

Airway Cells after Swimming Outdoors or in the Sea in Nonasthmatic Athletes

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ABSTRACT

BONSIGNORE, M. R., G. MORICI, L. RICCOBONO, M. PROFITA, A. BONANNO, A. PATERNÒ, R. DI GIORGI, L. CHIMENTI, P. ABATE, F. MIRABELLA, A. M. VIGNOLA, and G. BONSIGNORE. Airway Cells after Swimming Outdoors or in the Sea in Nonasthmatic Athletes. *Med. Sci. Sports Exerc.*, Vol. 35, No. 7, pp. 1146–1152, 2003. **Background:** Marathon runners and elite swimmers showed increased inflammatory cells in the airways at baseline. Although airway neutrophils increase further after a marathon race, the airway response to swimming is unknown. The aim of this study was to assess the effects of swimming on airway cells. To avoid the concomitant effects of chronic exposure to chlorine, the study was conducted in seven nonasthmatic swimmers [mean age (SD): 23.3 ± 7.7 yr, training: 32 ± 15 km·wk⁻¹] habitually training in an outdoor pool (OP), i.e., a low-chlorine environment. **Methods:** Spirometry, exhaled nitric oxide (NO), induced sputum, and peripheral blood samples were obtained at baseline, after a 5-km trial in OP, and after a 5-km race in the sea (S), i.e., hypertonic airway exposure. **Results:** Airway neutrophil differential counts at baseline were higher in swimmers than in sedentary controls (*N* = 10), but cell counts, neutrophil elastase, and eosinophil cationic protein were unaffected by 5-km swimming. After swimming, L-selectin expression on airway cells decreased, suggesting exercise-induced cell mobilization into the airways and/or direct effects of hyperventilation on airway cells. After S, airway eosinophil differential counts increased slightly. Exhaled NO concentration was 19 ± 6 ppb at baseline, 8 ± 4 ppb after OP, and 21 ± 7 ppb after S (*P* < 0.005 for OP vs baseline and S). **Conclusions:** In swimmers not chronically exposed to high chlorine concentrations, data obtained at baseline suggest a direct relationship between airway neutrophilia and endurance training. The low L-selectin expression by airway cells postexercise suggests hyperventilation-induced cell recruitment or modulation of cell function. Hypertonic exposure of airways during exercise may slightly increase airway eosinophils and exhaled NO. Overall, 5-km swimming exerted smaller effects on airway cells than running a marathon. **Key Words:** ENDURANCE EXERCISE, EXHALED NITRIC OXIDE, INDUCED SPUTUM, ADHESION MOLECULES

An increase in inflammatory cells in the airways has been documented, isolated or in association with asthma and/or airway hyperresponsiveness in athletes of different endurance sports (4,15–17,20). However, the functional significance of such findings is unknown, as well as the possible link between inflammatory cells and occurrence of respiratory symptoms in athletes. Few endurance sports have been examined in detail, which differed for the degree of hyperventilation, temperature and humidity of inspired air, and exposure to pollutants/irritants during exercise, all factors that potentially modulate airway responses. Finally, the long-term respiratory consequences of endurance sports are largely unknown.

Available data in athletes have been mostly obtained at rest. The possibility that acute exercise could worsen airway inflammation in athletes is supported by recent findings in nonasthmatic well-trained runners who showed intense neutrophilia of induced sputum at baseline, which increased further, together with exhaled nitric oxide (NO) concentration, a few hours after a marathon race (4). These changes, however, were not associated with respiratory symptoms after the race or evidence of activation of airway neutrophils (4). Therefore, exercise-related airway inflammation may be tightly regulated, as already shown for the systemic pro-inflammatory effects of endurance exercise (24).

