic asthma, that is, doctor-diagnosed asthma without concurrent aeroallergen sensitization, was similar to that of atopic asthma, but statistical significance was not reached. Adolescents living in a house with a heating system with wood consistently showed higher frequencies of sensitization against pollen (sensitization against at least one of the pollen antigens tested) and against pollen from weeds, trees, and grass, but not against house dust mite or all aeroallergens (sensitization against at least one of the tested allergens).

The results of the lung function measurements and determinations of biomarkers in exhaled air, NALF, and serum are provided in Table 3. Wood fuel use was not associated with changes in lung function measures or in the levels of exhaled NO. There were also no differences in the levels of CC16 or urea in NALF. Pairwise analyses on the entire study population showed that albumin in NALF was significantly higher in the group with wood fuel use. Multivariate analysis confirmed

TABLE 1— Characterization of the Sub-Populations With or Without Wood Fuel Use

	Wood fuel use all study subjects			Wood fuel use matched study subjects		
	No	Yes		No	Yes	
	n = 514	n = 230	P-value	n = 226	n = 226	P-value
Personal/family characteristics						
Female gender, n (%)	289 (56)	124 (54)	0.56	125 (55)	122 (54)	0.78
Age, median (IQR, years)	15.3 (14.8–16.0)	15.2 (14.7–15.8)	0.12	15.2 (14.7–15.8)	15.2 (14.7–15.8)	0.61
BMI, median (IQR), kg/m^2	20.2 (18.7–22.2)	19.8 (18.5–21.9)	0.19	20.2 (18.7–22.5)	19.8 (18.5–22.0)	0.31
Birth weight, median (IQR), kg	3.32 (3.00–3.60)	3.32 (3.00–3.70)	0.77	3.32 (3.00–3.62)	3.32 (3.00–3.70)	0.68
Parental allergy or asthma, n (%)	229 (45)	96 (42)	0.48	95 (42)	94 (42)	0.92
High parental education, n (%)	388 (75)	154 (67)	0.02	155 (69)	151 (67)	0.69
Maternal smoking during pregnancy, n (%)	62 (12)	22 (10)	0.32	29 (13)	22 (10)	0.30
Day care attendance, n (%)	258 (50)	91 (40)	0.01	108 (48)	89 (39)	0.07
Having older siblings, n (%)	298 (58)	133 (58)	0.97	122 (54)	129 (57)	0.51
Environment						
House in rural area, n (%)	332 (65)	206 (90)	2.E−12	204 (90)	204 (90)	1.00
Chlorinated pool attendance (>500 hr), n (%)	211 (41)	111 (48)	0.07	92 (41)	108 (48)	0.13
House built after 1950, n (%)	337 (66)	134 (59)	0.04	131 (58)	133 (59)	0.85
Having a furred pet, n (%)	384 (75)	180 (78)	0.30	180 (80)	177 (78)	0.73
Low room ventilation, n (%)	98 (19)	32 (14)	0.09	46 (20)	31 (14)	0.06
Molds on walls at home, n (%)	35 (7)	13 (6)	0.55	16 (7)	12 (5)	0.44
Cleaning with bleach, n (%)	141 (27)	50 (22)	0.10	58 (26)	49 (22)	0.32
Use of air fresheners, n (%)	133 (26)	56 (24)	0.66	56 (25)	55 (24)	0.91
Environmental tobacco smoke, n (%)	182 (35)	69 (30)	0.15	84 (37)	67 (30)	0.09

Data are provided for the sub-populations comprising all study subjects and for the matched sub-populations. Number of cases (%) or median (interquartile range), P-values from chi-squared test (frequencies), or t-test (continuous variables), comparisons relative to corresponding group without the exposure to wood smoke.

