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Effects of exercise training on airway responsiveness and airway cells in healthy subjects

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Scichilone N, Morici G, Zangla D, Chimenti L, Davì E, Reitano S, Paternò A, Santagata R, Togias A, Bellia V, Bonsignore MR. Effects of exercise training on airway responsiveness and airway cells in healthy subjects. *J Appl Physiol* 109: 288–294, 2010. First published June 10, 2010; doi:10.1152/japplphysiol.01200.2009.—Airway responsiveness to methacholine (Mch) in the absence of deep inspirations (DIs) is lower in athletes compared with sedentary individuals. In this prospective study, we tested the hypothesis that a training exercise program reduces the bronchoconstrictive effect of Mch. Ten healthy sedentary subjects (M/F: 3/7; mean ± SD age: 22 ± 3 yr) entered a 10-wk indoor rowing exercise program on rowing ergometer and underwent Mch bronchoprovocation in the absence of DIs at baseline, at *weeks 5* and *10*, as well as 4–6 wk after the training program was completed. Exercise-induced changes on airway cells and markers of airway inflammation were also assessed by sputum induction and venous blood samples. Mean power output during the 1,000 m test was 169 ± 49 W/stroke at baseline, 174 ± 49 W/stroke at 5 wk, and 200 ± 60 W/stroke at 10 wk of training ($P < 0.05$). The median Mch dose used at baseline was 50 mg/ml (range 25–75 mg/ml) and remained constant per study design. At the pretraining evaluation, the percent reduction in the primary outcome, the inspiratory vital capacity (IVC) after inhalation of Mch in the absence of DIs was 31 ± 13%; *at week 5*, the Mch-induced reduction in IVC was 22 ± 19%, $P = 0.01$, and it further decreased to 15 ± 11% at *week 10* ($P = 0.0008$). The percent fall in IVC 4–6 wk after the end of training was 15 ± 11% ($P = 0.87$ vs. end of training). Changes in airway cells were not associated with changes in airway responsiveness. Our data show that a course of exercise training can attenuate airway responsiveness against Mch inhaled in the absence of DIs in healthy subjects and suggest that a sedentary lifestyle may favor development of airways hyperresponsiveness.

rowing; methacholine; neutrophils

AIRWAY SMOOTH MUSCLE tone-driven airway narrowing responses are of major importance in obstructive airway diseases. In the absence of deep inspirations, healthy subjects develop substantial bronchoconstriction with methacholine (19, 21, 42) because of the absence of the bronchoprotective and bronchodilatory effects of lung inflation. With avoidance of deep inspirations one can, therefore, study how airway tone develops and influences healthy airway function. Studying healthy airway behavior is paramount in understanding abnormalities that are seen in asthma and other obstructive airway conditions.

Recently, we used methacholine provocations conducted in the absence of deep inspirations and discovered substantial differences among healthy individuals. Notably, we found that two groups, well-trained athletes and individuals who do not exercise vigorously in their daily life, had significantly different airway responsiveness to inhaled methacholine: in the absence of deep inspirations, the former exhibited lower responsiveness compared with the latter (35). This observation raised the hypothesis that long-term vigorous exercise reduces the bronchoconstrictive effects of parasympathetic stimulation. To test this hypothesis, we designed a prospective study in which healthy subjects entered a 10-wk indoor rowing exercise program and underwent methacholine bronchoprovocation in the absence of deep breaths at baseline, at the middle and the end of their training, as well as 4–6 wk after the training program was completed. Rowing was chosen because it is an ideal model to assess the effects of very intense, short-lived exercise, as it involves a large muscle mass and causes intense hyperpnea (44).

Our group has also shown that endurance athletes develop neutrophilia in airway fluids (4, 5). The physiological consequences of this phenomenon, if any, are not known, but the possibility exists that neutrophilia may lead to inflammatory changes that, in the case of our study, may prevent or modify the hypothesized ability of exercise to reduce parasympathetically induced bronchoconstriction. To examine the influence of exercise-induced changes on airway cells, we included sputum induction procedures in our study. Markers of airway inflammation were assessed by Clara cell protein 16 in serum (CC-16) and interleukin-8 (IL-8) in sputum supernatants.

MATERIALS AND METHODS

Subjects. We studied 10 healthy subjects (M/F: 3/7; mean ± SD age: 22 ± 3 yr, body mass index: 22.2 ± 2.3 kg/m²). No subject was a former or current smoker or reported symptoms suggestive of airway disease nor did any subject have a prior history of chronic airway disease. Two subjects had a history of allergic rhinitis but were untreated and asymptomatic at the time of the study. No subject reported upper respiratory infections in the 4 wk preceding enrollment. Physical activity before enrollment was moderate (<4 h/wk in the previous 6 mo), and no subject practiced regular training of any form. In the days when tests were performed, exercise, as well as

coffee or tea, was not allowed in the morning. The study was approved by the University of Palermo Ethics Committee, and all subjects gave written informed consent.

Training program. All subjects underwent a training indoor rowing program on rowing ergometer (Concept II, Morrisville, VT) consisting of two to three sessions per week for 10 wk (total 25 sessions). Each session lasting 60–90 min included a warm-up period for 20 min (running and stretching) and a specific training on rowing ergometer of 40–70 min. A 1,000-m all-out rowing test was first performed to document power output, strokes, and mean maximal speed (29, 30).

