Swimming is practiced extensively in Western countries (Vaz et al. 1999). Despite the benefits of physical activity, health concerns are growing because swimming in pools involves exposure to disinfectants and disinfection by-products (DBPs), such as trihalomethanes (THMs), one of the classes of DBPs at highest concentration in swimming pools, and trichloramine, a known irritant (World Health Organization 2006). A range of acute symptoms has been described among bathers after accidental exposure to high levels of chlorine in swimming pools, including mucosal and ocular irritation, cough, rash, dyspnea, and lung function decline (Bonetto et al. 2006; Grasemann et al. 2007). Subjects exposed chronically to the swimming pool environment, such as pool workers, showed irritant eye, nasal, and throat symptoms (Jacobs et al. 2007; Massin et al. 1998). Cases of occupational asthma and trichloramine sensitization have been described in pool lifeguards (Thickett et al. 2002). Although an increased asthma risk among children attending pools has been suggested but not confirmed (Font-Ribera et al. 2009; Goodman and Hays 2008), respiratory symptoms and asthma are consistently more prevalent among competitive swimmers compared with other athletes (Goodman and Hays 2008). However, one of the unsolved questions is what are the biological mechanisms behind these health effects (Bonetto et al. 2006; Grasemann et al. 2007).

The development of methods to evaluate respiratory and systemic biomarkers in blood, exhaled breath condensate (EBC), and exhaled breath has allowed the assessment of pathobiological mechanisms underlying respiratory disorders (Bonetto et al. 2006) and the detection of early subclinical respiratory effects after acute or chronic environmental exposures, including swimming pool attendance. Lung surfactant proteins, such as Clara cell secretory protein (CC16) or surfactant protein D (SP-D), are secreted in the lung epithelium and move passively across the epithelial barrier into the serum down a strong gradient (Broeckaert et al. 2000). A change in the concentration of lung surfactant proteins in serum has been proposed as a marker to detect early permeability changes in the lung epithelium (Broeckaert et al. 2000). Fractional concentrations of orally exhaled nitric oxide (FeNO) is a marker of eosinophilic airway inflammation (Choi et al. 2004), and has been shown to increase after short-term exposure to mold (Stark et al. 2005). Soluble molecules can be detected in EBC, including proinflammatory cytokines, growth factors, and oxidative stress biomarkers, and have been used to monitor different aspects of diseases such as asthma or chronic obstructive pulmonary disease, as well as the effects of environmental stressors or physical exercise (Bonsignore et al. 2003; Carbonnelle et al. 2002, 2008; Massin et al. 1998; Nanson et al. 2001).

Proposed mechanisms of respiratory damage related to swimming pool exposure include airway inflammation (Bonetto et al. 2006; Grasemann et al. 2007; Moreira et al. 2008; Pedersen et al. 2009), oxidative stress (Varraso et al. 2004, 2006), and hyperpermeability of the lung epithelium (Bonetto et al. 2006; Carbonnelle et al. 2002, 2008). Increased permeability of the lung epithelium has been evaluated extensively, and some authors suggest that it may result in increased airway inflammation and higher risk of sensitization.
where the swimming pool was located but separated from the swimming pool area. **Respiratory biomarkers. 8-Isoprostane and cytokines.** EBC was obtained approximately 70 min before swimming began and 35 min after swimming ended using an EcoScreen condenser (Jaeger GmbH, Würzburg, Germany) following American Thoracic Society/European Respiratory Society Task Force recommendations (Horvath et al. 2005). Samples were obtained through breathing at normal frequency and tidal volume until one total expiratory volume of 180 L was achieved. After collection, the condensing device was centrifuged at 4°C, and the resultant total EBC volume (~ 4 mL) was transferred into Eppendorf tubes and rapidly frozen in liquid nitrogen. All samples were lyophilized and stored at ~80°C before analysis. 8-Isoprostane was analyzed through an enzyme-linked immunosorbent assay (ELISA; Cayman Chemical, Ann Arbor, MI, USA). Using the BD Cytometric Bead Assay kit, cytokine levels were determined in EBC. Levels were characterized as picograms per millilitre of EBC.

**Cytokines.** The following cytokines and growth factors were measured in EBC: RANTES (regulated upon activation, normal T-cell expressed, and secreted), vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF), interleukin (IL)-12p70, IL-4, IL-10, interferon-gamma (IFN-γ), and IFN-γ-induced protein 10 (Ip10). Levels were expressed as parts per billion.

**Lung function.** Forced expiratory volume in 1 sec (FEV₁) and forced vital capacity (FVC) were measured 30 min before and 60 min after participants swam, with an EasyOne portable spirometer (ndd Medical Technologies, Zürich, Switzerland) following standard recommendations (Miller et al. 2005). FEV₁ and FVC were expressed as the percentage from the predicted value by age, sex, and height (Roca et al. 1986).

