



Risks of new-onset allergic sensitization and airway inflammation after early age swimming in chlorinated pools

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ABSTRACT

Rationale: Irritant chlorination products in swimming pools can cause respiratory problems in swimmers but their possible implication in allergies development is still unclear.

Objectives: To assess prospectively whether early-life attendance at chlorinated pools increases the risks of IgE sensitization and of airways inflammation later during childhood.

Methods: We conducted a two-year prospective study among 196 kindergarten children (mean age of 5.7 years, 54% of boys). We measured exhaled nitric oxide (eNO) and aeroallergen-specific IgE in nasal mucosa. Parents completed a questionnaire about the child's health, chlorinated pool attendance and potential confounders.

Main Results: Ever swimming at indoor or outdoor chlorinated pools before the age of three years was associated with higher odds for new-onset IgE sensitization to house dust mite (adjusted odds ratio [aOR] 2.93, 95% confidence interval [CI] 1.14–7.55) and for new-onset increased eNO (>15 ppb; aOR, 4.54, 95% CI 1.48–13.9). For both outcomes, aORs increased dose-dependently with time spent in chlorinated pools with values reaching, respectively, 3.60 (95% CI 1.21–10.7) and 5.92 (95% CI 1.72–20.5) when the cumulative pool attendance exceeded 60 h. These risks appeared independently of each other, of parental history of allergies and of pre-existing diseases, including eczema, which at baseline was more prevalent in early swimmers (aOR, 2.91; 95% CI 1.23–6.89). Such associations were not seen with IgE sensitization to pollen or cat allergens.

Conclusion: Attendance at chlorinated swimming pools in early life is associated with higher risks of new-onset airways inflammation and IgE sensitization to house dust mite, independently of other risk factors.

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Introduction

Atopic eczema, rhinitis and asthma are the most prevalent chronic diseases in children of the developed world (Mallol et al., 2012). These diseases have usually their onset during early life and then they develop sequentially along a pathway called the allergic or atopic march (Ker and Hartert, 2009; Spergel, 2010; Zheng et al., 2011). Although the links between these different types of allergic disease are not fully understood, the prevailing paradigm assumes that the atopic march starts with atopic dermatitis, then proceeds to allergic rhinitis to end with asthma (Martin et al., 2011).

Factors driving the atopic march and the epidemic of allergies are largely unknown. Current hypotheses postulate that these factors are related to the hygiene practices of the Western world. According to the classical hygiene hypothesis (Strachan, 1989), the increase of allergic diseases in developed countries would be the

consequence of the reduced exposure to certain microbial agents during early life when the immune system is maturing (Strachan, 1989, 2000). The hygiene hypothesis postulates that an insufficient stimulation of the immune system early in life shifts the immune system from a Th1- to a Th2-cell response, which leads to IgE-mediated diseases such as eczema, asthma and hay fever (McGeady, 2004). Some recent studies, however, give reasons for questioning the validity of the hygiene hypothesis as an unifying explanation for the worldwide rise of allergies. Among these, there is the emerging concept that allergic diseases are primarily epithelial disorders driven by airway barrier dysfunction caused by environmental insults (Holgate et al., 1999; Cookson, 2004). Also challenging the hygiene hypothesis, recent studies have failed to confirm the protective role of infectious exposures, older siblings or Bacillus Calmette-Guérin (BCG) vaccination toward the risk of allergic outcomes (Cramer et al., 2012; Flohr et al., 2012; Brunekreef et al., 2012).

Another hypothesis closely linked to hygiene is the “chlorine or chlorination hypothesis”. This hypothesis proposes that the rise of allergic diseases in Westernized countries is, at least partly, driven

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by the increasing and largely uncontrolled exposure of children to chlorination products, especially in swimming pools (Bernard et al., 2003; Bernard, 2007). Because of their strong oxidizing potential, chlorine and its derivatives such as chloramines can open tight junctions of epithelial barriers and thereby promote the allergen delivery to dendritic cells and the mounting of the Th2 immune response. Several studies have indeed shown that chlorinated pool attendance is associated with increased risks of allergic diseases including hay fever, allergic rhinitis and asthma (Bernard, 2007; Bernard et al., 2006; Kohlhammer et al., 2006; Bernard et al., 2009; Cotter and Ryan, 2009; Ferrari et al., 2011). These risks, which increase dose-dependently with the cumulative pool attendance (CPA), appear to largely stem from interactions with the atopic status (Bernard et al., 2006, 2009). To be triggered, these pool chlorine/atopy interactions require thus a pre-sensitization to aeroallergens as well as a cumulative exposure to chlorinated pools lasting at least a few years. These two conditions, with perhaps some misclassification biases (Bernard et al., 2011; Klootwijk and Krul, 2011), might explain the apparent inconsistencies between studies in young children (Schoefer et al., 2008; Font-Ribera et al., 2009, 2011) and those conducted in adolescents or adults (Bernard et al., 2009; Cotter and Ryan, 2009; Ferrari et al., 2011).

