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Airway inflammation in nonasthmatic amateur runners

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Bonsignore, Maria R., Giuseppe Morici, Loredana Riccobono, Giuseppe Insalaco, Anna Bonanno, Mirella Profita, Alessandra Paternò, Cristina Vassalle, Angela Mirabella, and A. Maurizio Vignola. Airway inflammation in nonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol* 281: L668–L676, 2001.—Elite athletes show a high prevalence of symptoms and signs of asthma, but no study has assessed the acute effects of endurance exercise on airway cells in nonasthmatic athletes. We measured exhaled nitric oxide (NO) and collected samples of induced sputum after 3% NaCl aerosol administration for 20 min in nonasthmatic middle-aged amateur runners after the Fourth Palermo International Marathon and 6–9 wk later (habitual training period) at baseline. After the marathon, exhaled NO (n = 9 subjects) was higher [27 ± 9 parts/billion (ppb)] than at baseline (12 ± 4 ppb; $P < 0.0005$). Polymorphonuclear neutrophil (PMN) counts in induced sputum were much higher in runners (91.2 ± 3.6% of total cells postmarathon and 78.7 ± 9.1% at baseline) than in sedentary control subjects (9.9 ± 5.9%; $P < 0.001$). Expression of L-selectin and CD11b/CD18 in sputum PMNs was lower after the race than at baseline and inversely related to the amount of exhaled NO ($r = -0.66$ and -0.69, respectively; $P < 0.05$). Our data indicate that sputum PMNs are increased in nonasthmatic runners both after a marathon and at baseline and suggest that NO may modulate exercise-associated inflammatory airway changes.

polymorphonuclear neutrophil; nitric oxide; adhesion molecules; elastase

COMPETITIVE TRAINING IN ATHLETES is often associated with symptoms and signs of airway inflammation. A questionnaire study reported a high prevalence of physician-diagnosed asthma in elite athletes compared with sedentary control subjects (8). More recently, an increased prevalence of airway hyperresponsiveness (AHR) was found in a large group of elite athletes involved in different sports (16). These changes were interpreted as secondary to repeated exercise-induced hyperventilation and/or increased airway exposure to inhaled allergens or pollutants. In elite cross-country skiers, AHR was documented at the end of an intense training period, although endobronchial biopsies showed high T-lymphocyte, macrophage, eosinophil, and PMN counts irrespective of the presence of AHR, atopy, or symptoms of asthma (11). Therefore, intense endurance training appears associated with changes in airway cells, but their relationship with asthma is unclear.

Intense and prolonged exercise also causes systemic inflammation, which appears to be tightly regulated (26, 31). Running a marathon markedly increases circulating white blood cell and PMN counts (24) and plasma levels of pro- and anti-inflammatory cytokines (26). Exercise modifies PMN adhesion molecules by decreasing the expression of L-selectin, likely in relation to mobilization of the PMN marginated pool (37) and by increasing the expression of CD11b/CD18 (34, 37). Running a marathon causes PMN activation and degranulation as indicated by an increased serum elastase level after the race (4).

In the days after a race, a high frequency of upper respiratory infections was reported by marathon runners, suggesting that intense and prolonged endurance exercise may increase the susceptibility of the respiratory tract to pathogens (25). Nasal mucociliary clearance in runners was low for several days after a marathon race (22), whereas PMN counts and chemotactic activity were increased in nasal lavage fluid (21), suggesting a role of PMNs in the pathogenesis of upper airway infections. However, no study specifically addressed the question of exercise-induced PMN involvement in lower airways or the possible relationship between the respiratory and systemic effects of exercise.

In this study, we asked whether running a marathon affects nasal or exhaled nitric oxide (NO), considered a noninvasive marker of airway inflammation (13, 14, 17). In addition, we collected samples of induced sputum to study airway cells and venous blood to explore the relationship between the effects of the race at the systemic and airway levels. To our knowledge, our study is the first on the effects of a marathon race on airway cells in healthy, nonasthmatic amateur runners.