**Biomarkers of exposure.** The four THMs—chloroform, bromodichloromethane, dibromochloromethane, and bromoform—were measured in exhaled breath before swimmers entered the swimming pool (80 min before swimming) and just after they swam (5 min after leaving the pool) (Figure 1), as markers of individual exposure to DBPs. Exhaled breath samples were collected using a portable system for end-exhaled breath sampling, which has been described previously (Lourenzetti et al. 2010). Briefly, volunteers were required to breathe through the sampling device equipped with an adsorption cartridge packed with Tenax TA (Supelco, Bellefonte, PA, USA). A total volume of 1 L was collected per person. The air passed through a stainless-steel cartridge (0.5 cm diameter and 9 cm long) containing 1.8 g Tenax TA (60/80 mesh). Chloroform, bromodichloromethane, dibromochloromethane, and bromoform were determined by an Automatic Thermal Desorption System (ATD 400; Perkin-Elmer, Shelton, CT, USA) coupled to an Autosystem gas chromatograph with electron capture detection (Perkin-Elmer). Concentrations were expressed as micrograms per cubic meter.

**Materials and Methods**

**Design.** The study has a crossover design involving 50 nonsmoking adults who were recruited through open advertisements on the Internet and at local universities. A screening questionnaire was used to verify eligibility among subjects (nonsmoking adults, 18–50 years of age, without respiratory diseases such as ever asthma or having had a cold in the preceding 3 weeks). Participants were requested to avoid swimming pools during the week before the session and to avoid taking a shower the day of the swimming experiment. The study was approved by the ethics committee of the research center following the international regulations, and all volunteers signed an informed consent before participation.

A single, indoor, 25-m-long chlorinated swimming pool in Barcelona, Spain, was used for the study. Every day, one to four participants were evaluated between 0900 and 1400 hours (before lunch) in May, June, September, or October 2007. Before and after the subjects swam in the chlorinated pool for 40 min, a battery of measurements and biological samples was collected to evaluate respiratory biomarkers according to a strict schedule (Figure 1). Biological samples and measurements before and after the swim were obtained in a room inside the sports center.
Environmental measurements.

Environmental measurements were taken to characterize the swimming pool and to complement the exposure assessment to DBP. Free chlorine, THMs, and mono-, di-, and trichloramine were measured in pool water. A 1-L composite water sample was collected at four different points of the pool for each participant while he or she was swimming. A single value for free chlorine, monochloramine, dichloramine, and trichloramine was obtained for each participant as measured by N,N-diethyl-p-phenylenediamine (DPD) procedure with a portable photometer (DINKO Instruments, Inc., Barcelona, Spain). The methods for water and air THM analyses have been described elsewhere (Lourencetti et al. 2010). Briefly, for THM analyses, 5 mg sodium thiosulfate was added to a 40-ml glass vial with screw cap and polytetrafluoroethylene-lined silicone septa. Water samples were stored at 4 °C until laboratory analysis on the same day. THMs in water were determined using a SOLATek 72 Multi-Matrix Vial Autosampler (Tekmar Dohrman, Mason, OH, USA) coupled to a purge-and-trap concentrator (Tekmar 3100; Tekmar, Cincinnati, OH, USA), which transfers the sample directly to a gas chromatograph coupled to a mass spectrometer (Voyager MS; ThermoQuest Finnigan, Manchester, UK) and had a coefficient of variation between 0.98% and 5.6%. An indoor air sample for THM measurements was collected for each participant with a pump located 60 cm above the floor and 1.5 m from the pool border, at 7 ml/min flow rate for 20 min through an adsorption cartridge filled with Tenax TA. Quality control was assured by daily calibration of the pump. The four THMs were measured as described for exhaled breath samples and were expressed as micrograms per cubic meter.

Additional air samples were collected to measure trichloramine in a subset of the days (6 days). Air was collected with a constant flow sampling pump (flow rate of 1.2 L/min for an mean ± SD of 115 ± 32 min), within 1 m from the water and at a height of 60 cm above the floor level. The instrumental analyses were performed at the Institute for Risk Assessment Sciences at Utrecht University (Utrecht, the Netherlands) following the method described by Hery et al. (1995); further details are available elsewhere (Jacobs et al. 2007). Trichloramine measurements were used for comparison with other swimming pools but were not used as personal exposure estimates because only 2 of the 6 trichloramine measurement days coincided with the experimental study involving only two participants.

Other information collected.

Questionnaires were used to collect information on personal and family history of atopic diseases, exposure to environmental tobacco smoke, diet, sociodemographic data, frequency and duration of swimming pool attendance and other physical activity, and way of commuting to the swimming pool facility. Weight and height were measured with standard procedures. Exercise intensity during swimming was estimated using the distance swum by each participant during the 40 min. Energy expenditure (in kilocalories) was estimated using the swimming speed and the weight of the participant, assuming that swimming at 46 m/min equals 11 metabolic equivalent tasks (METs; kilocalories per kilogram per hour):

\[
\text{kcal} = \text{weight (kg)} \times \text{speed (m/min)} \\
\times 40 \text{ min} \times 1 \text{ hr}/60 \text{ min} \\
\times 11 \text{ (kcal/kg/hr)/46 (m/min)}
\]

(Ainsworth et al. 2000). Atopic status was measured with the Phadiatop test (Pharmacia & Upjohn, Uppsala, Sweden), a qualitative test for serum-specific immunoglobulin E to a mixture of common allergens (Vidal et al. 2005). A single-nucleotide polymorphism in the \(CC16\) gene (rs3741240), known to modify gene expression, was genotyped using Sequenom (CÉGEN-Santiago, San Diego,

**Table 1.** Physicochemical parameters in water, air, and exhaled breath and exercise intensity performed by participants (\(n = 48\)).

