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Abstract

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Fractional Exhaled NO and Serum Pneumoproteins after Swimming in a Chlorinated Pool

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$^1$School of Public Health, Faculty of Medicine, Catholic University of Louvain, Brussels, BELGIUM; $^2$Departments of Human Physiology and Critical Care Medicine, School of Medicine, Flinders University, Adelaide, AUSTRALIA; $^3$Department of Pulmonary Diseases, Heart Lung Centre Utrecht, St. Antonius Hospital, Nieuwegein, THE NETHERLANDS; $^4$Institute of Physical Education and Rehabilitation, Faculty of Medicine, Catholic University of Louvain, Louvain-la-Neuve, BELGIUM

ABSTRACT

CARBONNELLE, S., A. BERNARD, I. R. DOYLE, J. GRUTTERS, and M. FRANCAUX. Fractional Exhaled NO and Serum Pneumoproteins after Swimming in a Chlorinated Pool. Med. Sci. Sports Exerc., Vol. 40, No. 8, pp. 1472–1476, 2008. Purpose: The purpose of this study was to examine whether a swimming session performed in a pool sanitized with chlorine-based agents induces lung inflammation, modifies lung epithelium permeability, and alters lung function. Methods: Eleven volunteers performed two standardized swimming sessions: one in a nonchlorinated indoor swimming pool and the other one in a chlorinated indoor pool. Lung inflammation was assessed by fractional exhaled nitric oxide (FE\textsubscript{NO}). Changes in lung epithelium permeability were estimated by measuring the surfactant-associated proteins Type A and Type B (SP-A and SP-B), the Clara cell protein (CC16), and the Krebs von den Lungen-6 protein (KL-6). Lung function tests were also performed. All measurements were carried out in basal conditions, after training completion and 3 h postexercise. Nitrogen trichloride (NCl\textsubscript{3}), the most concentrated gas derived from pool water chlorination, was measured in each pool during the swimming sessions. Results: NCl\textsubscript{3} ranged from 160 to 280 \textmu g m\textsuperscript{-3} in the air of the chlorinated pool and was undetectable in the nonchlorinated one. Lung function was affected neither by the exercise session nor by the type of sanitation. Serum pneumoproteins were unchanged excepted SP-A which decreased by 8% after exercise in the chlorinated pool ($P < 0.05$). FE\textsubscript{NO} increased by 34% ($P < 0.05$) after exercise in the nonchlorinated pool, whereas it was unaffected in the chlorinated one. Conclusions: At concentrations lower than 300 \textmu g m\textsuperscript{-3}, NCl\textsubscript{3} in an indoor chlorinated pool, does not produce short-term changes in lung function or in epithelial permeability. The unchanged FE\textsubscript{NO} found in the chlorinated pool after exercise suggests that chlorination might inhibit NO-induced vasodilation observed during exercise. Key Words: NITROGEN TRICHLORIDE, CHLORINE-BASED DISINFECTANTS, CC16, SP-A, SP-B, KL-6

Swimming pools are mainly sanitized with chlorine-based agents, which release hypochlorous acid, a powerful oxidant working as an active biocide. When reacting with organic matter brought by swimmers, hypochlorous acid releases various by-products, among which the most concentrated in the air of swimming pools is trichloramine or nitrogen trichloride (NCl\textsubscript{3}) (22), a gas with high oxidative properties at the origin of the typical smell of chlorinated pools. Depending on the chlorine dose, pool occupancy, swimmers’ hygiene, water and air temperature, and renewal, NCl\textsubscript{3} concentrations in public pools are extremely variable. Average concentrations typically vary between 300 and 500 \textmu g m\textsuperscript{-3}, but concentrations up to 2000 \textmu g m\textsuperscript{-3} can be found in some recreational or poorly ventilated pools, particularly at times of maximal occupancy (8,20–22,27).

There is mounting evidence that chlorine-based disinfectants can cause detrimental effects on the airways of swimmers and other pool attendees. Several studies documented a higher prevalence of bronchial hyperreactivity (15,34) and sensitization to aeroallergens (34). A higher prevalence of respiratory problems was found in lifeguards and other pool workers compared to the general population (21,25). Studies recently conducted in schoolchildren showed that the cumulated pool attendance was associated with an increased permeability of the lung epithelium, a loss of its integrity, and a risk to develop asthma (4).