The influence of chlorinated pools on the development of allergic diseases might, however, not be limited to interactions promoting the clinical manifestations of atopy (Bernard et al., 2011). Some observations suggest that chlorinated pool attendance might also increase the risks of allergen sensitization and therefore contribute to the development of the atopic status. The attendance at indoor or outdoor chlorinated pools during infancy or early childhood has been associated with an increased risk of house dust mite (HDM) sensitization (Bernard et al., 2008; Jacobs et al., 2012), which is one of strongest predictors of childhood rhinitis and asthma (Lodge et al., 2011). Studies among infant swimmers have also shown that this practice is associated with an increased risk of eczema, one of the earliest signs of the atopic march (Font-Ribera et al., 2009; Chaumont et al., 2012).

As all these findings were based on cross-sectional studies with a retrospective assessment of swimming pool attendance, the possibility of recall bias, exposure misclassification or even of reverse causation cannot be formally excluded. The aim of this prospective study was thus to further assess whether attendance at chlorinated pools early in life may increase the risks of aeroallergen sensitization as well as of increased exhaled nitric oxide (eNO), a predictor also of rhinitis and asthma in children (Olin et al., 2010; Malinovschi et al., 2012).

Materials and methods

Study population

This study was conducted in the frame of a larger study intended to assess the impact of air pollution on the respiratory health of children (Voisin et al., 2010). We aimed to recruit all children in third kindergarten in 30 schools located in the areas of Brussels and Liège in Belgium. An informed consent document and a questionnaire were provided to the parents. Of the 839 children who received these documents, 430 returned the questionnaire and the consent document signed by the parents. These children were examined in schools between December 2007 and March 2008. Two years later, between December 2009 and March 2010, we visited the schools in the same order to re-examine these children. Of the initial baseline population, 128 children had left the schools, which left for the follow-up a population of 302 children. Among these, 236 (78.1%) participated to the study and of them 196 could perform successfully all the tests. Participants and non-participants did not

differ significantly regarding studied outcomes, chlorinated pool attendance and the main risk factors of allergies. All children were examined only with the written informed consent of their parents. In addition, just before performing the tests in schools, we verbally assured to have the assent of each child. The ethics committee of the Faculty of Medicine of the Catholic University of Louvain approved the study protocol, which complied with applicable requirements of the international regulations.

Questionnaire

Parents completed a self-administered questionnaire addressing aspects related to social and medical characteristics of the child and its family, the in- and out-house environment and recreational activities including swimming. For swimming practice, parents were asked to specify the type of swimming pool attended by their child, the type of disinfection method used (some children had access to copper-silver sanitized pools), the frequency of attendance and the age at which their child started to attend the pool on a regular basis. This information served to calculate the cumulative attendance of indoor and/or outdoor chlorinated pools, before the age of 3 years (what was referred to as early swimming), during the two-years follow-up and over lifetime, at the mean age of 5.7 or 7.5 years. The questionnaire also included questions asking whether the child had ever been diagnosed for most common respiratory or allergic diseases, including bronchiolitis, eczema, asthma, hay fever and allergic rhinitis.

Exposure to chlorination and its by-products

In Belgium, public swimming pools (all indoor in the studied areas) are legally required to monitor the microbial and chemical quality of the water by maintaining several parameters within regulatory limits, including free chlorine (0.5–1.5 ppm) and combined chlorine (<0.8 ppm) in water and trichloramine in pool air (<500 µg/m³ in air sampled 1.5 m above the pool surface). There are no specific regulations for privately owned swimming pools, which are disinfected according to the instructions of the chlorine supplier (recommended free chlorine, 1–2 ppm). An additional potential source of exposure to chlorination products for our children was via the use of tap water, in particularly for showering or bathing. Data about chlorine levels in tap water for the years 2003 were provided by the Public Department of Wallonia, the Vivaqua Company and the Brussels Institute for Management of the Environment. Data were allocated to each child according to the postcode of residence of the 63 municipalities studied. The mean concentration of free chlorine in tap water (regulatory limit, 200 µg/l) of our study participants was 65 µg/l, a value more than 10 times lower than levels in swimming pools. The concentration of THM, the major chlorination by-product in tap water, did not exceed 10 µg/l.

Samples collection and analyses

Children were examined in their schools between 9:00 and 13:00. The protocol comprised the measurement of height and body weight, a screening of aeroallergen sensitization using the Rhinostick test, a non-invasive test having a similar sensitivity but a greater specificity than skin-prick tests (Marcucci et al., 2004). The Rhinostick test was calibrated with serum standards and considered positive at specific IgE ≥ 0.35 kIU/l (Marcucci and Sensi, 1989). The concentration of nitric oxide (NO) was measured in exhaled air with the NIOXTM analyzer (Aerocrine AB, Solna, Sweden) by following the guidelines of the American Thoracic Society (1999). For diagnosing increased eNO values, we used a cut-off of 15 ppb

(Buchvald et al., 2005), which in our study corresponded to the 95th percentile of baseline values in non-atopic children.