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METHODS

Subjects and study design. Nine amateur male runners were studied (age 40.1 ± 10.7 yr; body mass index at rest 22.8 ± 1.8 kg/m^2). All subjects were nonsmokers and clinically healthy and had no history of recent infection or other disease. No subject used nonsteroidal anti-inflammatory agents before or during the study period. No subject reported a clinical diagnosis of asthma, asthmalike symptoms, or habitual use of β_2-agonists. Two subjects with a history of allergic rhinitis were asymptomatic and under no treatment at the time of the study; induced sputum was not obtained in them. The study was approved by the local Ethical Committee, and all subjects gave written informed consent. One subject refused to continue the study after the measurements on the day of the marathon; in addition, his blood sample underwent hemolysis. Complete sets of measurements were obtained in the rest of the subjects.

The mean racing experience of the runners was 14 ± 10 yr. On average, they ran 77 ± 15 km/wk in an extraurban environment (La Favorita Park, Palermo, Italy), but training was gradually tapered during the 2 wk before the race. The Fourth Palermo International Marathon was held on December 8, 1998 (start time 9 AM). The circuit comprised urban and extraurban roads at sea level. No traffic was allowed in the circuit for the entire duration of the race, accounting for low levels of air pollutants (Table 1). The weather was cloudy and windy, and at times, there was light rain. The runners completed the marathon in 179 ± 24 min (range 154–221 min). Water intake during the race was allowed ad libitum.

After the race, the subjects were taken to the Italian National Research Council Institute of Respiratory Pathophysiology (Palermo, Italy) where all tests were performed. Six to nine weeks later, the subjects returned to the laboratory to repeat the protocol under baseline conditions. In the interval between measurements, they had resumed their usual weekly training, but no subject had participated in any competition. Baseline measurements were obtained at the same time of day (early afternoon) as in the postrace condition, 24–28 h after the last training session.

Induced sputum samples from the runners were compared with samples previously obtained in a group of sedentary control subjects studied under baseline conditions (39). This group comprised 10 normal subjects, ranging from 25 to 39 yr of age (mean 30.2 ± 4.3 yr), who were lifelong nonsmokers and had pulmonary function within the normal range. No subject of this group trained regularly or participated in running competitions. Subjects from this group were also examined for nasal and exhaled NO as well as for plasma NO metabolites.

Table 1. *Weather and air quality at start and finish line of the 4th International Palermo Marathon*

Variable	Recorded Value	Attention Threshold
Barometric pressure	1,015 mbar	
Temperature	8°C	
Wind	2.3 m/s	
Humidity	54%	
Ozone	26 μg/m^3	200 μg/m^3
CH_4	1,114 μg/m^3	2,000 μg/m^3
SO_2	6 ppb	200 ppb
NO_2	72 ppb	200 ppb
CO	0.8 mg/m^3	15 mg/m^3
PM_{10}	15.9 ppm	200 ppm

Data are means of hourly measurements from 8 AM to 1 PM taken by the City Weather Bureau of Palermo, Italy, on December 8, 1998. PM_{10}, particulate matter <10 μm.

Measurements. In each subject and condition, a 20-ml blood sample was drawn from the antecubital vein for complete blood cell counts and preparation of plasma and serum aliquots. These were stored at −20°C for subsequent determination of cortisol, elastase, albumin, IgA, α_1-protease inhibitor (α_1-PI), histamine, and NO metabolite concentrations.

Pulmonary function was measured by spirometry. The lung diffusion capacity for CO ($D_{L_{CO}}$) was measured only on the day of the race and corrected for hemoglobin content.

Exhaled and nasal NO levels were determined by chemiluminescence (Sievers Instruments, Boulder, CO). Ambient NO was undetectable at all times. Exhaled NO was measured in triplicate after fast inhalation maneuvers to total lung capacity at a constant expiratory flow of 100 ml/s against an expiratory resistance of 20 cmH_2O (12). Nasal NO was sampled directly from both nostrils and reported as the mean of six measurements.