The 1,000-m all-out test was preceded by a 20-min warm-up: after 10 min of low-intensity exercise (heart rate 120–140 beats/min, 22–26 strokes/min), the subject performed two to three sprints of five to seven strokes at submaximal intensity (heart rate 140–160 beats/min, 27–30 strokes/min). Active rest (2 min of light rowing, 22–24 strokes/min) was maintained between sprints. The warm-up ended with two to three trials at maximal intensity preceded and followed by short active rest periods. On the basis of the performance, watts per stroke and strokes per minute recorded in the 1,000-m all-out test, a certified European rowing coach created personalized training programs. Rowers undertook a 10-wk training program of 20% of all training time at maximal power output, 10% of all training time at 75–90% of maximal power output, and 70% of all training time at 60–65% of maximal power output. At *weeks 0, 5,* and *10,* 1,000- and 2,000-m all-out rowing tests were performed to evaluate performance and reassess the individualized workload. A certified training instructor and a pulmonologist supervised all sessions.

Pretraining clinical evaluation. At the beginning of the study and before the training program began, in addition to providing a clinical history, each subject underwent a functional evaluation to exclude the presence of respiratory disease. Functional respiratory assessment included conventional spirometry and measures of static and dynamic lung volumes, using the helium dilution technique and a computerized water-sealed spirometer (Biomedin; Padua, Italy). Measurements were made in accordance with the American Thoracic Society (ATS) acceptability and reproducibility criteria (1).

Study design. At the pretraining evaluation (*week 0*), modified spirometry (see below) was first conducted followed by determination of airway responsiveness to methacholine in the absence of deep inspirations; in addition, induced sputum and venous blood samples were collected at rest on the following day. The same protocol was repeated at *weeks 5* (midtraining) and *10* (end of training), as well as 4–6 wk after the end of the training program (recovery). During the recovery period subjects were asked not to engage in any training activity.

Assessment of airway responsiveness in the absence of deep inspirations. To evaluate airway responsiveness in the absence of deep inspirations, we employed the single-dose methacholine bronchoprovocation procedure that we have previously used and validated in several studies (35, 36, 38). The protocol for the single-dose methacholine provocation is depicted in Fig. 1. At baseline, three acceptable, modified (see below) spirometric maneuvers are obtained. Subjects are then instructed to avoid deep inspirations for 20 min and are observed for compliance during this period. Thereafter, subjects inhale a single methacholine dose with five tidal breaths, and 3 min later, a single modified spirometry is repeated.

Because the deep inspiratory maneuver that is embedded in conventional spirometry can alter the response to methacholine (11, 39), a modified spirometry technique is used in our studies (Fig. 1, *inset*). The modified spirometry incorporates a partial forced expiratory maneuver (from end tidal inspiration to residual volume) immediately before the maximal maneuver. Inspiratory vital capacity (IVC), i.e., the volume from the end of the forced partial expiratory maneuver to the end of maximal inspiration, is recorded and is used as the primary outcome of the methacholine provocation,because it offers the advantage of not being affected by a preceding deep inspiration. The usage of IVC does not eliminate our ability to also measure forced expira-
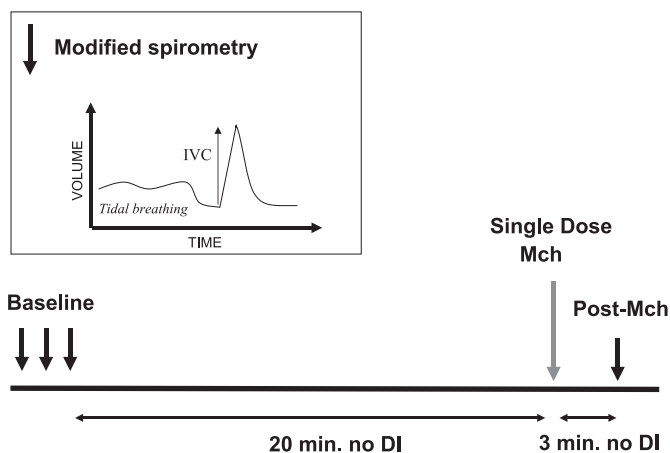

Fig. 1. Single-dose methacholine (Mch) bronchoprovocation protocol. *Inset*: modified spirometric maneuver that was used in the study. This consists of a partial forced expiratory immediately followed by a maximal inspiratory maneuver. Inspiratory vital capacity (IVC) is the volume from the end of the forced partial expiratory maneuver to the end of maximal inspiration and represents the primary outcome of the methacholine provocation. DI, deep inspiration.

tory volume in 1 s (FEV_1) and forced vital capacity (FVC) since a maximal maneuver always follows the partial one. The methacholine-induced effect on IVC is recorded as the percent difference between the post-methacholine IVC and the best IVC from the three baseline, modified spirometric measurements.

The dose of methacholine used in the pretraining bronchoprovocation was individualized as the inhaled concentration attaining at least 15% reduction in IVC from baseline and was determined for each subject by a series of increasing single-dose methacholine bronchoprovocations carried out earlier, also in the absence of deep breaths. If the first concentration (7.5 mg/ml) was ineffective, the provocation was repeated with a higher concentration (25 mg/ml) and, if needed, with the highest concentration (75 mg/ml). These single-dose methacholine bronchoprovocations were repeated on the same day (at least 2 h apart) if the preceding provocation reduced IVC by <5% from baseline or on the following day if IVC had dropped by >5%. The concentration of methacholine that was used for the single-dose challenge in the pretraining bronchoprovocation was used in all subsequent bronchoprovocations.