Swimming has been considered as an ideal activity for asthmatics, because the high humidity of inspired air and low-grade exposure to allergens and pollutants may prevent exercise-induced symptoms (3). However, in elite swimmers, increased neutrophil and eosinophil differential counts in induced sputum, and a high prevalence of bronchial hyperreactivity to histamine, were found (16). The relative role of endurance exercise as opposed to exposure to irritants in swimming pools (8,23,25) in the pathogenesis of airway inflammation in swimmers is unclear. Airway inflammation and respiratory symptoms subsided in swimmers who stopped competing compared with active athletes

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(18). However, no study assessed the acute effects of swimming on the airways. It can be hypothesized that differences in ventilatory pattern and environmental conditions could result in different responses of airway cells to running and swimming.

To fill this gap, we studied a group of nonasthmatic competitive swimmers by analysis of exhaled NO, and inflammatory cells and markers in induced sputum and peripheral blood. We took advantage of the favorable climate of Sicily, which allows training all year-round in an outdoor heated pool (OP), i.e., environmental conditions of low chlorine exposure (8). Such an experimental setting may help define exercise-dependent changes in airway cells in swimmers without the concomitant effect of chronic exposure to high concentrations of chlorine derivatives. All athletes were studied at rest and after a 5-km trial in OP.

The same athletes were also studied after a real 5-km competition in the Mediterranean Sea (S). Because this condition implies intense exercise in a hypertonic environment, swimming in S could be a model to study the effects of hyperosmolarity of the airway surface layer (12), a mechanism likely to be involved in inflammatory activation of airway epithelium (13,14) and exercise-induced asthma (2,28). No such data have ever been collected in either humans or animals after exercise.

METHODS

Subjects. We studied seven swimmers with a mean age and racing experience of 23.3 ± 7.7 (SD) and 11.3 ± 3.8 yr, respectively. Their average training volume was 32 ± 15 km·wk⁻¹. All subjects had regularly trained outdoors in a heated pool in the last 3 yr. No subject referred a history of atopy or asthma, or habitual or on-demand use of inhaled beta-2-agonists. All were nonsmokers, did not refer recent infection or other significant disease, or use of steroidal/nonsteroidal anti-inflammatory agents before or during the study. The protocol was approved by the local ethical committee, and all subjects gave written informed consent.

Data previously obtained in 10 sedentary subjects at baseline were used as controls for induced sputum (34). All subjects in this group (age: 30.2 ± 4.3 yr) were lifelong nonsmokers, had normal pulmonary function, and did not train regularly or participate to competitions.

Protocol. Measurements were obtained: a) at baseline, at least 24 h after a training session, b) after a 5-km trial in OP, and c) after a 5-km race in S. Experiments were performed in late morning, at 2-wk intervals between mid-May and mid-June 2000, in the following order: baseline, OP, and S. The 5-km S race (First “Memorial Piero Ciancimino,” Palermo) was held on June 18, 2000, starting at 11:00 a.m. Table 1 reports the environmental conditions during OP and S swimming.

The OP trial took place at the Palermo Olympic outdoor pool (50-m long, 25-m wide, 2-m deep), where the subjects trained habitually. In this pool, chlorine is continuously added at the bottom, and free chlorine in the water is automatically checked every 2 h. During the trial, two water

TABLE 1. Environmental conditions during OP and S swimming.

	Outdoor Pool (OP)	Sea (S)
Air temperature (°C)	31.5	29.0
Humidity (%)	40	45
Barometric pressure (mbar)	1010	1015
Water temperature (°C)	27	24
Chlorine (mg·L ⁻¹)	<0.1	NA
NaCl (%)	NA	2.22

samples were collected at 20–30 cm below the water surface and kept in air-tight containers at 4°C; chlorine concentration was determined by colorimetric kit (Lovibond, Aqua-Merck) and high-pressure liquid chromatography. During the race in S, two water samples were similarly collected in the racing field, and NaCl concentration was measured as atomic absorbance by mass spectrometry.