Little is known about the exact chlorine compounds responsible for these effects and even less about the exposure level from which these chemicals might affect respiratory tract. Currently, the most suspected causative agent is NCl\textsubscript{3} that was identified as a cause of asthma in lifeguards (31).
and of respiratory ailment in pool workers (21). In a previous study, we found that concentrations ranging from 355 to 490 μg m⁻³ induced a transient leakage of pneumocytes in serum in trained and recreational swimmers (8). With the objective to establish a safe exposure level to this gas, we have examined whether similar effects can be induced by a swimming session performed in a pool atmosphere containing less than 300 μg m⁻³ NCl₃, a level that might serve as a basis of a guideline.

METHODS

Subjects. The study protocol was approved by the ethical committee of the Medicine Faculty of the Catholic University of Louvain, Brussels, Belgium, and the experiments were conducted according to the principles and rules laid down in the Declaration of Helsinki and its subsequent amendments. Volunteers were healthy young females (n = 7, 22.0 ± 1.3 yr, 56.2 ± 6.4 kg, and 164.9 ± 5.2 cm) and males (n = 4, 21.9 ± 2.0 yr, 72.5 ± 6.8 kg, and 179.1 ± 4.1 cm) practicing sports on a regular basis (about 4.1 cm) for about 1 h wk⁻¹) but not specifically trained for swimming exercise (about 1 h wk⁻¹). All subjects were nonsmokers, did not experience any infection of the respiratory tract during the week preceding the experiments, and none of them had ever complained of asthma. All subjects gave a written informed consent before participating to the study.

Protocol. The study was conducted in a cross-over fashion. All subjects participated twice to a standardized 45-min swimming session consisting of 11 exercise bouts, representing a total of 1300 m. One session took place in a nonchlorinated indoor pool sanitized by a copper silver process. Briefly, a low-potential electric current that circulates between electrodes produces copper and silver ions ensuring both flocculation and disinfection of water. The formation of a copper hydroxide flocculate eliminates colloids in suspension (organic matter). The copper concentration in water ranged from 400 to 600 μg L⁻¹, and the silver concentration ranged from 10 to 15 μg L⁻¹, values largely below the drinking water quality guideline of WHO. The other pool was an indoor pool sanitized by sodium hypochlorite. The two sessions were separated by 1 wk and were performed at the same time of the day to avoid diurnal variations. For obvious reasons, the subjects were not blinded regarding the pool atmosphere. Due to time constraints and limited accessibility of one of the swimming pools, the exposure conditions were not randomized. Subjects swam first in the nonchlorinated pool. They were asked to abstain from exercising for at least 48 h before each experimental session. Before exposure to the pool air, a blood sample was taken, the fraction of nitric oxide was measured in exhaled air (FeNO) and lung function was tested by spirometry. Then, the volunteers performed the standardized swimming session. Blood sampling, measurements of FeNO, and lung function tests were performed again after completion of the swimming session and 3 h postexercise. The measurements were not carried out in the swimming pool but in a room outside the swimming pool.

NCl₃ in pool air. Mean NCl₃ concentrations in the pool atmosphere were measured during the experiments using the method described by Hery et al. (20). In this case, air samples were collected at 20 cm above the water surface for 105 min. Ambient air was aspirated through glass fiber filter paper (37 mm diameter; GF/C Whatman) impregnated with a solution of sodium carbonate and trioxide diarsenic, with small vacuum pumps (du Pont S-2500; du Pont De Nemours & Co, Kennett Square, PA). The flow rate was 1.0 L min⁻¹. The impregnated filter was desorbed in 10 mL twice distilled water, and the final solution passed through a cation exchange resin to eliminate carbonate that impairs the analysis. NCl₃ concentration of the solution was measured by liquid chromatography, with NCl₃ accounting for about 90% of measured chloramines. The detection threshold of this HPLC method was typically 20 μg m⁻³.

Lipopolysaccharides in water and in air. Lipopolysaccharides (LPS; building up bacteria wall) were measured in water and in air of the chlorinated pool by the kinetic quantitative chromogenic Limulus amebocyte lysate assay (Kinetic-QCL™ Kit; BioWhittaker, Verviers, Belgium) to exclude a confounding effect due to the presence of endotoxins. Height and duration of air sampling were the same as for NCl₃ (13).