Sputum induction and processing. Sputum was induced and processed in accordance with the "plugs" method (23) with slight modifications (33). After washing the oral cavity with saline, subjects inhaled a hypertonic (5%) saline aerosol for 20 min administered with an ultrasonic nebulizer (median particle diameter 2.5 μm, output 1 ml/min; Fisoneb, Fisons Italchimici Spa, Rome, Italy). They were then asked to cough and expectorate sputum into 50-ml sterile containers. Samples were spread in a Petri dish, and mucus plugs were selected and weighted. PBS was added to the plugs in a 4:1 weight ratio. Samples were then gently mixed and centrifuged (800 *g* for 20 min) and supernatants were aspirated and frozen at −20°C for analysis of IL-8.

The cell pellets from the mucus plugs were resuspended in 0.1% dithiothreitol-saline solution (DTT, Sigma, St. Louis, MO) at a 4:1 weight ratio and gently mixed and agitated at 25°C for 20 min. The samples were then filtered (nylon filter, 70 μm), and centrifuged (800 *g* for 10 min). Cell pellets were again resuspended in 2% human serum albumin-saline solution. Total cell count and viability were determined with a standard hemocytometer and Trypan blue exclusion. Cells were then cytocentrifuged (Cytospin 2, Shandon Instruments, Runcorn, UK) and stained with Diff-Quick (Merz-Dade, Dudingen, Switzerland). The slides were read by two investigators

who counted at least 400 cells/slide. Slide readers were unaware of the sample's origin (subject ID or time point).

Biochemical analysis on plasma, serum, and sputum supernatants. Clara cell protein 16 (CC-16) is a 16-kDa protein released from nonciliated pulmonary epithelia cells (Clara cells) in the lungs (18). The rationale for using CC-16 lies in the fact that it has been used as a marker of increased lung epithelial permeability (18), which may occur after intense exercise. Indeed, CC-16 has been found to be increased during (8), or shortly after, exercise (31). Because of its small size, CC-16 diffuses across the bronchoalveolar/capillary barrier and can be detected in serum (18). We reasoned that changes in markers of lung damage would impact on the beneficial effect of exercise. CC-16 was measured in serum with an ELISA sensitive to 2 ng/ml (BioVendor). Sputum supernatants were analyzed for IL-8, known to mediate recruitment of neutrophils (2, 22), with an ELISA sensitive to 26 pg/ml (R & D Systems).

Statistical analysis. Data are reported as means ± SD. Different time points and experimental conditions were compared by ANOVA; the Bonferroni test was used for post hoc comparisons. Nonparametric tests were used for nonnormally distributed variables. The statistical package we employed was StatView 5.0.1 (Abacus Concept, Berkeley, CA). Statistical significance was accepted at $P < 0.05$.

RESULTS

The functional characteristics of the study sample are summarized in Table 1. On average, subjects attended 18 ± 3 training sessions (71 ± 15% of the sessions required by protocol). Performance was evaluated during training by 1,000- and 2,000-m all-out rowing tests at 5 and 10 wk. Mean power output during the 1,000-m test was 169 ± 49 W/stroke at *week 0*, 174 ± 49 W/stroke at 5 wk, and 200 ± 60 W/stroke at 10 wk of training ($P < 0.05$); similarly, mean power output in the 2,000-m test increased from 148 ± 37 to 175 ± 61 W/stroke from *weeks 1* to *10* ($P < 0.05$). This improvement in performance was as expected and confirmed the effectiveness of the training program.

Spirometry and airway responsiveness. Lung volumes and spirometric outcomes at rest were unaffected by training (Table 1). The median methacholine dose used in the group of subjects at baseline was 50 mg/ml (range 25–75 mg/ml) and remained constant per study design. At the pretraining evaluation, the percent reduction in IVC after inhalation of methacholine in the absence of deep inspirations was 31 ± 13%, in agreement with previous data obtained in healthy subjects (35). The reduction in FEV_1 during the same evaluation was 38 ± 14%, and the reduction in the FEV_1/FVC ratio was 21 ± 8.8%. At *week 5* (midtraining), the methacholine-induced reduction in IVC was 22 ± 19% ($P = 0.01$ compared with the pretraining provocation). At the end of training (*week 10*), the magnitude of the methacholine-induced reduction in IVC further

Table 1. *Functional characteristics of the study sample before and at the end of training*

	Pretraining	Posttraining	*P* Value
FEV_1, % predicted	111 ± 12	110 ± 12	0.64
FVC, % predicted	110 ± 12	106 ± 13	0.08
TLC, % predicted	106 ± 12	110 ± 11	0.09
FRC, % predicted	109 ± 31	101 ± 31	0.08
RV, % predicted	104 ± 19	99 ± 23	0.25