Induced sputum production and processing. Sputum induction and processing were according to the method of Fahy et al. (5) with slight modifications (39). Briefly, after the collection of samples of saliva, the subjects were exposed to a hypertonic saline (3%) aerosol for 20 min. The aerosol was administered with an ultrasonic nebulizer (median particle diameter 2.5 μm, output 1 ml/min; Fisoneb, Fisons Italchimici Spa, Rome, Italy). The subject regularly interrupted the procedure to cough and expectorate sputum into 50-ml sterile ampoules. The oral cavity was washed with saline before the sputum was expectorated, and salivary contamination was minimized by blowing the nose. After the procedure, the absence of bronchospasm was confirmed by spirometry.

Sputum and saliva samples were added to an equal volume of a 0.1% dithiothreitol-saline solution (Sigma, St. Louis, MO). They were gently mixed with a vortex mixer and placed in a water bath at 37°C for 15 min, with periodic removal from the water bath for further brief gentle mixing. Homogenized samples were centrifuged at 800 *g* for 10 min, and the supernatants were aspirated and frozen at −20°C for subsequent biochemical analysis.

The cell pellets were resuspended in saline, and the total cell count and viability were assessed in 10-μl aliquots with a standard hemacytometer and Trypan blue exclusion, respectively. The cells were then cytocentrifuged (Cytospin 2, Shandon Instruments, Runcorn, UK) and stained with Diff-Quik (Merz-Dade, Dudingen, Switzerland). The slides were blindly read by two investigators (L. Riccobono and A. M. Vignola) who counted at least 400 cells/slide. The squamous cell count was subtracted from the total cell count, and the differential counts are expressed as corrected percentages. Sputum samples were considered adequate if the sputum volume was at least 1 ml with <50% squamous cells on differential count.

Expression of adhesion molecules on sputum PMNs. After cytocentrifugation, the cell pellets were resuspended in saline, and slides were prepared, dried at 20°C for 30 min, fixed with acetone-methanol at 4°C for 10 min, and incubated at 37°C for 1 h with monoclonal antibodies for against intercellular adhesion molecule (ICAM)-1, L-selectin, CD11a/CD18 (lymphocyte function-associated antigen-1), and CD11b/CD-18 (DAKO) at dilutions of 1:2, 1:2, 1:100, and 1:100, respectively. Immunoreactivity was revealed with the labeled streptavidin-biotin-alkaline phosphatase technique. Four hundred cells per slide were counted by two investigators (M. Profita and A. M. Vignola), and the results are

Table 2. *Blood cell counts and biochemical analyses on plasma and serum*

	Postmarathon	Baseline Conditions
WBC counts, cells/ml	17,400 ± 3,200	6,200 ± 2,100*
Neutrophils, %	89.6 ± 2.0	62.3 ± 6.9*
Lymphocytes, %	7.2 ± 2.2	30.3 ± 8.7*
Monocytes, %	3.2 ± 2.0	7.1 ± 4.1†
Neutrophils, cells/ml	15,728 ± 3,252	3,864 ± 1,087*
Cortisol, mg/dl	55.7 ± 32.0	21.1 ± 6.5*
Albumin, g/100 ml	4.54 ± 0.21	4.26 ± 0.19†
Histamine, mmol/ml	2.27 ± 1.03	6.03 ± 2.07*
Elastase, μg/l	136.3 ± 29.2	39.6 ± 9.7*

Values are means ± SD. WBC, white blood cell. Significantly different from postmarathon: *$P < 0.01$; †$P < 0.05$.

expressed as percentages of positive PMNs. Interinvestigator agreement was excellent ($\kappa = 0.94$).