Statistical analyses

Results were reported as mean $\pm$ SD or as median with interquartile range for non-normally distributed variables. Differences between early swimmers and controls were assessed using the Chi-squared test for binary variables and the Student's *t*-test or the Mann–Whitney *U*-test for continuous variables. Associations between early swimming and outcomes were assessed by logistic regression analyses. We adopted a backward approach that consisted to include all potential control variables and to remove the least significant predictor until the model only contained variables with a *p*-value of <0.20 . We tested 17 potential predictors including age, gender, maternal smoking during pregnancy, preterm birth, delivery by cesarean, breastfeeding, day care attendance, number of older siblings, probiotics, rural versus urban area, vicinity to a busy road (<100 m), parental asthma, parental allergies, high parental educational level (father and/or mother graduate of university or high school), parental smoking, house cleaning with bleach, double-glazed windows, mold on bedroom walls, air fresheners and chlorinated pool attendance (all types of pools combined). We analyzed exposure-response relationships by stratifying children in categories of increasing cumulative pool attendance (CPA). For the attendance at chlorinated pools before the age of three (referred to early swimming), we used as referents children who never attended chlorinated pools before the age of 3 and we dichotomized early swimmers at CPA cut-off values (30 or 60 h) that corresponded approximately to the median (32 h) or twice the median of their CPA. We also selected these two cut-off values because they were respectively equivalent to a 30 min swimming session fortnightly or weekly during three first years of life. As there were only 10 children who never visited chlorinated pools, the CPA during the follow-up or over lifetime was stratified in tertiles of increasing CPA. Results were considered as statistically significant at *p*-values below 0.05. All statistical analyses were performed using StatView (v5.0.1, 3rd ed., a business unit of SAS, 2001).

Results

Table 1 compares the characteristics of children who had attended chlorinated pools before the age of three with their peers who did not, both at baseline and at the end of the two-year follow up period. Early swimmers had on average spent a total of 33 h in indoor or outdoor chlorinated pools, which over a period of three years corresponds approximately to a 30 min swimming session fortnightly. These early swimmers continue to swim more frequently than their peers later so that their CPA during the follow-up and over lifetime was more than twice that of controls. Except for chlorinated pool exposure, early swimmers and their controls were well matched for gender, parental history of atopic diseases and the exposure to indoor or outdoor pollutants. Proportions of children who had been breastfed or had attended a day care center were however higher among early swimmers than in their peers.

At baseline, early swimmers and controls were well matched for sensitization to aeroallergens, the two groups having almost the same prevalences of sensitization to HDM (13.6 vs. 14.3%) and pollen (17.9 vs. 16.1%). There prevalence of elevated eNO in early swimmers (12.1%) was almost twice lower than that of controls (23.2%) but after adjustment for other covariates this difference was not statistically significant. The two groups also did not show any meaningful difference in the baseline prevalences of doctor-diagnosed respiratory or allergic diseases except for eczema

which much more frequent in early swimmers (aOR 2.91, 95% CI 1.23–6.89, $p=0.02$).

Table 3 shows the prevalences of studied outcomes at the end of the two-year follow-up period. Compared to controls, early swimmers were significantly more likely to develop new-onset HDM sensitization (aOR, 2.93, 95% CI 1.14–7.55, $p=0.03$) and increased eNO (aOR, 4.54, 95% CI 1.48–13.9, $p=0.008$). These associations were largely independent of each other, being moderately affected by the mutual exclusion of the two outcomes (aOR for new-onset HDM without increased eNO; 2.95, 0.96–9.02, $p=0.06$; aOR for new-onset increased eNO with no HDM sensitization, 2.96, 0.93–9.44, $p=0.07$). Excluding children with a history of eczema at baseline who were overrepresented among early swimmers did not weaken either these associations between early swimming and new-onset HDM sensitization (aOR, 3.27, 95% CI 1.04–10.3, $p=0.02$) or increased eNO (aOR, 3.52, 95% CI 1.07–11.5, $p=0.04$). Nor were these associations weakened by the exclusion of bronchiolitis, asthma and other pre-existing diseases. There was a trend toward a lower prevalence of new-onset sensitization to pollen among early swimmers but this trend was largely abolished by the exclusion of cases of eczema at baseline (aOR, 0.47, 95% CI 0.12–1.77, $p=0.26$). Because of the pre-existing differences at baseline, this pattern of changes did not really emerge when considering all cases at the end of follow-up. The increase in the prevalence of HDM sensitization in early swimmers was of borderline significance ($p=0.056$) and the increase in eNO completely lost its statistical significance. Whether considering all or new-onset cases, there were no significant differences between early swimmers and controls in the prevalences of doctor-diagnosed allergic rhinitis, hay fever and asthma at the end of the follow-up.