TABLE 2— Frequencies of Respiratory Symptoms and Diseases and Aeroallergen-Specific Sensitization With Respect to Wood Fuel Use, and the Associated Crude and Adjusted Odds Ratios [OR, Provided as Odds Ratio (95% Confidence Interval)]

	Wood fuel use all study subjects					Wood fuel use matched study subjects		
	No (n = 514)	Yes (n = 230)	OR	Adj. OR		No (n = 226)	Yes (n = 226)	OR
	n (%)	n (%)	(95% CI)	(95% CI)	*P*-value	n (%)	n (%)	(95%CI)
Respiratory symptoms								
Wheezing	56 (11)	27 (12)	1.1 (0.7–1.8)			25 (11)	27 (12)	1.1 (0.6–2.0)
Chest tightness	20 (4)	7 (3)	0.8 (0.3–1.8)			8 (4)	7 (3)	0.9 (0.3–2.5)
Cough crisis	73 (14)	35 (15)	1.1 (0.7–1.7)			34 (15)	34 (15)	1.0 (0.6–1.7)
Nasal problems	225 (44)	96 (42)	0.9 (0.7–1.3)			97 (43)	93 (41)	0.9 (0.6–1.4)
Shortness breath	35 (7)	11 (5)	0.7 (0.3–1.4)			17 (8)	11 (5)	0.6 (0.3–1.4)
Respiratory diseases								
Doctor-diagnosed asthma	44 (9)	31 (13)	1.7 (1.0–2.7)	1.9 (1.1–3.4)	0.02	14 (6)	31 (14)	2.4 (1.3–4.8)
Atopic asthma	33 (6)	22 (10)	1.5 (0.9–2.7)	1.9 (1.0–3.8)	0.049	9 (4)	22 (10)	2.6 (1.2–6.1)
Non-atopic asthma	11 (2)	9 (4)	1.9 (0.7–4.6)			5 (2)	9 (4)	1.8 (0.6–6.1)
Allergic rhinitis	77 (15)	46 (20)	1.4 (0.9–2.1)	1.4 (0.9–2.3)	0.10	29 (13)	45 (20)	1.7 (1.0–2.8)
Hay fever	64 (12)	54 (24)	2.2 (1.4–3.2)	2.3 (1.4–3.6)	<0.001	26 (12)	53 (23)	2.3 (1.4–4.0)
Pneumonia	80 (16)	28 (12)	0.8 (0.5–1.2)	0.7 (0.4–1.1)	0.10	32 (14)	27 (12)	0.8 (0.5–1.4)
Bronchitis	224 (44)	103 (45)	1.1 (0.8–1.4)			100 (44)	101 (45)	1.0 (0.7–1.5)
Bronchiolitis	70 (14)	33 (14)	1.1 (0.7–1.7)			30 (13)	32 (14)	1.1 (0.6–1.9)
Frequent colds (≥4/year)	153 (30)	77 (34)	1.2 (0.9–1.7)			69 (31)	75 (33)	1.1 (0.8–1.7)
IgE test (positive: >0.35 kIU)								
Aeroallergens (at least 1)	191 (37)	93 (41)	1.2 (0.8–1.6)			78 (35)	92 (41)	1.3 (0.9–1.9)
Pollen (at least 1)	92 (18)	65 (28)	1.8 (1.3–2.6)	1.9 (1.3–2.8)	0.001	36 (16)	65 (29)	2.1 (1.3–3.4)
Weed pollen	23 (4)	20 (9)	1.2 (0.9–1.7)	1.9 (1.0–3.6)	0.06	11 (5)	20 (9)	1.9 (0.9–4.2)
Tree pollen	40 (8)	33 (14)	2.0 (1.1–3.8)	1.8 (1.1–3.0)	0.03	16 (7)	33 (15)	2.2 (1.2–4.3)
Grass pollen	75 (15)	56 (24)	2.0 (1.2–3.3)	2.0 (1.3–3.0)	0.001	23 (10)	56 (25)	2.9 (1.7–5.0)
House dust mites	132 (26)	68 (30)	1.9 (1.3–2.8)			55 (24)	67 (30)	1.3 (0.9–2.0)

Adjusted odds ratios are only provided for total study population and if the exposure to wood smoke was retained in the optimized model, *P*-value given for adjusted OR.