Biochemical analysis on plasma, serum, and sputum supernatants. Plasma cortisol and histamine concentrations were assessed by radioimmunoassay (Immunotech, Marseille, France). The detectability threshold for histamine was 0.2 nM. Total elastase was measured with a homogeneous enzyme immunoassay specific for human PMN elastase (Ecoline, Kit Merck, Darmstadt, Germany) (23), with a detectability threshold of 20 μg/l. α_1-PI, IgA, and albumin were measured by a nephelometric assay (Beckman array protein system) using specific monoclonal antibodies (Beckman Immunochemistry Systems) (28). The assay detected levels of α_1-PI, IgA, and albumin >2.8, 11.0 and 6.0 μg/ml, respectively. Plasma NO concentrations were estimated by a colorimetric assay based on the Griess reaction (6). Nitrates were converted to nitrites by nitrate reductase, and then Griess reagents were added to convert nitrites to a chromophore compound. Nitrite concentration was estimated by spectrophotometry absorbance at 540 nm.

Sputum supernatants were analyzed to determine the concentrations of total elastase, α_1-PI, albumin, IgA, and histamine by using the same methods as for plasma samples.

Statistics. Data are reported as means ± SD. Data obtained after the marathon and at baseline were compared by paired *t*-test (blood cell counts and biochemical measurements on plasma and serum) or unpaired *t*- or Mann-Whitney test as appropriate (sputum cell counts and biochemical analysis and NO measurements). A Kruskal-Wallis test was used to compare sputum cells in the runners after the race and at baseline and in sedentary control subjects. Relationships between variables were analyzed by linear regression or Spearman rank coefficient test. For all tests, a statistical package was used (StatView 4.5, Abacus Concept, Berkeley, CA). A level of $P < 0.05$ was chosen as indicating significance.

RESULTS

Clinical data. No subject reported respiratory symptoms during immediate recovery after the marathon race or symptoms of respiratory infections in the weeks after the race when returning to the laboratory for baseline measurements.

Blood, plasma, and serum samples. White blood cell and PMN counts were very high after the marathon, with decreased percentages of lymphocytes and monocytes (Table 2). No significant difference was found between postmarathon and baseline samples for red blood cell and platelet counts, hematocrit, hemoglobin concentration, or absolute lymphocyte or monocyte counts (data not shown). Circulating cortisol and elastase levels were high, whereas the histamine level was low after the marathon (Table 2). Serum albumin concentration showed a small significant increase after the race. Plasma α_1-PI and IgA concentrations were normal and unaffected by the race (data not shown). The mean plasma NO concentration in the runners was 69 ± 10 μM after the marathon and 72 ± 20 μM at baseline [not significant (NS)], both values being, as expected (10), significantly higher than the plasma NO concentration in the sedentary control subjects (51 ± 14 μM; $P < 0.05$; $n = 7$).

Pulmonary function tests and NO measurements. Spirometry was performed on average 103 ± 29 min after the runners finished the race. Forced expiratory volume in 1 s and forced vital capacity were similar after the race and at baseline (Fig. 1). D_{LCO} after the marathon was in the normal range (105.4 ± 11.0% of predicted value).

NO was measured on average 175 ± 50 min after the end of the race. Exhaled NO (Fig. 2, *top*) after the marathon was twice as high [26.9 ± 8.6 parts/billion (ppb); $n = 9$ subjects] than at baseline (12.0 ± 3.8 ppb; $P < 0.0005$; $n = 8$ subjects), with no difference between rhinitic and nonrhinitic runners. Baseline values in the runners were similar to those obtained in sedentary control subjects (13.8 ± 4.9 ppb; $n = 4$). Nasal NO (Fig. 2, *bottom*) was higher after the race (101 ± 58 ppb; $n = 9$ subjects) than at baseline (86 ± 43 ppb; $n = 8$ subjects), the difference between measurements becoming significant after excluding the rhinitic subjects