The athletes performed at their best in OP (70.2 ± 4.6 min) and S (54.2 ± 2.7 min). The experimental protocol included in the following order: blood sampling, spirometry, exhaled NO measurement, and sputum induction and collection. It started at the laboratories of the IBIM-CNR 84 \pm 8 min after the OP trial, and 96 ± 15 min after the S race, and was completed in less than 1 h in all subjects and conditions.

Blood samples. Blood was drawn from the antecubital vein for blood cell counts. Plasma/serum aliquots were prepared and stored at -20°C for subsequent determination of cortisol, muscle enzymes, and neutrophil elastase (see below).

Slides were prepared for analysis of adhesion molecules on circulating neutrophils. For this purpose, whole blood was collected from the swimmers into ACD-containing tubes at baseline, OP, and S. Each sample was centrifuged at 800 g (Beckman GPR centrifuge) for 10 min, then serum was removed and discarded. The lower layer was mixed with 6% dextran T-500 (Pharmacia, Uppsala, Sweden) prepared in isotonic saline solution and allowed to sediment for 20 min. The upper fraction, containing predominantly neutrophils, was removed and centrifuged at 400 g. Erythrocytes were removed by hypotonic saline solution (0.2% NaCl) and a rebalancing solution (3.98 g NaCl; 0.5 g sucrose). After washing with phosphate-buffered saline, neutrophils were purified by Ficoll-Hypaque centrifugation. The top layer and interface were removed, and the neutrophil-containing pellet was washed twice, counted, and resuspended for slide preparation. The total cell yield was determined by counting a portion of the final volume. Total cell counts and cell viability were assessed by hemocytometer and Trypan blue exclusion, respectively. Cytospin were made with 2×10^5 cells for subsequent immunostaining, as described below for induced sputum cells.

Measurements. At all experimental time points, standard spirometry was obtained. Exhaled NO was determined by chemiluminescence (Sievers Instruments Inc, Boulder, CO) and measured in triplicate after fast-inhalation maneuvers to total lung capacity, at a constant expiratory flow of 100 mL·s⁻¹ against an expiratory resistance of 20 cm H₂O (30).

Standard methacholine (Mch) challenge tests were performed in the morning on a different day after baseline measurements were obtained. The ampul-dosimeter used (median particle diameter: 1.53–1.61 μm) was activated by inspiration to deliver 5 μL of solution (Mefar Elettromedicali, Bovezzo, BS, Italy). After saline control, Mch was administered in doubled increasing amounts beginning with 2 μg . FVC and FEV₁ measurements (1) were obtained 2 min after each inhalation (27), the value measured after saline was taken as baseline. The challenge was stopped at the dose decreasing FEV₁ by 20% of baseline, or at the cumulative Mch dose of 2350 μg (corresponding to a concentration $\geq 8 \text{ mg}\cdot\text{mL}^{-1}$, taken as the lower limit of normal bronchial reactivity).

Induced sputum production and processing.

Sputum induction and processing were according to the method of Fahy and coworkers (10,11), with slight modifications (4,34). Subjects were exposed to hypertonic saline (3%) aerosol for 20 min (ultrasonic nebulizer Fisoneb, Fisons Italcchimici Spa, Rome, Italy; median particle diameter: 2.5 μm , output: 1 $\text{mL}\cdot\text{min}^{-1}$) and expectorated into sterile ampoules. Samples were added an equal volume of 0.1% dithiothreitol saline solution (Sigma Chemical Co, St. Louis, MO), gently mixed, placed in a water bath at 37°C for 15 min, and centrifuged at 800 g for 10 min. Supernatants were frozen at -20°C. Cell pellets were resuspended in saline, and total cell count (standard hemocytometer) and viability (Trypan blue exclusion) were assessed in 10- μL aliquots. The cells were cytocentrifuged (Cytospin 2, Shandon Instruments, Runcorn, UK) and stained (Diff Quick, Merz-Dade, Dudingon, Switzerland). Slides were blindly read by two investigators (LR, AMV), who counted at least 400 cells per slide ($K = 0.92$). Differential counts were expressed as corrected percentages, after subtraction of squamous cells. Samples were considered adequate if sputum volume was $\geq 1 \text{ mL}$.