Because remission of allergic sensitization is rather frequent during early childhood, especially in case of mono-sensitization (Matricardi et al., 2009), we examined whether chlorinated pool attendance could influence this phenomenon as well as the possible remission of increased eNO and other outcomes. During the follow-up, sensitization to HDM and to pollen resolved in 15 and 26 children, respectively, and there were 12 cases of remission for increased eNO. Remissions rates for these outcomes were not influenced by the time spent in chlorinated pools during the follow-up (all *p* for trend >0.20) and they did not differ significantly between early swimmers and their controls (57.9 vs 50%; 76.0 vs 77.8% and 38.5 vs 41%, respectively, all $p>0.50$). No associations were seen also between swimming in chlorinated pools and the remission of other outcomes (results not shown).

We pursued our analyses by examining the exposure-response relationships between new-onset cases of allergic sensitization or increased eNO and the early swimming practice using as exposure metric the number of hours children had spent in chlorinated pools before the age of three years. We categorized early swimmers in low versus high or low versus very high CPA using as cutoff points the values of 30 h or 60 h, which approximated respectively the median (32 h) or twice the median of the CPA values of early swimmers before the age of three years. Table 4 shows that for both types of stratification, the adjusted prevalences of new-onset HDM sensitization and elevated eNO increased dose-dependently with the cumulative exposure to chlorinated pools during early life. There were yet some differences between the two outcomes in that the exposure-response relationship emerged at higher CPA for elevated NO than for HDM sensitization. Quite remarkably, all these associations were strengthened by the exclusion of children with reported eczema at baseline or with a parental history of allergic diseases. The same analyses done with new-onset pollen sensitization, which tended to be lower in early swimmers – Table 2, did not reveal any relationship with the time spent of chlorinated pools before three the age of three years (*p* for trend = 0.54 and 0.77 for stratification at a CPA of 30 h or 60 h, respectively). Because

Table 1

Characteristics of children at baseline and follow-up.

	Baseline			Follow-up		
	Controls	Early swimmers	P	Controls	Early swimmers	
N	56	140		56	140	
Boys, N (%)	30 (53.6)	77 (55.0)	0.86			
Age, mean $\pm$ SD, years	5.78 $\pm$ 0.43	5.64 $\pm$ 0.34	0.02	7.54 $\pm$ 0.42	7.45 $\pm$ 0.35	0.08
BMI, mean $\pm$ SD, kg/m ²	21.2 $\pm$ 3.80	20.8 $\pm$ 3.45	0.45	16.8 $\pm$ 2.24	16.7 $\pm$ 2.45	0.64
Parents						
Higher education, N (%)	39 (69.6)	112 (80.0)	0.12			
Parental allergies, N (%)	23 (41.1)	63 (45.0)	0.62			
Parental asthma, N (%)	7 (12.5)	18 (12.9)	0.95			
Early life care						
Smoking during pregnancy, N (%)	4 (7.1)	20 (14.3)	0.17			
Preterm birth, N (%)	12 (21.4)	35 (25.0)	0.60			
Cesarean delivery, N (%)	9 (16.0)	35 (25.0)	0.18			
Breastfeeding, N (%)	40 (71.4)	119 (85.0)	0.03			
Number of older siblings, mean $\pm$ SD	1.12 $\pm$ 1.11	0.87 $\pm$ 0.94	0.11			
Day-care attendance, N (%)	21 (37.5)	96 (68.6)	<0.001			
Environment and lifestyle						
Living in a rural area, N (%)	29 (51.8)	89 (63.6)	0.09	26 (46.4)	58 (41.4)	0.52
Living less than 100 m from a busy road, N (%)	28 (50)	89 (63.6)	0.08	28 (50)	81 (74.3)	0.32
Exposure to furry pets, N (%)	29 (51.8)	71 (50.7)	0.89	27 (48.2)	76 (54.3)	0.44
Probiotics consumption, N (%)	27 (48.2)	54 (38.6)	0.22	35 (66.0)	81 (60.9)	0.51
Parental smoking at home, N (%)	16 (28.6)	38 (27.1)	0.84	14 (25.0)	38 (27.1)	0.76
House cleaning with bleach, N (%)	12 (21.4)	24 (16.1)	0.48	18 (32.1)	34 (24.3)	0.26
Chlorinated pool attendance						
Before 3 years, median (IQR), hours		33 (14.0–81.2)			33 (14.0–81.2)	
Lifetime, median (IQR), hours	26.5 (7.8–70.5)	139 (61.0–240)	<0.001	98.5 (31.5–286)	305 (166–533)	<0.001

early swimmers continued to attend more frequently chlorinated pools than their peers later during their childhood, we repeated the above analyses by testing as independent variable the time spent in chlorinated pool during the follow-up period or after the age of three years. Whether tested alone or together with the CPA before the age of three years, the attendance at chlorinated pools during the follow-up or after the age of three years was never retained as a significant predictor for any studied outcome at a *p*-value threshold of 0.20. Last, we examined whether these risks were different when attending indoor or outdoor chlorinated pools. Given the rather limited size of our population, the numbers of subjects having attended exclusively each type of pool before the age of 3 were small. Nevertheless, it can be seen from [Table 5](#)

that odds for new-onset HDM sensitization and increased eNO were increased by attending outdoor or indoor chlorinated pools (although not always significantly because of the low numbers). As observed when combining both types of pools, associations persisted when excluding children with eczema at baseline and they were noticeably strengthened by the exclusion of children with parental allergies.