| Measurement | Mean ± SD | Median | Minimum | Maximum |
|-------------|-----------|--------|---------|---------|
| Water Free chlorine (mg/L) | 1.17 ± 0.4 | 1.10 | 0.5 | 2.17 |
| | Dichloramine (mg/L) | 0.43 ± 0.1 | 0.46 | 0.16 | 0.65 |
| | Temperature (°C) | 27.2 ± 0.4 | 27.4 | 26.5 | 27.7 |
| | pH | 7.3 ± 0.1 | 7.3 | 6.9 | 7.5 |
| | Chloriform (µg/L) | 16.1 ± 3.4 | 16.7 | 8.5 | 20.8 |
| | Bromodichloromethane (µg/L) | 12.3 ± 2.3 | 11.9 | 9.3 | 22.8 |
| | Dibromochloromethane (µg/L) | 10.9 ± 3.1 | 10.5 | 6.5 | 22.6 |
| | Bromoform (µg/L) | 6.1 ± 2.4 | 5.7 | 3.0 | 18.2 |
| | Total THMs (µg/L) | 45.4 ± 7.3 | 45.5 | 35.2 | 75.2 |
| Air | | | | |
| | Chloriform (µg/m³) | 35.0 ± 12.3 | 31.4 | 19.5 | 61.6 |
| | Bromodichloromethane (µg/m³) | 14.6 ± 5.0 | 13.0 | 7.5 | 23.4 |
| | Dibromochloromethane (µg/m³) | 13.2 ± 4.3 | 12.4 | 6.0 | 26.2 |
| | Bromoform (µg/m³) | 11.2 ± 5.2 | 8.4 | 4.4 | 22.6 |
| | Total THMs (µg/m³) | 74.1 ± 23.7 | 68.9 | 44.0 | 124.9 |
| Exhaled breath (after swimming) | | | | |
| | Chloriform (µg/m³) | 4.5 ± 1.7 | 4.6 | 1.1 | 8.1 |
| | Bromodichloromethane (µg/m³) | 1.8 ± 0.5 | 1.6 | 0.7 | 3.2 |
| | Dibromochloromethane (µg/m³) | 1.2 ± 0.5 | 1.2 | 0.3 | 2.8 |
| | Bromoform (µg/m³) | 0.5 ± 0.2 | 0.4 | 0.1 | 1.3 |
| | Total THMs (µg/m³) | 7.9 ± 2.8 | 7.7 | 3.0 | 14.0 |
| Exercise intensity | | | | |
| | Distance swum (km) | 0.90 ± 0.4 | 0.95 | 0.05 | 1.75 |
| | Energy expenditure (kcal) | 248.5 ± 120.6 | 241.9 | 16.8 | 603.3 |

\(n = 47\).

**Table 2.** Spearman correlation coefficients (\(r\)) between the different exposure indicators measured (\(n = 47\)).

| Medium (concentration) | Water (µg/L) | Concentration in exhaled breath after swimming (µg/m³) | Energy expenditure (kcal) |
|-----------------------|--------------|-----------------------------------------------------|--------------------------|
|                       | Free Cl | NHCl₂ | CHCl₂ | CHCl₂Br | CHCl₂Br₂ | CHBr₂ | CHBr₃ | TTHMs | Free Cl | NHCl₂ | CHCl₂ | CHCl₂Br | CHCl₂Br₂ | CHBr₂ | CHBr₃ | TTHMs | Free Cl | NHCl₂ | CHCl₂ | CHCl₂Br | CHCl₂Br₂ | CHBr₂ | CHBr₃ | TTHMs |
| Water (µg/L) | Free Cl | -0.28 | -0.22 | -0.44* | -0.44* | -0.50* | -0.37* | -0.26 |
| | NHCl₂ | -0.24 | 0.17 | -0.27 | -0.03 | -0.32 | 0.01 | 0.07 | -0.04 |
| | CHCl₂ | -0.03 | -0.31* | -0.40* | -0.05 | 0.25 | 0.24 | -0.17 | 0.08 |
| | CHBr₂ | -0.04 | -0.40* | 0.06 | 0.19 | 0.18 | 0.48* | 0.14 | 0.04 |
| | TTHMs | 0.19 | 0.04 | 0.64* | 0.40* | 0.13 | 0.29* | 0.52* | -0.001 |
| Air (µg/m³) | CHCl₂ | 0.24 | -0.29* | 0.22 | 0.35* | 0.28 | 0.55* | 0.30* | 0.12 |
| | CHCl₂Br | 0.38* | -0.13 | 0.55* | 0.48* | 0.34* | 0.43* | 0.53* | 0.03 |
| | CHCl₂Br₂ | 0.83* | 0.60* | 0.55* | 0.94* | 0.14 |
| | CHCl₂Br₃ | 0.80* | 0.72* | 0.94* | 0.18 |
| | CHCl₂ | 0.74* | 0.79* | 0.18 |
| | CHCl₂Br | 0.70* | 0.32* |
| | TTHMs | 0.19 |

Abbreviations: CHBr₂, bromoform; CHCl₂Br₂, dibromochloromethane; CHCl₂Br, bromodichloromethane; CHCl₂, chloroform; NHCl₂, dichloramine; TTHMs, total THMs.

*Kilocalories expended during the 40 min. *p < 0.05.
CA, USA). DNA was extracted from peripheral blood samples.