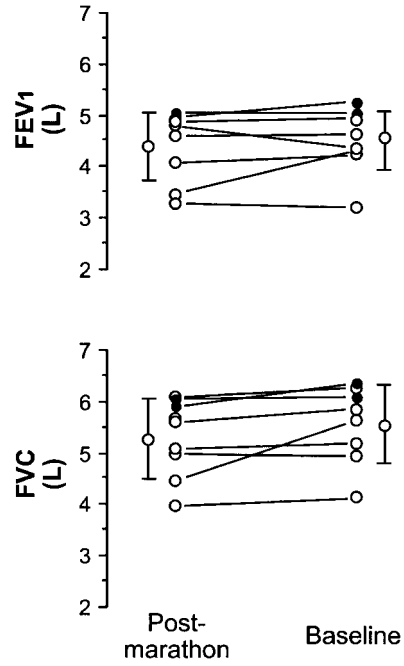

Fig. 1. Spirometric data. Forced expiratory volume in 1 s (FEV_1; *top*) and forced vital capacity (FVC; *bottom*) were similar after the race (postmarathon) and under baseline conditions (rest). ●, Two subjects with a history of allergic rhinitis. Mean ± SE values refer to all subjects.

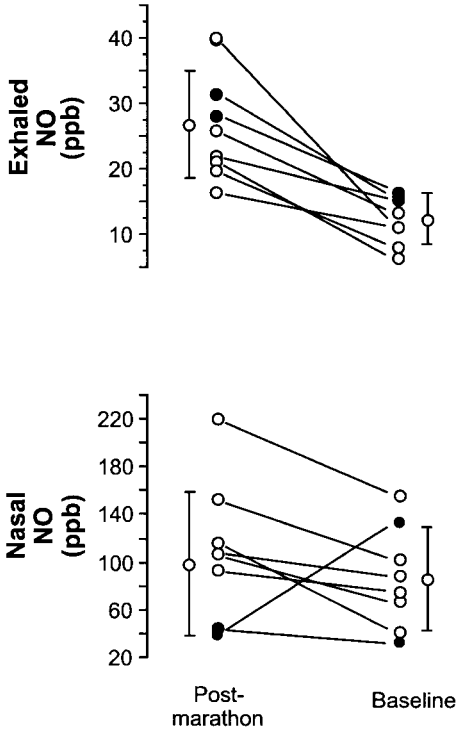

Fig. 2. Exhaled nitric oxide (NO; *top*) was higher after the race compared with baseline conditions ($P < 0.0005$). Nasal NO (*bottom*) showed a similar behavior only after exclusion of subjects with a history of allergic rhinitis (●; $P < 0.007$). Mean ± SE values refer to all subjects. ppb, Parts/billion.

($P < 0.01$). In the sedentary control subjects ($n = 4$), nasal NO was 71 ± 38 ppb (NS vs. nonrhinitic runners at baseline). Exhaled or nasal NO concentration in the runners was unrelated to plasma NO concentration.

Induced sputum. Induced sputum was obtained on average 202 ± 28 min after the end of the race. Technically adequate sputum samples were obtained only from five of seven subjects after the marathon and from five of six subjects at baseline. The samples rejected showed excess squamous contamination (both samples from the same subject) and low cell yield (postrace sample from another subject). Therefore, induced sputum samples were obtained only from four subjects on both occasions, and the data were analyzed by tests for unpaired comparisons.

The cells in the induced sputum from the runners were mostly PMNs (Fig. 3) as opposed to the prevalence of macrophages in the control subjects. However, the absolute macrophage count in the runners was in the normal range. Compared with postrace samples, a lower percentage of PMNs ($P < 0.05$) and a higher percentage of macrophages ($P < 0.05$) were found in the runners at baseline, but the absolute counts of both cell types did not differ significantly between experimental conditions. Eosinophil counts in the runners were low (0.4 ± 0.7% of total cells after the marathon and 1.0 ± 1.5% at baseline). Lymphocyte and bronchial