Discussion

The results of this study show that early-life attendance at chlorinated pools increases dose-dependently the risks of HDM sensitization and of airways inflammation during childhood.

Table 2

Prevalences and adjusted odds ratios for aeroallergen sensitization, airways inflammation and allergy or respiratory diseases in controls and early swimmers at the mean age of 5.7 years.

	Controls (N = 56)	Early swimmers (N = 140)	Adjusted ORs (95% CI)	P
Sensitization to aeroallergens				
At least one aeroallergen ^a	17 (30.4)	37 (26.4)	0.90 (0.43–1.86)	0.77
House dust mite ^b	8 (14.3)	19 (13.6)	1.12 (0.44–2.82)	0.81
Pollen ^c	9 (16.1)	25 (17.9)	1.18 (0.48–2.93)	0.72
Cat ^d	7 (12.5)	12 (8.6)	0.78 (0.28–2.21)	0.64
Airway inflammation				
Exhaled NO ₂ >15 ppb ^e	13 (23.2)	17 (12.1)	0.45 (0.19–1.09)	0.08
Allergic and respiratory diseases				
Eczema ^f	9 (16.1)	45 (32.1)	2.91 (1.23–6.89)	0.02
Allergic rhinitis ^g	8 (14.3)	16 (11.5)	0.79 (0.29–2.09)	0.63
Hay fever ^h	7 (12.5)	13 (9.3)	0.64 (0.23–1.79)	0.39
Asthma ⁱ	6 (10.7)	10 (7.1)	0.55 (0.17–1.77)	0.32
Bronchiolitis ^j	12 (21.4)	47 (33.6)	1.68 (0.73–3.86)	0.22

Letters indicate covariates for which ORs were adjusted.

^a Preterm birth.

^b Preterm birth, older siblings, probiotics, furry pets.

^c Preterm birth, living less than 100 m from a busy road, day care attendance, gender, parental smoking.

^d Breastfeeding, parental smoking, furry pets.

^e Preterm birth, furry pets, older siblings, day care attendance, raw milk.

^f Older siblings, parental smoking, furry animals, mold in bedroom.

^g Parental asthma, parental allergies, furry pets, day care attendance, bleach, doubled-glazed windows.

^h Preterm birth, BMI, bleach, parental asthma.

ⁱ Preterm birth, parental smoking, parental asthma.

^j BMI, cesarean delivery, probiotics, living less than 100 m from a busy road, breastfeeding, day care attendance, parental asthma.

Table 3
Prevalences and adjusted odds ratios for aeroallergen sensitization, airways inflammation and allergic diseases in controls and early swimmers at the mean age of 7.7 years.

	New-onset cases				All cases			
	Controls	Early swimmers	Adjusted OR (95% CI)	P	Controls	Early swimmers	Adjusted OR (95% CI)	P
Allergic sensitization								
At least one aeroallergen ^a	9/39 (23.0)	39/103 (37.9)	1.44 (0.70–2.95)	0.32	17/56 (30.4)	51/140 (36.4)	1.39 (0.70–2.75)	0.34
House dust mite ^b	7/48 (14.6)	35/121 (28.9)	2.93 (1.14–7.55)	0.03	11/56 (19.6)	43/140 (30.7)	2.15 (0.98–4.71)	0.056
Pollen ^c	9/47 (19.1)	10/115 (8.7)	0.40 (0.15–1.09)	0.07	11/56 (19.6)	16/140 (11.4)	0.49 (0.22–1.26)	0.11
Cat ^d	5/49 (10.2)	10/128 (7.8)	1.04 (0.28–3.81)	0.95	6/56 (10.7)	10/140 (7.1)	0.74 (0.23–2.33)	0.60
Airway inflammation								
Exhaled NO>15 ppb ^e	5/43 (11.6)	37/123 (30.1)	4.54 (1.48–13.9)	0.008	13/56 (23.2)	47/140 (33.6)	1.74 (0.81–3.72)	0.15
Allergic diseases								
Eczema ^f	3/47 (6.4)	4/95 (4.2)	0.80 (0.14–4.57)	0.80	6/56 (10.7)	24/140 (17.1)	1.60 (0.57–4.50)	0.38
Allergic rhinitis ^g	2/48 (4.2)	3/123 (2.5)	0.49 (0.07–3.21)	0.45	3/56 (5.4)	9/139 (6.5)	1.72 (0.37–7.92)	0.49
Hay fever ^h	5/49 (10.2)	7/127 (5.5)	0.75 (0.20–2.83)	0.67	10/56 (18.2)	14/140 (10.0)	0.55 (0.22–1.37)	0.20
Asthma ⁱ	2/50 (4.0)	2/130 (1.5)	0.35 (0.05–2.67)	0.50	5/56 (8.9)	9/140 (6.4)	0.79 (0.21–2.97)	0.73

Letters indicate the covariates for which ORs were adjusted.