Data are expressed as means ± SD. FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume.
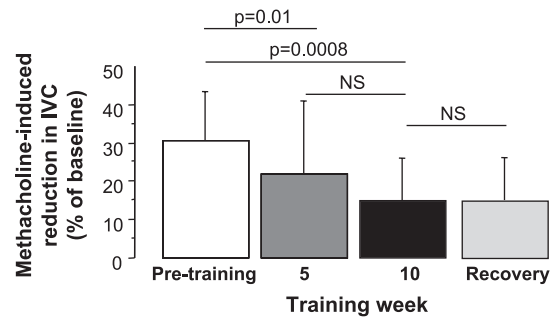

Fig. 2. Single-dose methacholine-induced reductions in IVC from baseline in the absence of deep inspirations, at baseline, after 5 and 10 wk of rowing exercise, and 4–6 wk after the exercise program had ended (recovery). Bars represent means ± SD. NS, not significant.

decreased to 15 ± 11% ($P = 0.0008$ compared with the pretraining provocation), although the difference between *weeks 5* and *10* did not reach significance ($P = 0.10$) (Fig. 2). Individual data points for percent reduction in IVC during training are also presented in Fig. 3. A similar trend was found for FEV_1: the percent fall in FEV_1 in the absence of deep breaths was 38 ± 14% at the pretraining provocation, 26 ± 21% at midtraining, and 20 ± 11% at end of training (repeated-measures ANOVA: $P = 0.008$). The methacholine-induced reduction in FEV_1 and in FEV_1/FVC at the end of training were significantly lower than that at baseline ($P = 0.004$ and $P = 0.04$, respectively).

Pretraining to posttraining changes in airway responsiveness, as assessed by the reduction in IVC in the absence of deep inspirations, were not associated with changes in lung volumes [for total lung capacity (TLC): $r = 0.20$, $P = 0.59$; for functional residual capacity (FRC): $r = 0.21$, $P = 0.55$], a finding that argues against the possibility of the reduction in airway responsiveness being secondary to modifications in individual lung volumes. Also, the change in the percent reduction in IVC between the beginning and the end of training did not relate to the level of adherence to the training program ($r = 0.02$, $P = 0.96$) nor with the airway response to methacholine at baseline ($r = 0.53$, $P = 0.11$).

To test whether any changes that occurred during the training period were persistent, further evaluation was conducted 4–6 wk after the end of training (recovery). The dose of methacholine used in this bronchoprovocation was the same as that used in all previous evaluations. The results did not differ from those obtained during the end of training provocation: the percent fall in IVC at recovery was 15 ± 11% ($P = 0.87$ vs.
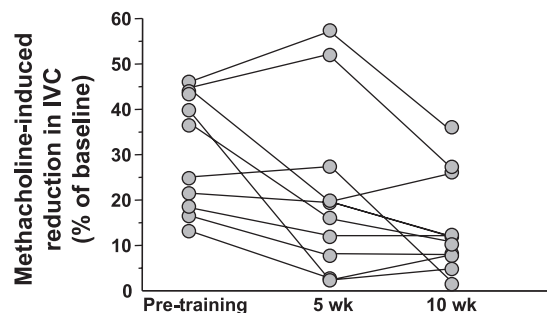

Fig. 3. Individual data for methacholine-induced percent reduction in IVC from baseline obtained at baseline and after 10 wk of rowing exercise training.

end of training) (Fig. 2); similarly, the percent fall in FEV_1 at recovery was $20 \pm 11\%$ (P = 0.95 vs. end of training).

Airway cells. Adequate sputum samples were obtained in eight subjects; two subjects were consistently unable to expectorate. Absolute and differential cell counts are reported in Fig. 4. The total number of cells collected in induced sputum was unaffected by training. The absolute neutrophil counts did not change from baseline to midtraining (*week 5*) but significantly increased from midtraining to end of training (*week 10* vs. *week 5, P < 0.004*) and were back to baseline at recovery. No difference was recorded between recovery and *week 10* (P = 0.13). The absolute neutrophil count at the end of training was not different from baseline (P = 0.57). Bronchial epithelial cells at 5 and 10 wk of training, as well as at recovery, were reduced from baseline with the latter two differences reaching statistical significance (P < 0.01).

We conducted simple regression analyses in the attempt to assess whether increased numbers of sputum neutrophils or changes in other airway cells were inversely associated with the reduction in airway responsiveness with training. These analyses did not show any relationship between the training-associated changes in airway responsiveness and changes in absolute neutrophil cell counts (r = 0.25, P = 0.49), macrophage (r = 0.02, P = 0.98), or bronchial cell counts (r = 0.12, P = 0.78).

Inflammatory mediators in induced sputum and blood. In the induced sputum supernatants, the concentration of IL-8 was significantly higher at end of training (P < 0.03) and returned back to baseline at recovery (468 ± 273 pg/ml at baseline, 356 ± 182 at midtraining, and 966 ± 600 at the end of training, Fig. 5). Serum CC-16 concentration did not change during training (pretraining: 4.4 ± 1.4 ng/ml; *week 5*: 4.1 ± 0.9 ng/ml; *week 10*: 5.2 ± 1.7 ng/ml; recovery: 5.3 ± 0.8 ng/ml, P = 0.86) (Fig. 5). No relationship was found between the training-induced changes from baseline in airway responsiveness at the end of training and those in sputum IL-8 or serum CC-16 (r = 0.32, P = 0.56 and r = 0.06, P = 0.90, respectively).