these findings. Wood fuel use was significantly associated with the albumin levels in NALF (slope factors of 0.19, 0.24, and 0.021 and *P*-values of 0.08, 0.02, and 0.04 for the albumin concentration, the albumin concentration adjusted for plasma/NALF urea ratio and the albumin recovery, respectively). When comparing the matched sub-populations, no differences for albumin in NALF could be detected. The concentrations of IgE, creatinine, and urea in serum did not show statistically significant differences. When comparing the non-matched sub-populations, lower levels of CC16 and higher levels of SPD were observed in the adolescents with wood fuel use. These differences persisted after adjustment for renal function using the creatinine concentration. As a consequence, the CC16 to SPD ratio in serum, integrating the changes of both biomarkers, was significantly decreased (median −10%). Multivariate analysis confirmed the difference of the CC16 levels in serum, but only a trend for SPD remained with slope factors of −0.07 and 0.06 (log-transformed) and *P*-values of 0.02 and 0.14, respectively. In the multivariate model of the CC16 to SPD ratio, wood fuel use had a slope factor of −0.13 and *P*-value of 0.01. When comparing the matched sub-populations, a similar pattern was observed. A small but statistically significant decrease of serum CC16 (median −3.5%), and a weak trend towards increased levels of serum SPD (median +4.5%). The CC16/SPD ratio in serum was statistically significantly lower (−9%) in the sub-population with wood fuel use.

As discussed above, wood fuel use showed significant and consistent associations with doctor-diagnosed asthma, hay fever, sensitization against pollen, and the CC16 to SPD ratio in serum. In order to further explore these relationships, we included multiplicative interaction terms combining wood fuel use with doctor-diagnosed asthma, hay fever, or sensitization against pollen in the initial multivariate model of the CC16 to SPD (log-transformed). Except for wood fuel use (slope −0.13, *P*-value = 0.007) and sensitization against pollen (slope 0.08, *P*-value = 0.13), none of the above-mentioned variables or interaction terms was retained in the model after optimization. Figure 1 illustrates this in another way by showing the CC16/SPD ratio in serum for the matched sub-populations with respect to wood fuel use and asthma (a), hay fever (b), or

TABLE 3— Lung Function Parameters and Biomarkers in Exhaled Air, Nasal Lavage Fluid, and Serum of Sub-Populations With or Without Wood Fuel Use

	Wood fuel use all study subjects			Wood fuel use matched study subjects		
	No (n = 514)	Yes (n = 230)	*P*-value	No (n = 226)	Yes (n = 226)	*P*-value
Lung function measurements						
FEV_1 % pred. values	100 (92–110)	99 (92–108)	0.44	98.5 (92–109)	99.5 (92–108.8)	0.81
FVC % pred. values	89 (80–97)	88 (79–97)	0.47	88 (80–95)	88 (79–97)	0.79
FEV_1/FVC ratio	1.15 (1.1–1.19)	1.16 (1.11–1.19)	0.96	1.15 (1.1–1.18)	1.16 (1.11–1.19)	0.97
Exhaled breath						
NO (ppb)	13.2 (9.4–19.9)	13.2 (9.9–21.0)	0.58	12.4 (8.9–21.7)	13 (9.7–21.0)	0.80
Nasal lavage fluid						
Volume (ml)	3.71 (3.03–4.09)	3.64 (3.04–4.18)	0.78	3.74 (3.17–4.08)	3.65 (3.1–4.19)	0.72
CC16						
Concentration (μg/L)	21.3 (8.4–71.4)	26.5 (9.0–72.9)	0.43	22 (8.1–68.8)	26.5 (8.9–68.8)	0.52
Adjusted for plasma/NALF urea ratio (μg/L)	3.7 (1.2–15.9)	4.3 (1.2–13.3)	0.43	4.3 (1.2–14.4)	4.3 (1.1–12.9)	0.60
Recovery (ng)	78 (31–206)	95 (32–233)	0.38	82 (30–193)	95 (31–228)	0.52
Albumin						
Concentration (mg/L)	8.6 (4.1–15.8)	10.2 (5.8–19.1)	0.02	10.3 (4.9–18.7)	10.0 (5.8–19.4)	0.32
Adjusted for plasma/NALF urea ratio (μg/L)	1.40 (0.60–3.38)	1.85 (0.91–3.59)	0.03	1.70 (0.72–3.60)	1.81 (0.91–3.63)	0.46
Recovery (μg)	28.1 (15–50.7)	36.7 (21–64.4)	0.01	34.6 (17.3–65.7)	36.7 (20.9–66.3)	0.35
Urea						
Concentration (mg/L)	40.7 (31.5–53.1)	43.3 (32.3–58.5)	0.27	43.7 (32.5–55.2)	43.3 (32.9–58)	0.95
Recovery (mg)	0.14 (0.11–0.18)	0.15 (0.12–0.19)	0.16	0.15 (0.12–0.2)	0.15 (0.12–0.19)	0.76
Serum						
Urea (mg/L)	248 (213–291)	257 (214–309)	0.23	259 (218–299)	258 (215–310)	0.72
Creatinine (mg/L)	8.7 (8.0–9.7)	0.88 (0.79–0.98)	0.54	0.87 (0.80–0.95)	0.88 (0.79–0.98)	0.74
Total IgE (kIU/L)	47 (17–145)	63 (20–170)	0.38	46 (16–132)	63 (21–166)	0.24
SPD (μg/L)	87 (63–120)	91 (63–137)	0.02	88 (64–120)	92 (63–138)	0.12
SPD/creatinine ratio ($\times 10^{-3}$)	9.6 (6.8–14.0)	10.3 (7.2–16.7)	0.02	10.1 (7.1–14.2)	10.4 (7.3–16.8)	0.17
CC16 (μg/L)	8.9 (6.7–11.9)	8.4 (6.3–10.5)	0.01	8.7 (6.7–11.9)	8.4 (6.3–10.5)	0.03
CC16/creatinine ratio ($\times 10^{-3}$)	1.00 (0.74–1.35)	0.96 (0.71–1.18)	0.02	1.01 (0.75–1.37)	0.96 (0.71–1.18)	0.01
CC16/SPD ratio ($\times 10^{-3}$)	102 (67–152)	92 (52–136)	0.001	100 (69–142)	91 (52–135)	0.01