**Statistical analysis.** The distribution of each biomarker was evaluated with a test for normality evaluating skewness and kurtosis. Mean or median values were reported accordingly to describe central tendencies. We calculated the individual change in the concentration of each biomarker after swimming in the pool (concentration after – before). Samples with cytokines under the detection limit before (30.1%) or after (27.1%) swimming were imputed half the detection limit. Those with undetectable levels before and after swimming were excluded from the statistical analysis. A nonparametric test was used to evaluate whether there was a significant change in the concentration of each biomarker. Linear regression models were fitted to calculate the association between changes in the concentration of each respiratory biomarker and the personal markers of DBP exposure and exercise intensity. The β-coefficient of a change in a unit in the concentration of each biomarker was tested once per month, including seven percentiles for DBP exposure and exercise intensity. The changes in the concentration of each respiratory biomarker was calculated the individual change in the concentration after swimming. Those with undetectable levels before swimming were imputed half the detection limit before (30.1%) or after (27.1%) swimming in the pool. The change in respiratory markers for an increase from 25th to 75th percentile in exposure parameters (95% confidence interval)

**Results** We recruited 50 subjects for the study. We excluded two subjects with history of asthma for the present analysis, resulting in a sample of 48 subjects. Most participants were women (65%) and were highly educated (92% with university studies), with an average age (± SD) of 30 ± 6.1 years, and 30% were positive to the Phadiatop test. Twenty percent were regular swimmers (at least once per month), and 54% practiced sport at least once a week. Regarding the CC16 genotype (A38G), frequencies were 39%, 12%, and 49% for AG, AA, and GG, respectively. The genotyping frequency was 91%, and it was in Hardy–Weinberg equilibrium (p = 0.365). Minor allele frequencies were similar to those described in the International HapMap Project for European individuals (International HapMap Consortium 2003). The mean (± SD) speed during swimming was 22.5 ± 9.7 m/min, and the mean energy expenditure was 248.5 ± 120.6 kcal. We had one missing value for energy expenditure, one for THM in exhaled breath, one for FeNO, and three for 8-isoprostanate in EBC.

Average free chlorine level in the pool water was 1.17 ± 0.4 µg/L. Average total THM concentration in water was 45.4 ± 7.3 µg/L (Table 1). The mean (± SD) level of THM in exhaled breath before swimming was 1.19 ± 0.40 µg/m³ for total THMs, 0.72 ± 0.28 µg/m³ for chloroform, 0.25 ± 0.09 µg/m³ for bromodichloromethane, 0.13 ± 0.06 µg/m³ for dibromochloromethane, and 0.10 ± 0.07 µg/m³ for bromoform. After swimming, THMs in exhaled breath increased on average about seven times. The increase was similar by age group, sex, or body mass index (data not shown). Chloroform levels in exhaled breath were significantly correlated with levels in the swimming pool’s air, but not with levels in water (Table 2). Dichlormethane in water was inversely and significantly correlated with brominated THMs but not with chloroform in water, air, and exhaled breath. Free chlorine in water was not significantly correlated to total THMs in water but was significantly correlated to total THMs in air and exhaled breath. The energy expenditure correlated significantly only with bromoform concentration in exhaled breath after swimming. Trihalomethane in water was detectable, and monochloramine correlated with the same DBPs as dichlormethane, so we show only dichlormethane in the tables.

The concentration of CC16 in serum was increased significantly after swimming, with an overall median increase of 0.47 µg/L (3.3% increase) (Table 3). We detected no significant changes in percent predicted

### Table 3. Level of respiratory markers before and after swimming: linear regression coefficients of the change after swimming for the exposure parameters.

| Parameter | FEV₁ (% predicted) | FVC (% predicted) | FEV₁/FVC | FeNO (ppb) | 8-Isoprostanate (µg/mL) | SP-D (µg/L) | CC16 (µg/L) |
|-----------|--------------------|-------------------|----------|------------|------------------------|------------|-------------|
| n         | 48                 | 48                | 48       | 47         | 45                     | 48         | 48          |
| Median (IGR) |                   |                   |          |            |                        |            |             |
| Before     | 97.3 (90.1 to 103.6)| 98.1 (90.0 to 105.4)| 0.83      | 13         | 1.6                    | 54.4       | 6.01        |
| After      | 95.9 (90.7 to 104.2)| 96.4 (90.5 to 106.0)| 0.93      | 12.5       | 15.3                   | 65.8       | 7.21        |
| Change     | (–2.5 to 2.4)      | (–5.1 to 3.9)     | (–0.02 to 0.04) | (–2.2 to 1) | (–0.8 to 1.1)          | (–3.7 to 6.6) | (–0.3 to 1.1) |
| p-Value (change ≠ 0) | 0.83 | 0.46 | 0.24 | 1.00 | 0.91 | 0.44 | 0.03 |

**Change in respiratory markers for an increase from 25th to 75th percentile in exposure parameters (95% confidence interval)**