Expression of adhesion molecules on cells in induced sputum. Cell pellets of induced sputum and blood samples (see above) were resuspended in saline. Slides were prepared, dried, fixed (acetone/methanol, 4°C, 10 min), and incubated (37°C, 1 h) with monoclonal antibodies (DAKO A/S, Denmark) anti-L-selectin, CD11a/CD18 (LFA-1), and CD11b/CD18 (MAC-1) at the dilutions of 1:2, 1:100, and 1:100, respectively. Immunoreactivity was revealed by the labeled streptavidin biotin (LSAB) alkaline-phosphatase technique. Two investigators (MP and AMV) counted 400 cells per slide ($Kappa: 0.94$). Results were expressed as percent of positive cells.

Biochemical analysis on plasma/serum and sputum supernatant. The systemic effects of swimming were assessed by measuring: a) serum muscle enzymes (lactic dehydrogenase and creatine kinase) by standard assays; b) plasma cortisol by radioimmunoassay (Immuno- tech, SA, Marseille, France); and c) total elastase by an enzyme immunoassay specific for human elastase (detection threshold: 20 $\mu\text{g}\cdot\text{L}^{-1}$; Ecoline, Kit Merck, Darmstadt, Germany).

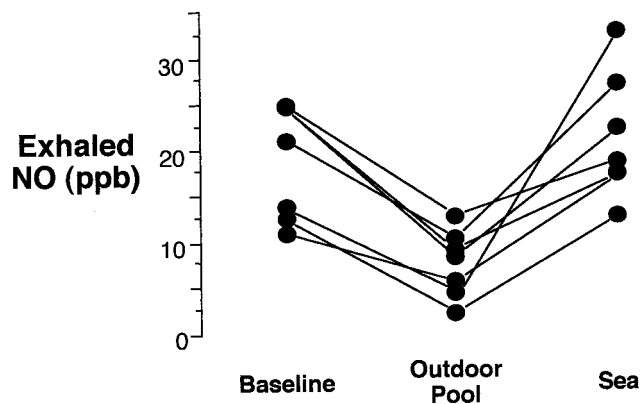


FIGURE 1—Exhaled nitric oxide in swimmers at baseline, and after 5 km in and outdoor pool and the sea.

Sputum supernatants were analyzed to determine the concentrations of: a) albumin by nephelometric assay (Beckman Array Protein System, detection threshold 6.0 $\mu\text{g}\cdot\text{mL}^{-1}$) using specific monoclonal antibodies (Beckman Immunochemistry Systems); b) histamine by radioimmunoassay (Immuno- tech, SA, detection threshold 0.2 nM); c) neutrophil elastase (same method as for plasma); and d) eosinophil cationic protein (ECP) by radioimmunoassay (Pharmacia Diagnostic AB).

Statistics. Data are reported as means and standard deviations, except for induced sputum cell counts reported as interquartile ranges. Baseline data in swimmers and sedentary controls were compared by unpaired t -, or Mann-Whitney test (cell counts in induced sputum). Comparisons among different experimental conditions in swimmers were by ANOVA with Bonferroni correction for *post hoc* analysis, or by Kruskal-Wallis test (cell counts in induced sputum). Statistical significance was at $P < 0.05$. Type II error analysis was employed to estimate the effects of sample size in case of borderline significance.

RESULTS

Spirometry, Mch challenge and exhaled NO.

Baseline FEV₁ and FVC were $4.73 \pm 0.75 \text{ L}$, or $116 \pm 19\%$ predicted, and $5.63 \pm 0.98 \text{ L}$, or $116 \pm 16\%$ predicted, respectively. FEV₁ and FVC were unchanged after OP or S. The Mch bronchial provocation test was negative in six subjects. One subject showed slight bronchial hyperresponsiveness (PD20 at the highest Mch dose tested). Exhaled NO decreased in all subjects after OP but was similar to baseline after S (Fig. 1).