Data presented as medians (inter-quartile range), statistics: unpaired *t*-test on log-transformed data.

sensitization against pollen (c). Two-way analysis yielded three similar graphs. The serum CC16/SPD ratio was consistently and significantly lower in the groups with wood fuel use, whereas the other investigated variables showed no association with the CC16 to SPD ratio. Similar to the multivariate analysis, the two-way analyses of the matched study populations did not detect a statistically significant interaction between 1 of the 3 variables and wood fuel use.

DISCUSSION

In this study, wood fuel use was associated with a decreased level of CC16 and an increased level of SPD in serum, and, consequently, a decreased serum CC16/SPD ratio. Wood fuel use was also associated with increased risks of asthma, hay fever, and sensitization against pollen allergens. Even when limiting the effects of potential confounders by using an automated matching of the sub-populations or multivariate models with step-wise optimizations, the above-mentioned associations were consistently observed. Although the albumin levels in NALF were significantly increased when considering the whole study population and an increased extravasation of albumin into the airways is a known effect of toxic lung injury,[30] this effect was not retained as a key finding of our study on the basis that it was completely abolished by the automated matching of study subjects.

It is worth noting that the changes described in this study resemble those reported by Robin et al.,[31] who observed in smokers a decrease of CC16 and an increase of surfactant protein B. Whereas the decrease of CC16 in serum could reflect a decreased production of the Clara cells due to the irritation/cytotoxicity of smoke, the increase of surfactant proteins in serum might be explained by an unchanged production by the type II pneumocytes accompanied by an increased permeability of the lung epithelial barrier.[18] The decrease of the CC16 levels as observed in the current study (median −6%) is not as strong as Robin et al.[31] observed in smokers (median −28%). This might be