- Free chlorine to water (mg/L): 0.54 (–2.44 to 0.02), 0.92 (–1.22 to 0.61), 2.93 (0.58 to 1.02)
- NHCl₂ to water (µg/L): 0.12 (–0.50 to 0.57), 0.01 (–2.42 to 2.67), 0.92 (–0.03 to 1.91), 0.59 (–0.71 to 0.51), 0.34 (–2.10 to 7.97), 0.87 (–1.38 to –0.33)
- CHCl₂Br to water (µg/m³): 0.01 (–0.71 to 1.07), 0.03 (–1.27 to 2.35), 0.43 (–1.07 to 3.15) 0.59 (–0.17 to 1.32), 0.71 (–5.69 to 6.37), 0.21 (–0.44 to 0.92)
- CHCl₂Br to water (µg/m³): 0.01 (–0.71 to 1.07), 0.03 (–1.27 to 2.35), 0.43 (–1.07 to 3.15) 0.59 (–0.17 to 1.32), 0.71 (–5.69 to 6.37), 0.21 (–0.44 to 0.92)
- CHBr₃ to exhaled breath (µg/m³): 0.02 (–3.16 to 3.06), 0.00 (0.00), 0.02 (–0.05 to 0.15), 0.05 (–1.52 to 1.38), 0.01 (–0.41 to 1.05), 0.01 (–3.09 to 9.30), 0.01 (0.59 to 1.82)
- TTHMs to exhaled breath (µg/m³): 0.02 (–2.24 to 2.81), 0.02 (–0.93 to 1.24), 0.02 (0.12 to 1.20), 0.02 (–4.57 to 10.6), 0.02 (–0.18)
- Energy expenditure (kcal): 0.00 (–1.70 to 2.07), 0.00 (0.00), 0.02 (–2.10 to 7.97), 0.02 (–1.38 to –0.33), 0.02 (–0.44 to 0.92), 0.02 (0.08 to 1.02)

**Abbreviations:** CHBr₂, bromofrom; CHCl₂Br, dibromochloromethane; CHCl₂Br, bromochloromethane; CHCl₃, chloroform; NHCl₂, dichloramine; TTHMs, total THMs. Percent predicted refers to percentage of that predicted by age to sex and height. SP-D and CC16 were measured in serum. 8-Isoprostanate was measured in EBC.

*Wilcoxon test. **Coefficients from linear regression models represent a change in the biomarker level for an increase from 25th to 75th percentile of the exposure parameter. FeNO models are adjusted for rhinitis; 8-isoprostanate models are adjusted for usual swimming pool attendance. The other models are crude.*p < 0.05. **p < 0.01.
FEV₁, percent predicted FVC, FEV₁/FVC, FeNO, serum SP-D, 8-isoprostanate (Table 3), or cytokines in EBC (Table 4). The increase in serum CC16 concentration was significantly correlated with different indicators of DBP exposure (negatively with dichloramine in water and positively with free chlorine in water and bromodichloromethane, dibromochloromethane, and bromoform in exhaled breath) and with energy expenditure (Table 3, Figure 2). In multivariate models, both energy expenditure and markers of DBP exposure remained significantly associated with the increase in CC16 after mutual adjustment (Table 5). An interquartile range (IQR) increase in energy expenditure was associated with a significant increase in 8-isoprostanate in EBC after swimming. We found an interaction with the change in 8-isoprostanate and swimming regularly (p-value = 0.04). 8-Isoprostanate decreased among those who swam regularly (median change, −1.0 pg/mL; SD = 1.2; p-value = 0.04), whereas it tended to increase among those who did not swim regularly (0.62 pg/mL; SD = 2.1; p-value = 0.09).

When we calculated the change in the biomarker concentration as a relative measure (levels after – before) levels before, we found the same patterns. Bivariate analyses showed that the changes in the levels of these respiratory biomarkers did not differ by sex, age, body mass index, or the time spent in active commuting (walking or cycling) to the swimming pool facility. Atopic participants had higher baseline FeNO concentrations than did nonatopic participants, and they tended to have a decrease in FeNO after swimming, whereas nonatopic subjects remained stable (Figure 3). The increase in CC16 concentration in serum was not modified significantly by atopic status. Furthermore, CC16 change was not different among CC16 genotypes, modeled as dichotomous (GG vs. AA/AG; p-value = 0.507) (data not shown).

**Discussion**

We detected a slight but significant increase in lung epithelial permeability, as estimated by serum CC16, in healthy adult volunteers after swimming in a chlorinated pool. Energy expenditure during swimming and change in THM concentrations in exhaled breath after swimming (indicating higher DBP exposure) were significant predictors of increases in serum CC16, suggesting that these exposures may have contributed to an increase in lung permeability. We observed no significant changes in lung function tests or markers of inflammation or oxidative stress in adults after swimming in a chlorinated pool.

The lack of an association between swimming and lung function and FeNO was consistent with previous studies with a comparable design (Carbonnelle et al. 2002, 2008; Moreira et al. 2008; Pedersen et al. 2009). However, evidence for serum CC16 is less consistent. Serum CC16 did not vary significantly in 11 young adults (Carbonnelle et al. 2008) and in 16 children (Carbonnelle et al. 2002), whereas it decreased (29% decrease) among 13 adults after swimming in a chlorinated pool, with a concentration of trichloramine in air between 160 and 280 µg/m³ in one study (Carbonnelle et al. 2008) and of 490 µg/m³ in the other (Carbonnelle et al. 2002). Carbonnelle et al. (2002) detected an increase in serum CC16 levels among 14 elite swimmers after they swam in a chlorinated pool (44% increase) and a nonchlorinated pool (52% increase), suggesting that the hyperpermeability of the lung epithelium after swimming could be caused by physical activity (Nanson et al. 2001). We showed in the present study that the both exercise intensity during swimming and markers of DBP exposure were associated with the increase in serum CC16 after mutual adjustment, supporting the hypothesis of independent effects of exercise and chemical exposure on the permeability of the lung epithelium.