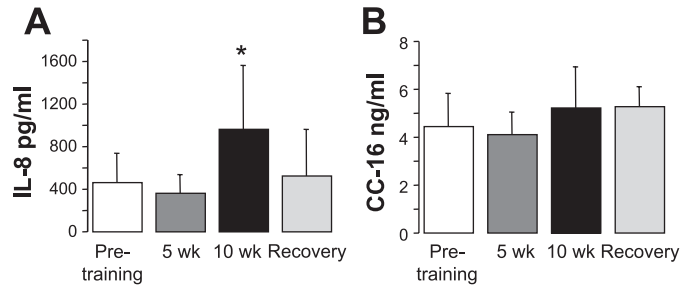Fig. 5. Absolute values for IL-8 and CC-16 during training and after recovery. Bars represent means $\pm$ SD. *P < 0.03, *week 10* vs. baseline.

DISCUSSION

This study was designed to test the hypothesis that long-term training reduces the bronchoconstrictive effects of inhaled methacholine. Our results clearly show that airway responsiveness to methacholine decreases with training in healthy subjects; this effect, an ~50% inhibition of the methacholine-induced drop in lung function, became apparent after 5 wk of training and persisted for at least 1 mo after training cessation. Our results are in line with those reported in other studies, albeit with different designs. For example, Freedman and colleagues (15) showed that exercise and voluntary hyperventilation similarly decreased airway resistance induced by methacholine inhalation in healthy subjects. Also, our group previously reported a state of hyporesponsiveness to methacholine in the absence of deep inspirations in healthy amateur runners compared with sedentary, age-matched control subjects (35). The attenuation of the methacholine-induced reduction in both IVC and FEV_1 following the training program, as well as in the FEV_1/FVC, suggests that exercise exerts its beneficial effect by preventing (or attenuating) airway closure and large airway narrowing (9).

The second goal of this study was to examine what influence, if any, do training-induced inflammatory changes in the
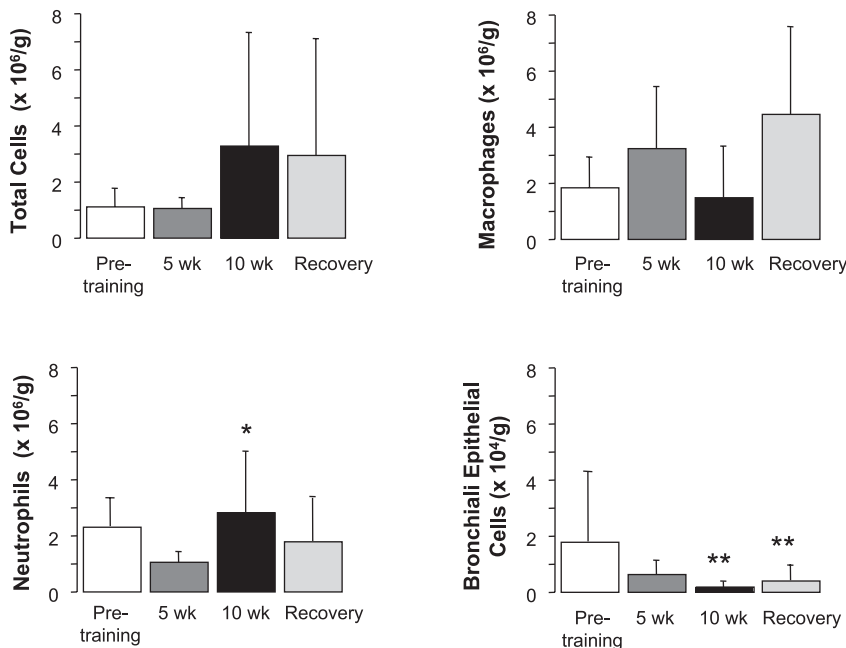
Fig. 4. Absolute cell counts in induced sputum at baseline, after 5 and 10 wk of rowing exercise training, and 4–6 wk after the end of the exercise program (recovery). *P < 0.004, *week 10* vs. *week 5*. **P < 0.01, *weeks 5* and *10* vs. baseline.

airways have on the beneficial effects of training on airway responsiveness. Several studies indicate that endurance exercise increases inflammatory cells in the airways. Increased airway neutrophil counts were described in endurance athletes of winter (20, 45) or summer sports (4, 6, 29), both at rest and after exercise. In trained mice, we previously reported increased inflammatory cells and damaged bronchial epithelium in small airways (10). Our results agree with those of Denguezli and coworkers (13), who reported a progressive increase in IL-8 in induced sputum during training. However, a long training history may be necessary before significant changes in airway inflammatory markers fully develop in young athletes (32), in line with the possibility that intensive prolonged training may eventually result in airway damage. Overall, the mild changes in airway cells observed during exercise training showed a different time course compared with changes in airway responsiveness. Our analyses do not support the hypothesis that these changes have any impact on the effect of training on airway responsiveness.