Pool water osmolality. Osmolality of pool water was determined by the freezing-point depression method using an automatic micro-osmometer (Type 13/13DR; Hermann Roebling Messtechnik, Berlin, Germany).

Spirometry. A Vitalograph spirometer (Buckingham, UK) was used to assess the vital capacity (VC), the forced vital capacity (FVC), the forced expiratory volume in 1 s (FEV₁), and the peak expiratory flow (PEF). Each testing session consisted of at least three measurements. VC, FVC, FEV₁, and PEF values were the best of the three acceptable ones according to the American Thoracic Society (30). Results are expressed as the percentage of predicted values calculated according to the equations recommended by the American Thoracic Society (30).

Fractional exhaled NO. FeNO was measured by chemiluminescence with a NIOX online analyzer (Aerocline, Solna, Sweden) with a single-breath online method according to the American Thoracic Society guidelines (1). Subjects inhaled NO-free air and exhaled through a dynamic flow restrictor. They were asked to exhale to residual volume, to insert the mouthpiece, to inhale to total lung capacity through an NO filter, and then to exhale into the device for 10 s at constant flow of 50 mL s⁻¹ with the aid of a visual feedback on a computer screen.

Serum pneumoproteins. Blood samples (7.5 mL) were obtained by venipuncture and collected on dry tubes. Each sample was allowed to clot for a minimum of 12 h at 4°C. Samples were then centrifuged at 2000g for 10 min,
TABLE 1. Lung function in basal conditions and after a standardized exercise session in a nonchlorinated and in a chlorinated swimming pool.

<table>
<thead>
<tr>
<th></th>
<th>Nonchlorinated Pool</th>
<th>Chlorinated Pool</th>
<th>ANOVA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Basal Condition</td>
<td>Postexercise</td>
<td>3 h Postexercise</td>
</tr>
<tr>
<td>VC (% pred.)</td>
<td>102 (82–120)</td>
<td>103 (84–117)</td>
<td>103 (85–114)</td>
</tr>
<tr>
<td>RVC (% pred.)</td>
<td>102 (79–115)</td>
<td>103 (82–111)</td>
<td>103 (86–115)</td>
</tr>
<tr>
<td>REV,VC (% pred.)</td>
<td>99 (86–111)</td>
<td>99 (92–108)</td>
<td>99 (88–113)</td>
</tr>
<tr>
<td>PEF (% pred.)</td>
<td>100 (87–119)</td>
<td>98 (79–112)</td>
<td>100 (83–120)</td>
</tr>
</tbody>
</table>

*Data are reported as arithmetic means (min–max).*

% pred. indicates percentage of predicted values.

and serum was decanted and stored at –18°C until protein analysis. Clara cell protein (CC16) concentration was determined by latex immunoassay using rabbit anti-Clara cell protein antibody (Dakopatts, Glostrup, Denmark) and CC16 purified in our laboratory as the standard (5). All samples were run in duplicate at two different dilutions. This assay has been validated by comparison with a monoclonal antibody-based ELISA (16). The between- and within-run coefficients of variation ranged from 5% to 10%. The concentrations of surfactant-associated proteins Type A and Type B (SP-A and SP-B) were determined with ELISA inhibition assays using polyclonal antibodies raised against alveolar proteinosis-derived SP-A and mature SP-B, respectively (12). These antibodies did not react with other plasma or alveolar epithelial lining fluid antigens and the assays had between- and within-run coefficients of variation lower than 10%. The level of Krebs von den Lungen-6 protein (KL-6) was measured by a sandwich-type ELISA technique using a KL-6 antibody kit (ED046; Eisai, Tokyo, Japan) (24).

All samples were run in duplicate, and mean values were used for subsequent analyses.

Statistics. Significance of differences observed between means was assessed by a two-way repeated-measures ANOVA design with swimming pool (chlorinated or not) as the first factor and time of testing (basal condition, postexercise, and 3 h postexercise) as the second one. When applicable, Bonferroni test was used as a post hoc analysis. Significance threshold was set at $P < 0.05$. The results are presented as means ± SEM.

RESULTS

NCl3 in pool air. NCl3 concentration during the experiments in the chlorinated swimming pool ranged from 160 to 280 μg m–3, whereas the air of the copper/silver pool contained less than 20 μg m–3, which was the detection limit of the analytical procedure.