Induced sputum. Induced sputum was obtained in all experimental conditions in six subjects, one subject being unable to expectorate. Viability was $68.0 \pm 5.8\%$ at baseline, $67.0 \pm 4.6\%$ after OP, and $57.4 \pm 10.9\%$ after S (NS by ANOVA). Figure 2 and Table 2 report differential and absolute sputum cell counts, respectively. Baseline samples showed a two- to threefold increase in neutrophil differential counts compared with controls ($P < 0.01$), but absolute counts were not significantly increased.

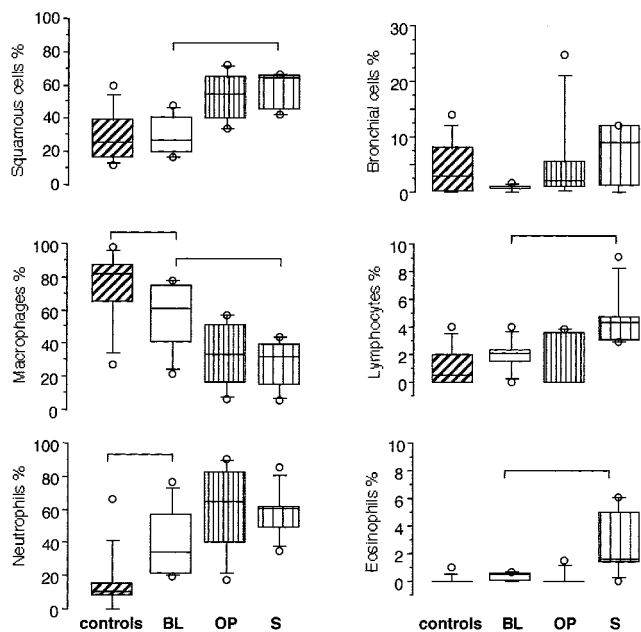


FIGURE 2—Box plots of induced sputum cell composition in sedentary controls (data from ref. 34) and in swimmers at baseline, and after OP trial and S race. Lines indicate significant differences between controls and swimmers at baseline, or among experimental conditions in swimmers.

After swimming, squamous cell contamination of induced sputum almost doubled compared with baseline ($P < 0.05$). Total corrected cell counts were in the normal range at baseline and tended to decrease after swimming. Neutrophil differential and absolute counts were comparable among experimental conditions. Macrophage absolute counts decreased after swimming ($P < 0.05$).

After S only, eosinophil and lymphocyte differential counts increased significantly compared with baseline or OP ($P < 0.05$ for both), with a similar trend for eosinophil absolute counts ($P = 0.07$). Type II error analysis (power of the test: 0.9) indicated that 10 paired observations would have shown a significant increase in eosinophil absolute counts after S, assuming mean and SD to remain the same after increasing sample size.

Mean albumin and histamine concentrations in induced sputum were unaffected by swimming in OP or S (not shown). The concentration of neutrophil elastase in sputum was $0.27 \pm 0.19 \mu\text{g}\cdot\text{mL}^{-1}$ at baseline, $0.10 \pm 0.06 \mu\text{g}\cdot\text{mL}^{-1}$ after OP, and $0.22 \pm 0.37 \mu\text{g}\cdot\text{mL}^{-1}$ after S (NS). Similarly, ECP concentration was at $28.9 \pm 17.8 \mu\text{g}\cdot\text{L}^{-1}$ at baseline and did not change significantly after OP ($21.5 \pm 17.3 \mu\text{g}\cdot\text{L}^{-1}$) or S ($13.2 \pm 8.1 \mu\text{g}\cdot\text{L}^{-1}$).