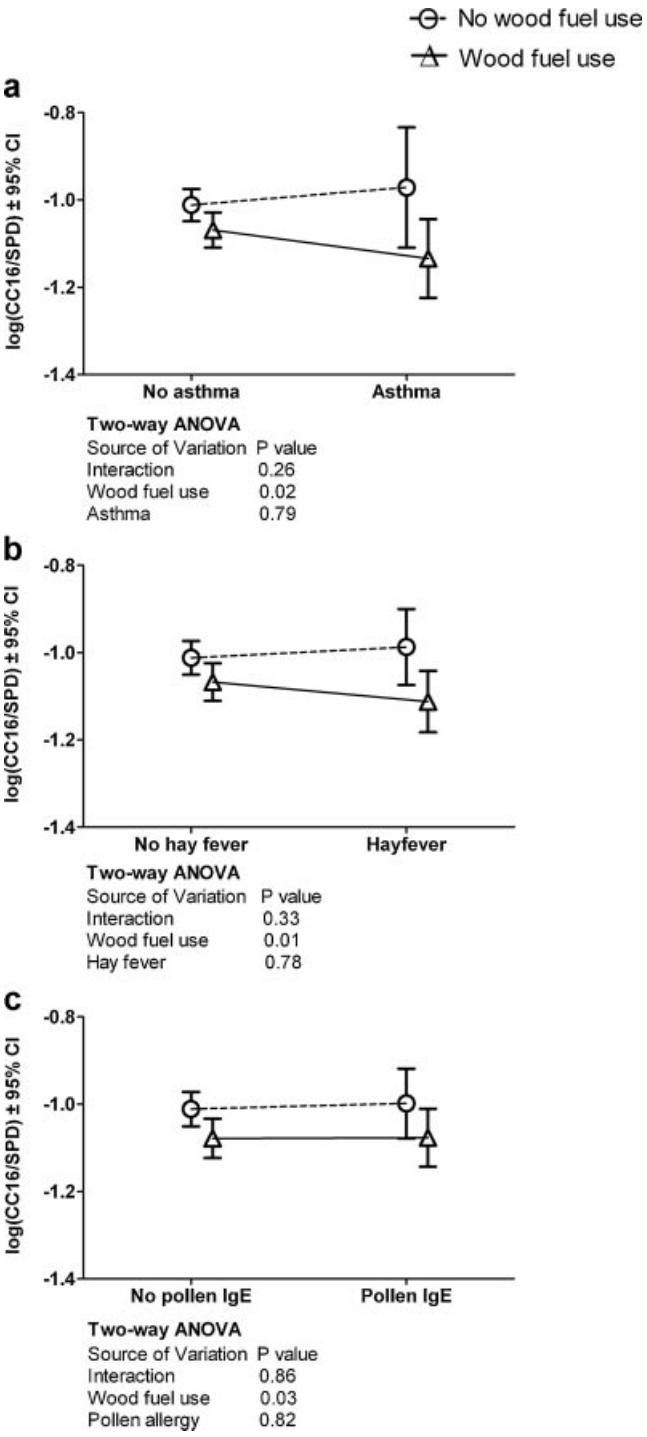

Fig. 1. Two-way analysis of the matched dataset investigating the relationships between the CC16 to SPD ratios in serum and asthma (a), hay fever (b), and sensitization against pollen (c) with or without concurrent wood fuel use.

explained by differences in study populations and in the exposure levels. Whereas the current study investigated non-smoking adolescents with a seasonal exposure to "wood fuel use," Robin et al.[31] investigated adult smokers with a median cumulative pack-years of 14.9. Moreover, even though exposure to combustion products is also involved in wood fuel use, a significant difference between the levels of exposure to hazardous chemicals in both studies is more than fair assumption.

The apparent paradox with the elevated serum levels of CC16 in the study by Barregard et al.[20] can be explained by the fact that wood smoke exposure in that study was acute and that the driving mechanism in that case was probably lung hyperpermeability and not the reduced production of CC16. As both wood smoke and tobacco smoke originate from the incomplete combustion of biomaterials, common mechanisms might underlie the observed changes. The findings in this study are in line with the growing amount of scientific data suggesting an adverse effect of in-house exposure to wood smoke on respiratory health. Wood smoke is known to contain compounds such as carbon monoxide, nitrogen oxides, sulphur oxides, aldehydes, polycyclic aromatic hydrocarbons, and fine respirable particulate matter. All of these have been shown to cause deleterious physiologic responses in laboratory studies in humans.[32] Barregard et al.[20] demonstrated that wood smoke at levels that can be found in smoky indoor environments can cause an inflammatory response and signs of increased oxidative stress in the respiratory tract, especially in the lower airways. Exposure to wood smoke has been linked to an increased incidence of respiratory symptoms,[11] respiratory infections, low birth weight, cardiovascular events, and all-cause mortality both in adults and children,[6,12] and to lung cancer.[10]