The unchanged concentration of 8-isoprostanate after swimming suggests the lack of association with oxidative stress in the airways. However, 8-isoprostanate tended to increase with energy expenditure, in accordance with a previous study showing that oxidative stress increases after physical exercise in healthy subjects (Moller et al. 1996). We detected no changes in the eight cytokines and one growth factor measured in EBC, in

**Figure 2.** Correlation between the change in serum CC16 concentration and 8-isoprostanate (8-iso-C18:2) in exhaled breath, energy expenditure during swimming, free chlorine in water, and dichloramine (NHCl₂) in water.

| Table 4. Concentration of eight cytokines and VEGF (pg/mL) in EBC before and after swimming. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Median (IQR)    | HANTES | Ip10 | VEGF | TNF | IL-12p70 | IL-10 | IL-8 | IFN-γ | IL-4 |
| **Before**      |        |      |      |     |          |       |      |       |      |
| β (p-value)     | 0.898  | 0.107 | 0.89 | 0.31 | 0.08     | 0.66  | 1.24 | 0.38  | 0.70 |
| **After**       |        |      |      |     |          |       |      |       |      |
| β (p-value)     | 0.740  | 0.477 | 0.107| 0.70 | 0.00     | 0.15  | 0.00 | 0.54  | 0.27 |
| **Change**      |        |      |      |     |          |       |      |       |      |
| β (p-value)     | 0.740  | 0.477 | 0.107| 0.70 | 0.00     | 0.15  | 0.00 | 0.54  | 0.27 |
| **p-Value**     | 0.631  | 0.683 | 0.879| 0.477| 0.107    | 0.740 | 0.898| 0.903 | 0.658 |

*Samples with undetectable levels were imputed half of the detection limit; participants with undetectable levels before and after swimming were excluded; Wilcoxon test.
accompanying a previous study that did not find changes in other markers of inflammation in 21 adolescents after they swam in a chlorinated pool (Pedersen et al. 2009). Although the concentrations of cytokines in EBC of our healthy study population were relatively low (< 1 pg/mL), they were detectable in most samples (< 80%). However, partly because of the lack of appropriate reference values, the validity of using these proteins in EBC as markers of acute inflammation in healthy subjects needs to be determined.

The present study has a larger sample size than previous studies with a similar design (n = 48, vs. 30 (Moreira et al. 2008), 29 (Carbonnelle et al. 2002), 21 (Pedersen et al. 2009), and 11 (Carbonnelle et al. 2008)). However, statistical power could still be limited for detecting minor changes in some biomarkers with statistically significance. We selected the timing of sample collection to account for the specific expression dynamics of the different biomarkers and highly controlled this timing during field work. However, available data on expression dynamics of some biomarkers were limited or inconsistent. For example, an increase in FeNO has been observed right after swimming (Carbonnelle et al. 2002) and also 6 hr after mold exposure (Stark et al. 2005). Therefore, undetected changes in some biomarkers due to inappropriate sample timing cannot be ruled out. Although THMs are not irritants and are not likely the putative agents for the respiratory effects associated to the swimming pool environment, we used their occurrence in exhaled breath as a surrogate for DBP dose because THMs are the most prevalent DBPs in swimming pools and are easy to measure in exhaled breath. The observed dichloramine concentrations (0.43 mg/L; Table 1) in water were low compared with other swimming pools using chlorine for disinfection (Weaver et al. 2009). Furthermore, we used the same DPD method for all participants; therefore, the influence of the biases should be minimal. The season when we conducted the study (spring–summer) probably represented lower levels of DBP exposures than would the rest of the year because the facility was highly ventilated with doors and windows opened. Trichloramine in the air ranged from 0.17 to 0.43 mg/m$^3$ (mean, 0.29 mg/m$^3$), which is below the World Health Organization (2006) recommendations of 0.5 mg/m$^3$ but comparable to the study by Carbonnelle et al. (2008).

The measurement of a battery of respiratory biomarkers allowed us to explore short-term respiratory changes that may reflect different mechanisms of respiratory effect in relation to swimming pool exposure. However, there is limited knowledge of the clinical significance to interpret the health impacts of the biomarkers measured.

The present study replicates with a higher sample size the methods of previous studies and provides new evidence on biomarkers not measured previously, including markers in EBC. It suggests for the first time that atopic status and CC16 genotype do not modify the effect of swimming in a pool on the permeability of the lung epithelium. Furthermore, we attempted to disentangle the effects of chemical exposure and exercise by measuring individual exposure to DBPs and energy expenditure during swimming.