^a New-onset cases: mold in bedroom, gender, probiotics; all cases: probiotics.

^b New-onset cases: preterm birth, mold in bedroom; all cases: probiotics, mold in bedroom, living less than 100 m from a busy road.

^c New-onset cases: probiotics; all cases: house cleaning with bleach, probiotics.

^d New cases: probiotics, mold in bedroom, urban area, double-glazed windows, bleach, day care attendance, smoking during pregnancy, parental asthma; all cases: bleach, probiotics; day care attendance.

^e New cases: preterm birth, older siblings, mold in bedroom, house cleaning with bleach, smoking during pregnancy, parental allergies, parental smoking; all cases, urban area, house cleaning with bleach, cesarean delivery, preterm birth, parental allergies, parental smoking.

^f New cases: BMI, bleach, gender, parental allergies; all cases, bleach, smoking during pregnancy, parental allergies, rural vs urban.

^g New cases, birth at term, furry pets; all cases, mold, traffic pollution, gender, parental asthma.

^h New cases, BMI, rural vs urban, day care; all cases: parental asthma, furry pets, parental smoking, living less than 100 m from a busy road, rural vs urban, probiotics.

ⁱ New cases, parental asthma; all cases, parental asthma, BMI, day care, birth at term, probiotics.

Children having spent more than 30 h in chlorinated pools before the age of three, i.e. the equivalent of a swimming session fortnightly, were respectively 3.3 and 4.7 times more likely to be sensitized to HDM and to have elevated eNO than their peers who had never attended chlorinated pools at the same age. Odds of developing these two outcomes increased up to 3.6 and 5.9, respectively, when the CPA before the age of three exceeded 60 h, which roughly corresponds to a weekly swimming session. These respiratory effects were largely independent of each other and also of traditional risks factors of allergic diseases, including parental allergies and eczema.

Our study confirms the observations of cross-sectional studies suggesting that the early attendance at chlorinated pools increases the risk of HDM sensitization (Bernard, 2007; Bernard et al., 2008; Jacobs et al., 2012). Selection bias is unlikely to explain our results as at baseline early swimmers and their controls were well matched for HDM sensitization as well as for the parental history of allergic diseases. Regarding the risk of elevated eNO, the situation is less clear-cut in that early swimmers at baseline showed a non-significant trend toward a lower prevalence for this outcome. Explanation for this difference, which persisted after adjustment for all possible confounders, is unclear. This difference might arise by chance but it might also be linked to early swimming since a decrease of exhaled NO has been observed after a swimming session in a chlorinated pool (Carbonnelle et al., 2008). This would mean that exhaled NO could present a biphasic response to chlorinated products, an early decrease due to the inhibition of NO production followed after prolonged exposure by an increase as a consequence of the airways inflammation. The case for implicating chlorinated pools in the higher prevalence of eczema in early swimmers at baseline is by contrast much stronger as this has already been reported in two cross-sectional studies (Font-Ribera et al., 2009; Chaumont et al., 2012). Furthermore, it is well known that chlorine and its by-products can irritate the skin and cause epithelial barrier defects that might facilitate skin sensitization or exacerbate eczema symptoms (Chaumont et al., 2012).

Our findings also highlight the importance of conducting prospective studies and above all of using appropriate exposure

and outcome indicators when investigating the respiratory effects of chlorinated pools. The comparison between early swimmers and non-swimmers at the mean age of 5.7 or 7.5 years did not show any significant increase in the prevalences of allergic sensitization or elevated eNO in early swimmers, despite the fact that risks for these two outcomes were multiplied by 3 to 4 in the course of the follow-up. But even in the prospective analysis of our data, effects of early swimming would have also passed undetected with endpoints based on doctor-diagnosed asthma or on the prevalence of atopy defined as sensitization to any airborne allergen. With respect to exposure, our study confirms the importance of a very precise estimation of CPA during early life as associations with allergic sensitization and other outcomes emerge after only a few tens hours of CPA. To be accurate, the estimated CPA must take into account the time spent in both indoor and outdoor chlorinated pools as chlorine-compounds irritating the airways of swimmers are present in the water and in the air at the surface of both types of pools. These critical issues regarding exposure and outcome indicators might explain the negative findings of some studies. For instance, the prospective study of Font-Ribera et al. (2011) based on data collected in the 1990s did not include any specific question about the attendance of chlorinated pools before the age of 4. Before the age of 4 years, children were thus classified as ever swimmer only when in open answer to a question about “other activities” the mother wrote “swimming” (information given in the supplemental material). The estimation of CPA between age of 4 and 7 was probably also incomplete as assessment was based only on swimming during term, thus with school. In addition, this prospective study did not look specifically to the risks of sensitization to HDM, the only type of allergy that so far has been associated with early swimming. These considerations might also apply to another study of the same group (Font-Ribera et al., 2009), in which attendance at outdoor or indoor swimming pools were examined independently of each other and not combined or mutually excluded in order to avoid exposure misclassification. Of course, inconsistencies between studies might be simply related to differences in exposure levels as standards for managing water and air quality of swimming pools greatly vary between countries (WHO, 2006). Observations in Germany (Schoefer et al., 2008) may