Despite reports suggesting the potential role of NO as a sensitive biomarker for respiratory disease, no significant association between exhaled NO and wood smoke exposure was observed. Possibly, the changes in this healthy, young population were too limited to be detected using this biomarker. Moreover, exhaled NO appears to be very sensitive to acute inflammatory changes as observed during acute asthma, or when the maintenance dose of inhaled steroids is reduced, but levels tend to normalize in case of stabilized situations, such as stable asthma after steroid treatment.[17] The same rationale probably holds true for the lack of significant associations between wood fuel use and the lung function measurements or respiratory symptoms. Barregard et al.[20] reported increased levels of NO in study subjects at 3 hr after an acute exposure to wood smoke, but no differences were detectable anymore the next morning. The latter situation probably corresponds best to the situation of the adolescents in this study who were examined at school. As such, both studies did not yield contradictory results.

Despite being consistently observed, changes in the serum levels of CC16 (−6%) and of the CC16/SPD ratio (−9%) were relatively small compared to those

observed in diseased individuals or in subjects chronically exposed to tobacco smoke or toxic atmospheres in the industry.[18] These changes reveal thus the existence of early subclinical effects in the airways epithelium, which are most likely caused by irritating agents in wood smoke.[18,33,34] A decrease of CC16 and an increase of the CC16 over SPD ratio in serum can be explained by a decreased production of CC16, a major protein in the lung with anti-inflammatory activity[23] and the increased lung permeability. As such the observed biomarker changes might thus be indications of a subclinical process capable of shifting the respiratory system towards a status of increased vulnerability to pro-inflammatory processes.

The associations between wood smoke exposure and asthma, hay fever or sensitization against pollen as observed in this study are not in line with the information which could be retrieved in scientific literature. A large meta-analysis by Po et al.[12] did not detect an association between the exposure to biomass fuels and asthma in women and children, and little epidemiologic data were found linking wood smoke exposure to atopic outcomes. It is however striking that the association between sensitization and wood smoke exposure was rather specific. The positive association was very consistent for pollen, whereas it was not found with house dust mites. It is unlikely that it is a mere consequence of the fact that wood stoves are more frequently used in a rural area as we tried to address this by using both matching and multivariate approaches. We think that this specific association between wood fuel use and pollen sensitization might be causal and result from a significant in-house exposure to pollen carried over by wood.

We could not detect a statistically significant association between the CC16 to SPD ratio in serum and the risk of asthma, hay fever, and sensitization against pollen (Fig. 1), despite the fact that all were significantly associated with wood fuel use. Possibly, the increased frequencies of asthma, hay fever, and sensitization against pollen are not related to the cytotoxic/irritating activity of wood smoke which we consider as the most likely explanation for a causal link between wood fuel use and the altered CC16 to SPD ratio in serum. A modifying effect of wood smoke exposure nevertheless cannot be excluded since Samuelsen et al.[16] observed in an in vivo experiment that particles from wood smoke can act as adjuvant.

The allocation of a study participant to a sub-population with respect to wood fuel use was based on the only available source of information, that is, the interview inquiring about a heating system with wood at home. The lack of a more detailed characterization of exposure is the main limitation of this study. Because the sample collection took place in March, it can be reasonably assumed that the exposure to wood smoke occurred regularly during the preceding months. Moreover, despite the limitation related to the exposure characterization, it looks to us unlikely that the associations with wood fuel use which were consistently observed both after multivariate and matching approaches and which also involved objective measures, that is, biomarkers, resulted merely from confounding.

In conclusion, wood fuel use was associated with altered levels of biomarkers reflecting the integrity of the terminal airways epithelium. CC16 in serum was decreased, whereas SPD in serum tended to be increased, and, consequently, the CC16 to SPD ratio in serum was decreased. Wood fuel use was also associated with higher risks of asthma, hay fever, and sensitization against pollen allergens. Albeit that this study did not allow to elucidate the mechanisms underlying these associations, the results consistently pointed towards a possible involvement of wood fuel use in the development of these respiratory effects.

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