The increase in serum CC16 after swimming in a well-maintained and highly ventilated indoor swimming pool confirms the high sensitivity of this assay to detect subtle changes in the concentration of this biomarker after environmental exposures. The fact that we detected no differences in serum CC16 levels by CC16 genotype further supports the hypothesis that

### Table 5. Multiple linear regressions between serum CC16 concentration (µg/L) in relation to a unit increase in indicators of DBP exposure and energy expenditure.

| Model | Variables | β (95% CI) | p-Value | R² | n |
|-------|-----------|------------|---------|----|---|
| 1     | CHClBr$_2$, exhaled breath (µg/m$^3$) | 1.68 (0.93 to 2.43) | < 0.001 | 0.45 | 46 |
|        | Energy expenditure (kcal) | 0.69 (0.09 to 1.28) | 0.024 | |
| 2     | CHCl$_2$, exhaled breath (µg/m$^3$) | 1.49 (0.65 to 2.33) | 0.001 | 0.46 | 46 |
|        | Energy expenditure (kcal) | 0.68 (0.08 to 1.27) | 0.027 | |
| 3     | CHClBr, exhaled breath (µg/m$^3$) | 0.33 (–0.35 to 1.01) | 0.336 | |
|        | Energy expenditure (kcal) | 0.45 (0.01 to 0.89) | 0.047 | 0.26 | 46 |
| 4     | CHClBr, exhaled breath (µg/m$^3$) | 0.35 (–0.09 to 0.78) | 0.117 | 0.34 | 46 |
|        | Energy expenditure (kcal) | 0.87 (0.22 to 1.51) | 0.010 | |
| 5     | CHBr$_3$, exhaled breath (µg/m$^3$) | 0.98 (0.31 to 1.66) | 0.005 | 0.33 | 46 |
|        | Energy expenditure (kcal) | 0.66 (–0.03 to 1.35) | 0.060 | |
| 6     | CHBr$_3$, exhaled breath (µg/m$^3$) | 0.82 (0.16 to 1.48) | 0.016 | 0.39 | 46 |
|        | Energy expenditure (kcal) | 0.61 (–0.05 to 1.28) | 0.070 | |
|        | Free chlorine (mg/L) | 0.69 (0.03 to 1.36) | 0.042 | |
| 7     | TTHMs, exhaled breath (µg/m$^3$) | 0.46 (–0.10 to 1.02) | 0.105 | 0.24 | 46 |
|        | Energy expenditure (kcal) | 0.97 (0.29 to 1.65) | 0.006 | |
| 8     | TTHMs, exhaled breath (µg/m$^3$) | 0.30 (–0.25 to 0.86) | 0.274 | 0.32 | 46 |
|        | Energy expenditure (kcal) | 0.88 (0.22 to 1.53) | 0.010 | |
|        | Free chlorine (mg/L) | 0.73 (0.08 to 1.49) | 0.030 | |
| 9     | Free chlorine (mg/L) | 0.85 (0.16 to 1.54) | 0.017 | 0.28 | 47 |

**Abbreviations:** CHClBr$_2$, dibromochloromethane; CHClBr, bromodichloromethane; CHBr$_3$, bromoform; CI, confidence interval; TTHMs, total THMs. No other variables were included in the models.

![Figure 3. Concentration of FeNO and serum CC16 before (B) and after (A) swimming, stratified by atopic status (median and IQR). The Phadiatop test was used to define atopic status. p-Value from a Mann–Whitney test between atopics and nonatopics: 0.022 for FeNO and 0.560 for CC16.](image-url)
the association is attributable to an increased permeability of the lung epithelium rather than an increase in CC16 synthesis, which may differ by genotypes (Laing et al. 2000). The higher molecular weight of SP-D (130 kDa) (Kishore et al. 2006) compared with CC16 (16 kDa) (Broekaert et al. 2000) probably explains the lack of increase in serum concentration of this protein after swimming because its higher molecular weight would not permit the passive diffusion of the molecule through the epithelial barrier. Previous studies that measured other surfactant proteins (SP-A and SP-B) in serum in similar settings found inconsistent results (Carboneille et al. 2002, 2008).

We assessed the role of atopy as an effect modifier because some epidemiologic studies have reported an increased asthma risk for swimming pool attendance among atopic children (Bernard et al. 2006, 2007, 2008, 2009). Baseline serum CC16 levels and the change after swimming were similar among atopics and nonatopics. Atopic status did not modify the effect of swimming pool exposure on the markers studied or on pulmonary function, in agreement with a previous study (Bonetto et al. 2006). However, atopic participants had higher baseline FeNO levels, and atopy modified the effect of swimming (p = 0.02). FeNO remained unchanged among nonatopics, whereas it tended to decrease among atopics. Moreira et al. (2008) found no changes on FeNO that did not vary by atopic status or asthma in 30 competitive swimmers after a training session.

Among the battery of respiratory biomarkers evaluated, only serum CC16 levels changed significantly after swimming. Given the moderate increase detected (3.3%), the risk of asthma during childhood. Environ Health Perspect 114:1567–1573.

Bernard A. 2007. Chlorination products: emerging links with allergic diseases. Curr Med Chem 14:1771–1782.

Bernard A, Carbonnelle S, de Burbure C, Michel O, Nickmilder M. 2006. Chlorinated pool attendance, atopy, and the risk of asthma during childhood. Environ Health Perspect 114:1567–1573.

Bernard A, Carbonnelle S, Dumont X, Nickmilder M. 2007. Infant swimming practice, pulmonary epithelium integrity, and the risk of allergic and respiratory diseases later in childhood. Pediatrics 119:1095–1103.

Bernard A, Nickmilder M, Voisin C. 2008. Outdoor swimming pools and the risks of asthma and allergies during adolescence. Eur Respir J 32:978–988.