Table 4
New-onset cases of sensitization to HDM and of increased exhaled NO according to the total time spent in chlorinated pools before the age of three years in all children and in children with no eczema at baseline and no parental history of allergic diseases.

	All			No eczema at baseline			No parental history of allergies		
	n/N (%)	Adjusted OR (95% CI)		n/N (%)	Adjusted OR (95% CI)		n/N (%)	Adjusted OR (95% CI)	
New-onset HDM sensitization^a									
Never	7/48 (14.6)	1.00		4/41 (9.8)	1.00		4/28 (14.3)	1.00	
1–30 h	15/57 (26.3)	2.60 (0.91–7.38)		8/39 (20.5)	2.38 (0.65–8.78)		7/33 (21.2)	2.35 (0.53–10.4)	
>30 h	20/64 (31.3)	3.27 (1.18–9.07)		15/47 (31.9)	4.08 (1.21–13.7)		13/37 (35.1)	4.12 (1.00–17.0)	
<i>P for trend</i>	0.047			0.01			0.049		
1–60 h	21/76 (27.6)	2.63 (0.98–7.12)		12/52 (23.1)	2.63 (0.77–8.98)		11/46 (23.9)	2.55 (0.63–10.3)	
>60 h	14/45 (31.1)	3.60 (1.21–10.7)		11/34 (32.4)	4.46 (1.24–16.0)		9/24 (37.5)	5.02 (1.11–22.7)	
<i>P for trend</i>	0.06			0.02			0.053		
New-onset exhaled NO > 15 ppb^b									
Never	5/43 (11.6)	1.00		4/37 (10.8)	1.00		2/25 (8.0)	1.00	
1–30 h	17/59 (28.8)	4.41 (1.33–14.7)		11/40 (27.5)	3.34 (0.92–12.1)		11/33 (33.3)	11.8 (1.62–85.7)	
>30 h	20/64 (31.3)	4.66 (1.42–15.2)		14/45 (31.1)	3.68 (1.02–13.2)		14/37 (37.8)	15.1 (2.04–111)	
<i>P for trend</i>	0.03			0.04			0.01		
1–60 h	21/78 (26.9)	3.88 (1.21–12.4)		13/51 (24.5)	2.92 (0.83–10.3)		13/45 (28.9)	9.54 (1.32–68.8)	
>60 h	16/45 (35.2)	5.92 (1.72–20.5)		12/34 (35.3)	4.64 (1.23–17.5)		12/25 (48.0)	27.9 (3.36–332)	
<i>P for trend</i>	0.01			0.02			0.002		

^a Adjusted for preterm birth, mold in bedroom.

^b Adjusted for preterm birth, older siblings, mold in bedroom, bleach, smoking during pregnancy, parental allergies, parental smoking.

also be explained by the fact that standards for free and combined chlorine (0.3–0.6 and <0.2 mg/l, respectively) are much lower in Germany than countries where respiratory effects of swimming pools have been reported.