LPS in water and in air. In the chlorinated swimming pool, LPS content was 0.60 ± 0.704 EU m–3 in air and 2.05 ± 0.200 EU m–3 in water. These low values indicated no significant contamination by this endotoxin potentially harmful to the lung.

Pool water osmolality. The osmolality of pool water was 15 mOsm L–1 in the nonchlorinated pool and 31 mOsm L–1 in the chlorinated pool.

Spirometry. In basal conditions, spirometry testing values were in the ranges accepted for young healthy subjects (30) (Table 1). In both pools, lung function was not modified throughout the experiment compared with the basal conditions. Each time point studied did not show any difference between pools.

Fractional exhaled NO. As shown in Figure 1 and in Table 2, FEENO did not change in the same way between pre and postswimming in the two pools (time \times pool: $P = 0.002$). Exhaled NO was clearly increased in the copper/silver pool (+34%, $P < 0.05$), whereas it did not change in the chlorinated pool. In both pools, FEENO was not different 3 h postswimming compared to preexercise conditions.

Serum pneumoproteins. Serum concentrations of pneumoproteins are presented in Table 2. CC16, SP-B, and KL6 did not change throughout the experiment, suggesting that neither the exercise session nor the environmental conditions affected the permeability of the pulmonary epithelium. SP-A evolved differently between pools as depicted by the significant interaction of time \times pool in the ANOVA ($P = 0.031$). SP-A decreased by 8% after swimming exercise in the chlorinated pool ($P < 0.05$), whereas it remained unchanged in the nonchlorinated pool. This decrease was, however, transient, because 3 h after exercise completion, SP-A concentration in serum recovered to baseline values.

DISCUSSION

This study shows that exercise performed in a pool air containing less than 300 μg m–3 does not induce any increase in the serum level of KL-6, SP-A, SP-B, and

FIGURE 1—Individual FEENO values after a standardized exercise session in a nonchlorinated (left panel) and a chlorinated swimming pool (right panel). (*) indicates a significant difference with basal conditions in the same swimming pool ($P < 0.05$); $\$, a significant difference with the nonchlorinated swimming pool at the same time; BE, basal condition; PE, postexercise.
CC16. KL-6 is a mucinlike glycoprotein expressed on alveolar Type II and bronchiolar epithelial cells, which can be a useful marker of pulmonary epithelial cell injury because of its overexpression in injured regenerating lung epithelium (23). The lack of change of this serum marker in both types of pools suggests that short-term exposure to chlorine compounds and/or a single swimming session is not associated with pulmonary cell damage. By contrast, the surfactant-associated proteins Type A and Type B (SP-A and SP-B) are lung secretory proteins that have been validated as markers of lung epithelium permeability (17). A modest decrease in SP-A was found (−8%) after exercise in the chlorinated pool, but this small change, not crucial in biological terms, was not confirmed by SP-B, a marker that usually shows the same pattern of response as SP-A (18). These results indicate that under the exposure conditions of the present study, chlorine compounds inhaled by exercising swimmers do not cause any detectable leakiness of the bronchoalveolar-capillary barriers. This contrasts with previous experiments conducted with higher levels of air pool NCl3 where a dose- and a time-dependent disruption of the epithelium was evidenced using the same markers (8). The 16-kDa Clara cell secretory protein is another lung marker that can be used to assess noninvasively the lung epithelium permeability (19). Changes in serum CC16 level reflect the number of Clara cells lining the airways or the epithelial barrier permeability depending on the type of toxicant and of exposure conditions. The absence of changes in this marker provides further evidence that a swimming session performed in a pool atmosphere containing less than 300 μg m⁻³ does not acutely affect the lung epithelium.

In both pools, lung function was clearly unchanged throughout the experiment, and no subject reported any symptoms of respiratory tract irritation.

The most significant finding of this study is the inhibition of the exercise-induced NO production observed when swimming in the chlorinated pool. Studies focused on changes in $\text{FE}_{\text{NO}}$ during exercise provided inconsistent results with increases, decreases, or even no changes of $\text{FE}_{\text{NO}}$ depending on the health status of the subjects, the nature, the duration, and the intensity of exercise (7,9,10,27,28).