Bernard A, Nickmilder M, Voisin C, Sardella A. 2009. Impact of chlorinated swimming pool attendance on the respiratory health of adolescents. Pediatr Pulmonol 44:1101–1112.

Bonetto G, Corradi M, Carraro S, Zanconato S, Alinovi R, Alivoni R, Foleasini G, et al. 2006. Longitudinal monitoring of lung injury in children after acute chlorine exposure in a swimming pool. Am J Respir Crit Care Med 174:566–574.

Bonipane MR, Morici S, Riccobone L, Profita M, Bonanno A, Patero A, et al. 2003. Airway cells after swimming outdoors or in the sea in nonasthmatic athletes. Med Sci Sports Exerc 35:1146–1152.

Broekaert F, Clippel S, Michels H, Bernards C, Bernard A. 2000. Clara cell secretory protein (CC16): features as a peripheral lung biomarker. AnnNY Acad Sci 923:68–77.

Carbonnelle S, Bernard A, Doyle IR, Grutters J, Francaux M. 2008. Fractional exhaled NO and serum pneumoproteins after swimming in a chlorinated pool. Med Sci Sports Exerc 40:1472–1476.

Carbonnelle S, Francaux M, Doyle I, Dumont X, de Burbure C, Morel G, et al. 2002. Changes in serum pneumoproteins caused by short-term exposure to nitrogen trichloride in indoor chlorinated swimming pools. Biomarkers 7:446–478.

Choi J, Hoffman LA, Rodway DW, Sethi JN. 2006. Markers of lung disease in exhaled breath: nitric oxide. Biol Res Nurs 7:241–255.

Font-Ribera L, Kogevinas M, Zock JP, Nieuwenhuijsen MJ, Heederik D, Villanueva CM. 2009. Swimming pool attendance and risk of asthma and allergic symptoms in children. Environ Res 109:1304–1310.

Goodman M, Hays S. 2008. Asthma and swimming: a meta-analysis. J Asthma 45:639–647.

Grassmann H, Tschiedel E, Grisch M, Kleeper J, Ratjen F. 2007. Exhaled nitric oxide in healthy children after accidental exposure to chlorine gas. Inhal Toxicol 19:895–898.

Hery M, Hecht G, Gerber JM, Gendre J, Hubert G, Rebuffaud J. 1999. Exposure to chloramines in the atmosphere of indoor chlorinated pools. Biomarkers 4:27–39.

Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, et al. 2005. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J 25:523–546.

International HapMap Consortium. 2003. The International HapMap Project. Nature Genet 34:1201–1204.

Jacobs JH, Spaan S, van Roor GB, Meleste F, Zaah VA, Rooyackers JM, et al. 2007. Exposure to trichloramine and respiratory symptoms in indoor swimming pool workers. Eur Respir J 29:490–498.

Kishore U, Greenbrown TJ, Waters P, Shrive AK, Gai R, Kamran MF, et al. 2006. Surfactant proteins SP-A and SP-D: structure, function and receptors. Mol Immunol 43:1293–1315.

Kowalska M, Villanueva CM, Font-Ribera L, Liviuc D, Bustamante M, Espinoza F, et al. 2010. Genotoxic effects in swimmers exposed to disinfection by-products in indoor swimming pools. Environ Health Perspect 118:1531–1537.

Laing IA, Hermans C, Bernard A, Burton PR, Goldblatt J, Le Douteux PN. 2000. Association between plasma CC16 levels, the A38G polymorphism, and asthma. Am J Respir Crit Care Med 161:124–127.

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

Louvencetti C, Ballester C, Fernandez P, Marzo E, Prado C, Peris geographic. J Clin Immunol 123:502–504.

Richardson SD, DeMarini DM, Kogevinas M, Fernandez P, Marzo E, Laursen C, et al. 2010. What's in the pool? A comprehensive identification of disinfection by-products and assessment of mutagenicity of chlorinated and brominated swimming pool water. Environ Health Perspect 118:1532–1537.

Roca J, Sanchis J, Agusti-Vidal A, Segarra F, Navajas D, Rodriguez-Roisin R, et al. 1986. Spirometric reference values from a Mediterranean population. Bull Eur Physiopathol Respir 22:293–303.

Stark HJ, Randell JT, Hirvonen MR, Purokivi MK, Roponen MH, Taksinen HO. 2005. The acute effect of swimming on airway inflammation in adolescent elite swimmers. J Allergy Clin Immunol 116:692–697.

Vaz da, Graca P, Alonso G, Américo A, Lappalainen R, Damkjaer S, 1999. Physical fitness and body weight in a nationally representative sample in the European Union. Public Health Nutr 2:105–113.

Vidal C, Sode F, Bouquete O, Fernandez-Merino MC, Meijeide LM, Rev J, et al. 2005. Evaluation of the phadiatop test in the diagnosis of allergic sensitization in a general adult population. J Invest Allergol Clin Immunol 15:124–130.

Venn WA, Li J, Wen J, Johnston J, Blatchley MR, Blatchley ER III. 2009. Volatile organic compounds and test analysis from chlorinated indoor swimming pools. Water Res 43:3308–3318.

World Health Organization. 2006. Guidelines for Safe Recreational Water Environments, Vol. 1. Swimming Environments. Geneva:World Health Organization. Available: http://www.who.int/water_sanitation_health/bathing/swimfull.pdf [accessed 28 October 2009].