The explanation for the different patterns of effects associated with chlorinated pools probably lies in the fact that allergic diseases develop in several steps, each step requiring a certain time to be realized, depending on the exposure to the triggers and the natural course of the disease. Current evidence suggests that chlorine-based irritants in swimming pools promote the development of allergic diseases by two different modes of actions (MOA). The first MOA is an interaction with atopic status promoting the clinical expression of allergic rhinitis and asthma. This interaction, which by definition can be triggered only in swimmers sensitized to aeroallergens, requires a CPA of at least several hundreds of hours. Such a CPA is unlikely to be achieved before adolescence in most recreational swimmers, explaining why early swimming was not associated with clinically diagnosed allergic diseases in very young children, in our study as well as in studies conducted by others (Schoefer et al., 2008; Font-Ribera et al., 2009, 2011). The second mode of action is an interaction between chlorinated pool attendance and allergen exposure, which promotes allergic sensitization. This second MOA can be triggered in early life after a CPA of a few tens of hours. However, to be effective, this MOA requires a concomitant and sufficient exposure to allergens, two conditions that are more easily met with perennial allergens such as HDM than with seasonal allergens linked to pollination. This probably explains why the increased HDM sensitization associated with early swimming is observed after a time lag time of a few years and also why sensitization to pollen is not increased in early swimmers. Differences in the exposure patterns between these allergens probably also explain why remission of sensitization observed during the follow-up was more frequent with pollen than with HDM. Even though they operate at different stages of the allergic march, these two MOA probably share the same basic mechanism i.e. which is a disruption of the airways epithelial barriers by chlorine-based oxidants (Bernard et al., 2003; Bernard, 2007; Carbonnelle et al., 2002; Lagerkvist et al., 2004; Bougault et al., 2012). The types of epithelial defects in epithelial barrier enhancing allergic sensitization and its clinical manifestations are largely unknown. There is, however, some evidence from biomarker studies that epithelial defects promoting allergic sensitization might consist in an increased epithelial hyperpermeability facilitating the allergen delivery to dendritic cells associated with a dysfunction or dedifferentiation of epithelial cells secreting immunomodulatory proteins, in particular the Clara cells (Sardella et al., 2012a,b).

Our study has some limitations. The main limitation is the relatively small size of the cohort and in particular of the group used as reference. Because of the increasing popularity of swimming in affluent countries and the widespread use of chlorine to sanitize swimming pools, it has become increasingly difficult to recruit children having never attended chlorinated pools during early life. These limitations, however, have been partly compensated by the prospective design of our study and the use of accurate and sensitive effect and exposure indicators. Another limitation is the lack of data regarding the concentrations of chlorine and its by-products in the outdoor and outdoor swimming pools attended by the children. By contrast, the fact that tap water in Belgium is disinfected with very low level of chlorine has probably eliminated a source of confounding. These methodological strengths along with the relatively high prevalences of studied outcomes explain why our study could evidence significant exposure-response relationships despite a relatively limited sample size.

In conclusion, attendance at chlorinated swimming pools in early life is associated independently with higher risks of developing IgE sensitization to HDM and an increased exhaled NO, which

Table 5

New-onset cases of sensitization to HDM and of increased exhaled NO associated with the attendance at indoor or outdoor chlorinated pools before the age of three years, in all children and in children with no eczema at baseline or with no parental history of allergic diseases.

	Attendance at chlorinated swimming pools before the age of three years							
	Never		Indoor pools only			Outdoor pools only		
	n/N (%)	OR	n/N (%)	OR (95% CI)	P	n/N (%)	OR (95% CI)	P
New-onset HDM sensitization ^a								
All subjects	7/48 (14.6)	1.00	11/40 (27.5)	2.70 (0.88–8.25)	0.08	9/30 (30.0)	3.06 (0.94–10.0)	0.06
No eczema at baseline	4/41 (9.8)	1.00	7/20 (35.0)	2.66 (0.69–10.2)	0.16	7/24 (29.2)	5.15 (1.24–21.3)	0.02
No parental allergies	4/28 (14.3)	1.00	7/24 (29.2)	3.10 (0.68–13.5)	0.15	4/17 (23.5)	2.71 (0.49–14.8)	0.25
New-onset exhaled NO>15 ppb ^b								
All subjects	5/43 (11.6)	1.00	17/40 (42.5)	8.36 (2.27–30.8)	0.001	5/28 (17.8)	1.90 (0.43–1.50)	0.40
No eczema at baseline	4/37 (10.8)	1.00	12/29 (41.4)	8.04 (1.87–34.6)	0.005	4/20 (20.0)	1.64 (0.32–8.28)	0.55
No parental allergies	2/25 (8.0)	1.00	11/13 (84.6)	60.0 (3.0–1400)	0.008	5/14 (35.7)	27.2 (1.45–509)	0.03

^a Adjusted for preterm birth, mold in bedroom.

^b Adjusted for preterm birth, older siblings, mold in bedroom, bleach, smoking during pregnancy, parental allergies, parental smoking.

are among the strongest predictors of rhinitis and asthma (Olin et al., 2010; Malinowski et al., 2012). If one adds to these findings observations from previous studies showing that pool chlorine interacts with atopic status to increase the risks of eczema, allergic rhinitis and asthma, this suggests that exposure to chlorine-based oxidants in swimming pools is a potent driver of the allergic march, promoting IgE sensitization at an early stage and later the clinical manifestations of atopy. These findings stress the importance of minimizing exposure of young children to chlorine and its by-products in swimming pools by a strict regulation of these chemicals or the use of alternative disinfection methods.

Author contributions

CV recruited the children, organized their examination in schools, performed the collection and analysis of data and contributed to the writing of the manuscript. AS was responsible for the Rhinostick tests both in the field and in the laboratory. AB provided the scientific guidance of the project, participating to the study design, the data analysis and interpretation and the writing of the manuscript.

Conflict of interest

None of the authors has a conflict of interest to disclose.

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