Expression of L-selectin by neutrophils in induced sputum decreased after swimming, whereas expression of LFA-1 or MAC-1 did not change (Table 3). Similarly, macrophages and eosinophils did not show expression of L-selectin after swimming, whereas the expression of other adhesion molecules did not change (Table 3).

Peripheral blood. Five-km swimming increased circulating white blood cells (baseline: 5.6 ± 0.6 , OP: 13.2 ± 4.2 , S: $10.9 \pm 1.9 \cdot 10^3 \cdot \mu\text{L}^{-1}$, $P < 0.0005$), neutrophils

(baseline: 2.9 ± 0.4 , OP: 10.8 ± 4.1 , S: $9.0 \pm 1.9 \cdot 10^3 \cdot \mu\text{L}^{-1}$, $P < 0.0001$), and plasma neutrophil elastase (baseline: 33.4 ± 7.1 , OP: 78.6 ± 36.9 , S: $61.6 \pm 11.3 \mu\text{g}\cdot\text{L}^{-1}$, $P = 0.005$). Muscle enzyme levels or cortisol were unchanged about 90 min after swimming (not shown).

Analysis of adhesion molecules on circulating neutrophils showed a slightly higher percentage of L-selectin-positive cells after swimming ($98.0 \pm 2.3\%$ after OP and $97.9 \pm 1.5\%$ after S) compared with baseline ($93.2 \pm 4.5\%$, $P < 0.05$ for both OP and S vs baseline). Conversely, the percentage of circulating neutrophils expressing LFA-1 and MAC-1 was unchanged. No correlation was found between L-selectin expression by sputum and blood neutrophils.

DISCUSSION

The aim of our study in competitive swimmers was to assess whether airway inflammatory cells: a) were increased at baseline and b) were modified by acute exercise. We found that baseline differential neutrophil counts in induced sputum were higher in swimmers compared with controls, similar to findings reported in elite athletes (16). However, differently from the data obtained in runners after a marathon race (4), 5-km swimming did not modify airway neutrophilia. Expression of L-selectin by airway cells significantly decreased after swimming, suggesting a possible explanation for the apparent lack of activation of neutrophils in athletes after exercise.

Our study differs from previous ones in swimmers, as our athletes habitually trained outdoors and were chronically exposed to low concentrations of irritants during exercise. Chlorine was undetectable in the OP water sampled during the trial, likely because of heightened evaporative loss outdoors (8). Indoor swimming, instead, causes exposure to high concentrations of irritants (23). Our study, therefore, provides unique information on the acute and chronic effects of swimming on the airways, without the concomitant confounding effect of heavy exposure to environmental respiratory irritants.

Nevertheless, airway neutrophil differential counts at baseline in our sample compared well with other reports (16,18), suggesting that endurance training may play a direct role in the pathogenesis of airway neutrophilia in athletes. Because repeated dry air challenges in dog airways were associated with progressively increasing neutrophil recruitment (6), we speculate that repeated bouts of exercise may cause similar changes in humans undergoing regular training. Therefore, airway neutrophilia at baseline may be an effect of chronic endurance exercise.

Studies in elite swimmers consistently found high eosinophil and lymphocyte counts in induced sputum at baseline (15,17), but we did not in our sample. Such difference in results may reflect a different level of training/performance, as our subjects were not elite athletes. Alternatively, eosinophilic inflammation in elite swimmers might be related to chronic exposure to irritants during indoor training, as supported by possible occurrence of asthma in people attending indoor pools (23).

TABLE 2. Absolute cell counts in induced sputum in swimmers and controls ($N = 10$, [34]).