Our study was conducted in healthy subjects. Although there are no known benefits of reducing airway responsiveness in people who do not have airway disease, increased airway responsiveness has been epidemiologically associated with sedentary lifestyle (40) and with obesity (41), conditions that predispose to various health problems. In the future, it may be of value to investigate the significance of the population variance in airway responsiveness, in the absence of overt airway disease. Since asymptomatic airway hyperresponsiveness has been associated with later development of asthmatic symptoms (27) or with accelerated decline in lung function (26), we can speculate that any factor reducing the degree of airway responsiveness theoretically would delay the occurrence of asthmatic symptoms or slow the lung function decline. From the methodological standpoint, it was important to first test our hypothesis in nonasthmatics. This allowed us to determine whether the anticipated effect of exercise alters the baseline, physiological responsiveness of the airways to methacholine. A similar study in mild asthma, to examine whether hyperresponsiveness to methacholine can be altered by exercise and whether this effect can have beneficial clinical consequences, should be conducted. In support of the possibility that exercise may benefit patients with asthma, we have recently documented decreased airway responsiveness to methacholine in children with mild asthma after 12-wk endurance training (7). However, the medical literature is inconsistent as to the beneficial effect of exercise in preventing induced bronchoconstriction and improving respiratory symptoms. In a study involving eight individuals with asthma (12), the authors showed impressive immediate reversal of airway narrowing during exercise, likely due to the mechanical stretching of the tidal breaths on the airway walls, although a direct effect of catecholamines on airway smooth muscle cannot be excluded. Intriguing findings on the effect of physical activity on airway hyperresponsiveness have been reported by a multicenter, cross-sectional European survey on 5,158 subjects (40). In this study, both frequency and duration of physical activity were inversely related to airway hyperresponsiveness. In addition, regular physical activity was associated with reduced risk of exacerbations in women with asthma in a longitudinal study (16). On the other hand, significantly higher prevalence of

airway hyperresponsiveness in elite athletes has been documented (25).

Several mechanisms can be advocated to explain the beneficial effects of physical exercise on airway responsiveness. Spirometric outcomes and lung volumes did not change after training, nor was there any correlation between individual changes in lung volumes and changes in methacholine responsiveness. This reasonably argues against the possibility that the observed changes in airway responsiveness are causally linked to changes in lung volumes. We favor the hypothesis that the intensive lung excursions associated with exercise result in airway smooth muscle stretch and may induce functional changes leading to reduction in smooth muscle contractility. In vitro studies have investigated the response of smooth muscle cells to stretch in great detail. Fredberg and colleagues (14) have demonstrated that the force generation of tracheal smooth muscle strips decreased with increasing amplitude and duration of the applied strain. The physiological range of tidal muscle stretch is ~4% of muscle length during spontaneous breathing and rises up to 12% during sigh and even more during exercise (14). Stretching airway smooth muscle by 3% of muscle length reduces force generation by 50% (17), possibly because of plastic reorganization and dynamic changes of the contractile apparatus. Functional studies indicate that adaptation of airway smooth muscle to different length change is a two-step process: first (within minutes) there is a decrease in the ability of muscle to generate force; this is followed by the structural reorganization of the contractile apparatus, that would lead to a full adaptation of the muscle to the new state (24, 34, 47). On the basis of this extensive work on airway smooth muscle function, we speculate that deep inspirations associated with regular physical exercise, such as in our protocol, could stretch airway smooth muscle and reduce its contractility, which remains attenuated during the state of deep inspiration avoidance. This is indirectly suggested by a study conducted in sheep that were chronically subjected to breathing at low lung volumes using a corset for 4 wk (28). The rate of stress generation of the airway smooth muscle significantly increased, with no change in the content or activity of myosin light-chain kinase. The authors proposed that the increased contractility was due to reorganization of the contractile apparatus of the smooth muscle. One should add that regular endurance training could potentiate the effectiveness of the skeletal muscle of the rib cage and the diaphragm, which would, in turn, amplify lung excursions and stretching of the airways. It is possible that exercise results in the release of bronchoprotective mediators, such as catecholamines and nitric oxide. In this regard, the lack of association between the level of adherence to exercise and the outcome of the study is in favor of the occurrence of humoral mechanisms. However, there is no evidence that these mediators have long-term effects as those observed in our study. Also, hyperpnea can cause bronchodilation even with normal levels of plasma catecholamines (3) and inhibition of NO synthase does not alter lung function in asthmatics during exercise (46). Finally, although the possibility of alveolar growth after intense exercise cannot be excluded (1a), we find unlikely that rowing had an effect on alveolar hyperplasia/hypertrophy in our study because the training program was shorter and less intensive than that reported in literature.

Whether the observed effect of training on airway responsiveness is specifically associated with the rowing exercise is

an interesting topic for further work. At this stage, we do not have information on the specificity of this phenomenon with regard to the type of exercise, nor do we know whether increased rates of diaphragmatic excursions in the absence of exercise, such as in incentive spirometry, would have a similar effect.

In conclusion, we have found that a 10-wk course of exercise training results in ~50% attenuation of the airway responsiveness against methacholine, inhaled in the absence of deep inspirations, in healthy subjects. Small changes in airway cells were detected, but with no association to the changes in airway responsiveness. Our data support the need for expanding this line of research in asthma. In addition, our observations imply that a sedentary lifestyle may favor development of airways hyperresponsiveness, in agreement with recent epidemiological data.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. **American Thoracic Society.** Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 152: 1107–1136, 1995.

1a. **Armour J, Donnelly PM, Bye PT.** The large lungs of elite swimmers: an increased alveolar number? *Eur Respir J* 6: 237–47, 1993.