In the present study, we report an increase in $\text{FE}_{\text{NO}}$ after the exercise session performed in the nonchlorinated pool (+34%, $P < 0.05$), whereas this rise was completely blunted in the chlorinated one (Fig. 1). The increase in $\text{FE}_{\text{NO}}$ after exercise in the nonchlorinated pool remained less than the values observed during severe lung inflammation (32) suggesting a physiological role of NO during exercise (2). Bonsignore et al. (7) reported a decreased $\text{FE}_{\text{NO}}$ after swimming in a chlorinated pool, whereas $\text{FE}_{\text{NO}}$ remained unchanged in seawater. The difference was explained by a higher osmolality of seawater. Because the osmolality of water in both chlorinated and nonchlorinated pools was low, this hypothesis may not be retained to explain the results of the present study. The destruction of NO by some chlorine compounds appears unlikely, especially because the subjects were examined in a room outside the swimming pool. A quite plausible explanation is supported by a recent study demonstrating that l-arginine chlorination results in the formation of a nonselective nitric oxide synthase inhibitor (33). Whatever the exact mechanism, the results of the present study suggest that chlorinated sanitation of swimming pools can interfere with the ability of the lung to produce NO. Our findings are consistent with the observations of Bonetto et al. (6) who reported a decreased $\text{FE}_{\text{NO}}$ in the acute phase after accidental exposure of children to high chlorine concentrations. On the contrary, other studies reported an increased $\text{FE}_{\text{NO}}$ after similar incident (14) or after long-term exposure to chlorine (3). The reason for these differences is unclear but may be related to the exposure conditions, in particular, the duration of exposure, the type, and the concentration of chlorine compounds in the pool air.

A potential limitation of the present results might be the relatively small size of the studied group ($N = 11$). The number of subjects was virtually the same as in our previous study in which higher concentrations of NCl3 were found to cause significant changes in lung epithelium biomarkers (8). Studies investigating the short-term effects of toxic air pollutants such as ozone are usually based on similar sample sizes (11,26). It should be stressed also that the cross-over design and the battery of sensitive tests used in the present study are conditions that can achieve confident results.

In conclusion, short-term exposure of swimmers to pool air with less than 300 μg m⁻³ of NCl3 is not associated with detectable effects on the airway epithelium as assessed by the assay of lung-derived proteins in serum. Our study suggests that this low level of chlorinated compounds might inhibit exercise-induced NO production, an observation that warrants further studies.

TABLE 2. Serum concentrations in CC16, SP-A, SP-B, and KL-6 and $\text{FE}_{\text{NO}}$ after a standardized exercise session in a nonchlorinated and in a chlorinated swimming pool.

<table>
<thead>
<tr>
<th></th>
<th>Nonchlorinated Pool</th>
<th>Chlorinated Pool</th>
<th>ANOVA</th>
<th>Pool × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal Condition</td>
<td>Postexercise</td>
<td>3 h Postexercise</td>
<td>Basal Condition</td>
</tr>
<tr>
<td>CC16 (μg L⁻¹)</td>
<td>9.5 ± 0.90</td>
<td>9.9 ± 0.94</td>
<td>7.5 ± 0.72</td>
<td>9.7 ± 0.96</td>
</tr>
<tr>
<td>SP-A (mg L⁻¹)</td>
<td>47.5 ± 54.9</td>
<td>453 ± 50.6</td>
<td>458.3 ± 46.6</td>
<td>461 ± 45.7</td>
</tr>
<tr>
<td>SP-B (mg L⁻¹)</td>
<td>577.5 ± 1004.6</td>
<td>5767 ± 1002.4</td>
<td>5829.9 ± 327.7</td>
<td>5712 ± 924.4</td>
</tr>
<tr>
<td>KL-6 (UI L⁻¹)</td>
<td>180 ± 24.3</td>
<td>164 ± 22.6</td>
<td>142 ± 20.1</td>
<td>189 ± 26.4</td>
</tr>
<tr>
<td>$\text{FE}_{\text{NO}}$ (ppb)</td>
<td>14.6 ± 1.27</td>
<td>19.6 ± 2.59</td>
<td>14.7 ± 1.98</td>
<td>17.2 ± 3.04</td>
</tr>
</tbody>
</table>

* Significant difference with basal conditions in the same swimming pool ($P < 0.05$).
** Significant difference with the nonchlorinated swimming pool at the same time.
REFERENCES


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