Condition	Total Cells × 10 ⁶ mL ⁻¹	Macrophages × 10 ⁴ mL ⁻¹	Neutrophils × 10 ⁴ mL ⁻¹	Eosinophils × 10 ⁴ mL ⁻¹	Lymphocytes × 10 ⁴ mL ⁻¹	Bronchial Cells × 10 ⁴ mL ⁻¹
Controls						
BL	1.35 (1.87)	110.4 (129.9)	15.2 (26.5)	0.04 (0.00)	1.79 (0.80)	5.5 (4.0)
Swimmers						
BL	0.65 (0.48)	33.0 (24.8)*	30.2 (48.2)	0.26 (0.27)	1.20 (1.45)	0.4 (0.5)
OP	0.36 (0.33)	11.3 (15.7)*	22.9 (23.9)	0.13 (0.00)	0.54 (1.25)	0.7 (0.3)
S	0.33 (0.17)	9.0 (12.4)*	19.8 (18.3)	0.88 (0.90)	1.38 (0.75)	2.1 (4.4)

Cell counts are reported after correction for squamous cells.

* $P < 0.05$ by Kruskal Wallis ranking test.

Data in parenthesis: interquartile ranges.

Acute exercise in OP did not modify airway cells significantly. This finding, at variance with previous results in runners, might reflect differences in inspired air during exercise, as the high-humidity environment of swimming may prevent airway cooling and dehydration (2). Along the same line of reasoning, we hypothesized that swimming in S could worsen airway inflammation, due to prolonged airway exposure to humid and salted environment (NaCl concentration >2% in water from the racing field). Hypertonicity of the airway surface layer was found to occur after hyperventilation with dry cold air in dog experiments, in which it triggered bronchoconstriction (12). Hypertonic airway exposure also increased bronchial epithelial cells in bronchoalveolar lavage in dogs (28) and caused inflammatory activation, i.e., expression of IL-8, in human bronchial epithelial cells in culture (13). Our study is the first one in humans trying to explore the effects of exercise in a hypertonic environment, but OP and S experiments gave similar results, the only difference being a slight increase in eosinophils and lymphocytes in induced sputum after S.

Several factors have been proposed to account for changes in airway cells or function after exercise. Unfortunately, few studies collected data shortly after exercise or directly measured some variables considered as possibly important in the recruitment of airway inflammatory cells into the airways (for example, degree of hyperventilation or changes in osmolarity of airway surface layer). Local factors, such as shear stress of airway surface during intense hyperventilation, may play a role (20). Because shedding of L-selectin occurs during exercise-associated systemic neutrophil mobilization (33), the low expression of L-selectin by airway neutrophils after exercise may indicate increased neutrophil recruitment and turnover in the airway compartment. Data obtained *in vitro* showed that bronchial epithelial cells released IL-8 and RANTES upon exposure to cooling-rewarming or a hyperosmolar medium, supporting that chemotactic pathways at the airway level can become activated during exercise (13,14). However, airway neutrophils did not increase significantly after swimming, possibly in rela-

tion with limited systemic neutrophilia and inflammatory response compared with the changes observed after a marathon race (4,9). Thus, our data support an overall lower impact of swimming on both systemic and airway compartments compared with running. Alternatively, the shorter exercise duration in swimming experiments compared with marathon running (1 vs 3–4 h) could play some role in modulating systemic and airway inflammatory response. Experiments are currently ongoing to assess the role of exercise duration on airway cell counts.

Neutrophil elastase and other inflammatory markers did not increase in the airways after swimming, similar to previous observations in runners (4). This finding may be at least partly linked to the low expression of L-selectin by airway neutrophils, macrophages, and eosinophils after swimming. We speculate that exercise hyperventilation in humans may not only increase the osmolarity of the airway surface layer (12) but also affect inflammatory cell function by modulating expression of adhesion molecules. Our hypothesis is based on the following evidence: a) hypertonic exposure of neutrophils *in vitro* caused cell shrinkage and shedding of L-selectin, partly through p38 kinase activation (26), a picture very similar to that described for bronchial epithelial cells exposed to a hypertonic medium (13); and b) neutrophils exposed to hypertonic environment became resistant to activation by endotoxin, with adhesion-independent shedding of L-selectin and inhibition of CD11b up-regulation (26). If a similar sequence of events applies to the effects of exercise hyperventilation on airway cells, we speculate it could explain why inflammatory cells are recruited but not activated during exercise in the airways of nonasthmatic athletes.