2. **Baggiolini M, Deranleau DA, Dewald B, Thelen M, von Tscharner V, Wymann MP.** [Properties and activation mechanism of neutrophilic leukocytes]. *Arzneimittelforschung* 39: 177–180, 1989.

3. **Barnes PJ, Brown MJ, Silverman M, Dollery CT.** Circulating catecholamines in exercise and hyperventilation induced asthma. *Thorax* 36: 435–440, 1981.

4. **Bonsignore MR, Morici G, Riccobono L, Insalaco G, Bonanno A, Profita M, Paterno A, Vassalle C, Mirabella A, Vignola AM.** Airway inflammation in nonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol* 281: L668–L676, 2001.

5. **Bonsignore MR, Morici G, Vignola AM, Riccobono L, Bonanno A, Profita M, Abate P, Scichilone N, Amato G, Bellia V, Bonsignore G.** Increased airway inflammatory cells in endurance athletes: what do they mean? *Clin Exp Allergy* 33: 14–21, 2003.

6. **Bonsignore MR, Morici G, Riccobono L, Profita M, Bonanno A, Paternò A, Di Giorgi R, Chimenti L, Abate P, Mirabella F, Maurizio Vignola A, Bonsignore G.** Airway cells after swimming outdoors or in the sea in nonasthmatic athletes. *Med Sci Sports Exerc* 35: 1146–1152, 2003.

7. **Bonsignore MR, La Grutta S, Cibella F, Scichilone N, Cuttitta G, Interrante A, Marchese M, Veca M, Virzi' M, Bonanno A, Profita M, Morici G.** Effects of exercise training and montelukast in children with mild asthma. *Med Sci Sports Exerc* 40: 405–412, 2008.

8. **Broeckaert F, Arsalane K, Hermans C, Bergamaschi E, Brustolin A, Mutti A.** Serum Clara cell protein: a sensitive biomarker of increased lung epithelium permeability caused by ambient ozone. *Environ Health Perspect* 108: 533–537, 2000.

9. **Brown RH, Pearse DB, Pyrgos G, Liu MC, Togias A, Permutt S.** The structural basis of airways hyperresponsiveness in asthma. *J Appl Physiol* 101: 30–39, 2006.

10. **Chimenti L, Morici G, Paternò A, Bonanno A, Siena L, Licciardi A, Veca M, Guccione W, Macaluso F, Bonsignore G, Bonsignore MR.** Endurance training damages small airway epithelium in mice. *Am J Respir Crit Care Med* 175: 442–449, 2007.

11. **Crimi E, Pellegrino R, Milanese M, Brusasco V.** Deep breaths, methacholine, and airway narrowing in healthy and mild asthmatic subjects. *J Appl Physiol* 93: 1384–1390, 2002.

12. **Crimi E, Pellegrino R, Smeraldi A, Brusasco V.** Exercise-induced bronchodilation in natural and induced asthma: effects on ventilatory response and performance. *J Appl Physiol* 92: 2353–23560, 2002.

13. **Denguezli M, Ben Chiekh I, Ben Saad H, Zaouali-Ajina M, Tabka Z, Abdelkrim Z.** One-year endurance training: effects on lung function and airway inflammation. *J Sports Sci* 26: 1351–1359, 2008.

14. **Fredberg JJ, Inouye D, Miller B, Nathan M, Jafari S, Raboudi SH, Butler JP, Shore SA.** Airway smooth muscle, tidal stretches, and dynamically determined contractile states. *Am J Respir Crit Care Med* 156: 1752–1759, 1997.

15. **Freedman S, Lane R, Gillett MK, Guz A.** Abolition of methacholine induced bronchoconstriction by the hyperventilation of exercise or volition. *Thorax* 43: 631–636, 1988.

16. **Garcia-Aymerich J, Varraso R, Anto JM, Camargo CA Jr.** Prospective study of physical activity and risk of asthma exacerbations in older women. *Am J Respir Crit Care Med* 179: 999–1003, 2009.

17. **Gump A, Haughney L, Fredberg J.** Relaxation of activated airway smooth muscle: relative potency of isoproterenol vs. tidal stretch. *J Appl Physiol* 90: 2306–2310, 2001.

18. **Hermans C, Bernard A.** Lung epithelium-specific proteins: characteristics and potential applications as markers. *Am J Respir Crit Care Med* 159: 646–678, 1999.

19. **Kapsali T, Permutt S, Laube B, Scichilone N, Togias A.** Potent bronchoprotective effect of deep inspiration and its absence in asthma. *J Appl Physiol* 89: 711–720, 2000.

20. **Karjalainen EM, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, Laitinen LA.** Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine. *Am J Respir Crit Care Med* 161: 2086–2091, 2000.

21. **King GG, Moore BJ, Seow CY, Pare PD.** Time course of increased airway narrowing caused by inhibition of deep inspiration during methacholine challenge. *Am J Respir Crit Care Med* 160: 454–457, 1999.

22. **Kobayashi Y.** The role of chemokines in neutrophil biology. *Front Biosci* 13: 2400–2407, 2008.

23. **Koller DY, Nething I, Otto J, Urbanek R, Eichler I.** Cytokine concentrations in sputum from patients with cystic fibrosis and their relation to eosinophil activity. *Am J Respir Crit Care Med* 155: 1050–1054, 1997.