In peripheral blood, the percentage of L-selectin positive neutrophils was slightly increased about 90 min postexercise, likely indicating bone marrow release of young neutrophils during recovery (32). Neutrophil expression of MAC-1 (CD11b/CD18), a marker of inflammatory activation (5), was not increased in blood after swimming, at variance with the increased MAC-1 expression in circulat-

TABLE 3. Percentage of cells expressing adhesion molecules in induced sputum of swimmers ($N = 6$).

Condition	LFA-1 + PMN %	L-sel + PMN %	MAC-1 + PMN %	LFA-1 + MΦ %	L-sel + MΦ %	MAC-1 + MΦ %	LFA-1 + Eos %	L-sel + Eos %	MAC-1 + Eos %
Baseline	71.6 ± 36.3	47.6 ± 25.3	61.1 ± 18.1	38.4 ± 22.0	33.1 ± 27.5	0	57.1 ± 53.5	42.9 ± 45.0	54.8 ± 45.9
Outdoor pool	86.9 ± 11.1	9.3 ± 20.9*	54.3 ± 36.9	11.4 ± 27.9	0*	5.6 ± 14.0	25.0 ± 41.8	0*	20.0 ± 44.7
Sea	81.3 ± 11.4	5.6 ± 13.6*	76.4 ± 10.5	10.2 ± 27.0	0*	21.4 ± 39.3	23.8 ± 41.8	0*	30.0 ± 44.7

Data reported as means ± SD.

* $P < 0.05$ vs baseline (ANOVA+ Bonferroni correction).

ing neutrophils reported after a marathon race (19). Altogether, the data support the interpretation that at least some of the differences found between 5-km swimming and marathon running might depend on different exercise intensity and/or duration.

Exhaled NO did not increase after swimming. NO in the airways might be involved in modulating bronchial tone, but its role is still uncertain. Some studies suggest NO involvement in postexercise bronchodilation (7,29,31), whereas other reports found high-exhaled NO associated with hyperventilation-induced bronchoconstriction in asthmatics (22). At the time that measurement of exhaled NO was obtained, spirometry was similar to baseline, ruling out exhaled NO as related to spirometric changes in our protocol. Exhaled NO is also a marker of inflammation in asthma (21). It could be a marker of airway neutrophilia in athletes, as suggested by the data obtained after a marathon race (4). After swimming in OP, exhaled NO decreased and airway neutrophils did not increase; after S, exhaled NO was similar to baseline, and associated with a slight increase in airway eosinophils. Our data therefore suggest a possible role of eosinophils in accounting for the small difference in exhaled NO between OP and S conditions. However, several cell types can release NO, and further studies are needed to confirm this hypothesis.

Our study shows some limitations. First, the number of subjects studied was small. This was justified by the complexity of collecting and processing induced sputum samples after exercise, which requires a major laboratory effort especially after a competition. Second, an unexpected, sys-

tematic increase in squamous cells in induced sputum was found after swimming, possibly increasing the variability of results. Nevertheless, data were consistent. Intrasubject variability was likely to be limited, as subjects were repeatedly studied over a short time period. Finally, we could have repeated the experiment after swimming indoors, to assess the acute effect of irritant exposure on the airways. This experiment, however, would give no information on chronic effects of airway exposure to chlorine during exercise, which represents a more relevant issue in regularly training competitive swimmers (23).

In conclusion, our study is the first report on airway cells and exhaled NO after exercise in nonasthmatic swimmers. The results support the hypothesis that endurance exercise may be the main factor responsible for airway neutrophilia in well-trained swimmers, as exposure to environmental irritants was low in our study. The changes acutely caused by swimming in our experimental settings were very modest compared with those caused by running, supporting that swimming exerts little pro-inflammatory effect on nonasthmatic airways.

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