24. **Kuo KH, Wang L, Pare PD, Ford LE, Seow CY.** Myosin thick filament lability induced by mechanical strain in airway smooth muscle. *J Appl Physiol* 90: 1811–1816, 2001.

25. **Langdeau JB, Turcotte H, Bowie DM, Jobin J, Desgagne P, Boulet LP.** Airway hyperresponsiveness in elite athletes. *Am J Respir Crit Care Med* 161: 1479–1484, 2000.

26. **Lange P, Parner J, Vestbo J, Schnohr P, Jensen G.** A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 339: 1194–1200, 1998.

27. **Laprise C, Boulet LP.** Asymptomatic airway hyperresponsivness: a three-year follow-up. *Am J Respir Crit Care Med* 156: 403–409, 1997.

28. **McClean MA, Matheson MJ, McKay K, Johnson PR, Rynell AC, Ammit AJ, Black JL, Berend N.** Low lung volume alters contractile properties of airway smooth muscle in sheep. *Eur Respir J* 22: 50–56, 2003.

29. **Morici G, Bonsignore MR, Zangla D, Riccobono L, Profita M, Bonanno A, Paternò A, Di Giorgi R, Mirabella F, Chimenti L, Benigno A, Vignola AM, Bellia V, Amato G, Bonsignore G.** Airway cell composition at rest and after an all-out test in competitive rowers. *Med Sci Sports Exerc* 36: 1723–1729, 2004.

30. **Morici G, Zangla D, Santoro A, Pelosi E, Petrucci E, Gioia M, Bonanno A, Profita M, Bellia V, Testa U, Bonsignore MR.** Supramaximal exercise mobilizes hematopoietic progenitors and reticulocytes in athletes. *Am J Physiol Regul Integr Comp Physiol* 289: R1496–R1503, 2005.

31. **Nanson CJ, Burgess JL, Robin M, Bernard A.** Exercise alters serum pneumoprotein concentrations. *Resp Physiol* 127: 259–265, 2001.

32. **Pedersen L, Lund TK, Barnes PJ, Kharitonov SA, Backer V.** Airway responsiveness and inflammation in adolescent elite swimmers. *J Allergy Clin Immunol* 122: 322–327, 2008.

33. **Popov TA, Pizzichini MM, Pizzichini E, Kolendowicz R, Punthakee Z, Dolovich J, Hargreave FE.** Some technical factors influencing the induction of sputum for cell analysis. *Eur Respir J* 8: 559–565, 1995.

34. **Pratusevich VR, Seow CY, Ford LE.** Plasticity in canine airway smooth muscle. *J Gen Physiol* 105: 73–94, 1995.

35. **Scichilone N, Morici G, Marchese R, Bonanno A, Profita M, Togias A, Bonsignore MR.** Reduced airway responsiveness in nonelite runners. *Med Sci Sports Exerc* 37: 2019–2025, 2005.

36. **Scichilone N, Kapsali T, Permutt S, Togias A.** Deep inspiration-induced bronchoprotection is stronger than bronchodilation. *Am J Respir Crit Care Med* 162: 910–916, 2000.

37. **Scichilone N, Permutt S, Bellia V, Togias A.** Inhaled corticosteroids and the beneficial effect of deep inspiration in asthma. *Am J Respir Crit Care Med* 172: 693–699, 2005.

38. **Scichilone N, Permutt S, Togias A.** The lack of the bronchoprotective and not the bronchodilatory ability of deep inspiration is associated with airway hyperresponsiveness. *Am J Respir Crit Care Med* 163: 413–419, 2001.

39. **Scichilone N, Togias A.** The role of lung inflation in airway hyperresponsiveness and in asthma. *Curr Allergy Asthma Rep* 4: 166–174, 2004.

40. **Shaaban R, Leynaert B, Soussan D, Anto JM, Chinn S, de Marco R, Garcia-Aymerich J, Heinrich J, Janson C, Jarvis D, Sunyer J, Svanes C, Wjst M, Burney PG, Neukirch F, Zureik M.** Physical activity and bronchial hyperresponsiveness: European Community Respiratory Health Survey II. *Thorax* 62: 403–410, 2007.

41. **Shore SA, Fredberg JJ.** Obesity, smooth muscle, and airway hyperresponsiveness. *J Allergy Clin Immunol* 115: 925–927, 2005.

42. **Skloot G, Permutt S, Togias A.** Airway hyperresponsiveness in asthma: a problem of limited smooth muscle relaxation with inspiration. *J Clin Invest* 96: 2393–2403, 1995.

44. **Steinacker JM, Both M, Whipp BJ.** Pulmonary mechanics and entrainment of respiration and stroke rate during rowing. *Int J Sports Med* 14: S15–S19, 1993.

45. **Sue-Chu M, Larsson L, Moen T, Rennard SI, Bjermer L.** Bronchoscopy and bronchoalveolar lavage findings in cross-country skiers with and without "ski asthma". *Eur Respir J* 13: 626–632, 1999.

46. **Suman OE, Beck KC.** Role of airway endogenous nitric oxide on lung function during and after exercise in mild asthma. *J Appl Physiol* 93: 1932–1938, 2002.

47. **Wang L, Pare PD, Seow CY.** Effects of length oscillation on the subsequent force development in swine tracheal smooth muscle. *J Appl Physiol* 88: 2246–22450, 2000.