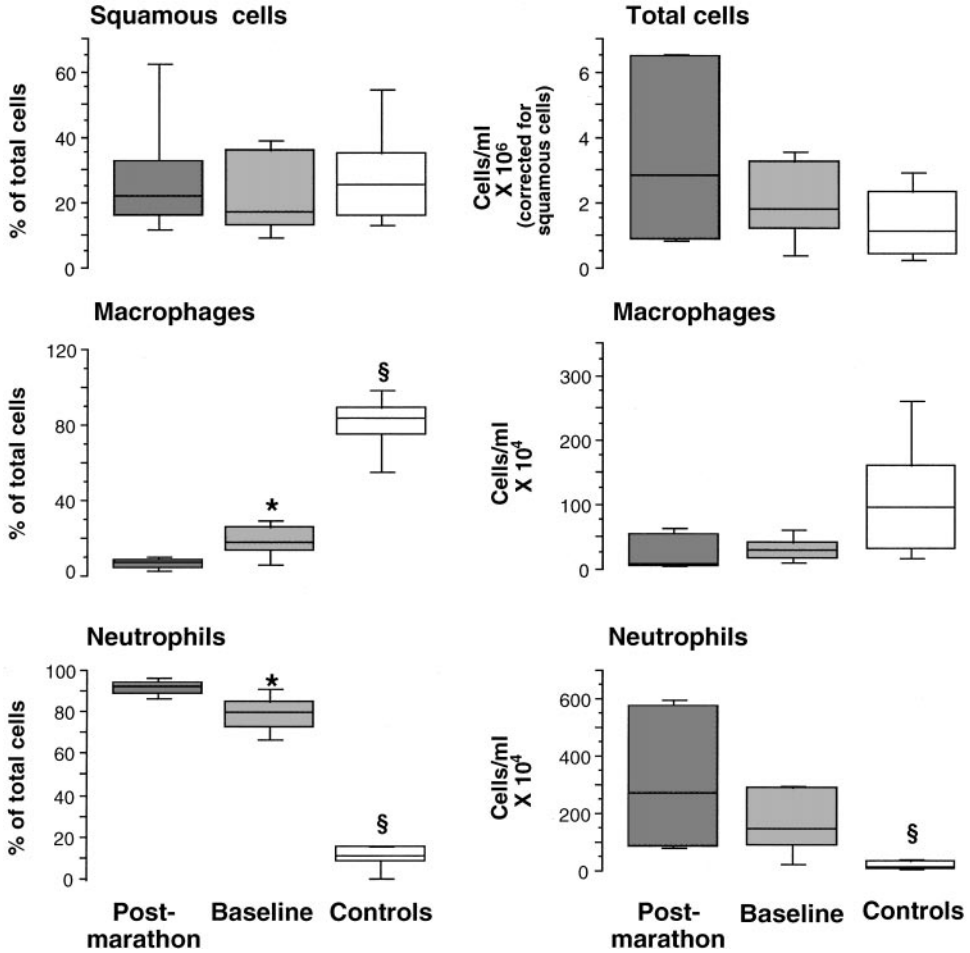

Fig. 3. Cells in induced sputum samples [$n = 5$ subjects under postmarathon and baseline conditions and 10 control subjects (37)]. *$P < 0.05$ for postmarathon vs. baseline (by Mann-Whitney test). §$P < 0.005$ for runners vs. control subjects (by Kruskal-Wallis test).

cell counts were similar in the runners and control subjects and in the postrace and baseline samples (data not shown). Although the sample size was small, analysis of all induced sputum samples showed positive correlations between absolute cell and PMN counts versus plasma cortisol concentration in the runners (Fig. 4). Absolute cell and PMN counts in the sputum were unrelated to exhaled NO.

After the race, few PMNs in the induced sputum were positive for L-selectin (12.3 ± 16.9%) and CD11b/CD18 (4.6 ± 7.3%; Fig. 5). Corresponding values at baseline were 71.8 ± 16.1 and 60.9 ± 27.6% ($P < 0.005$ for both adhesion molecules vs. postrace). All samples were negative for ICAM-1, whereas lymphocyte function-associated antigen-1 expression was unaffected by the race (58.9 ± 37.4% of positive cells after the marathon and 60.8 ± 30.9% at baseline). Analysis on all samples showed that exhaled NO correlated inversely with the percentage of sputum PMNs expressing L-selectin ($r = -0.66$; $P < 0.05$) or CD11b/CD18 ($r = -0.69$; $P < 0.05$). In addition, in induced sputum, the expression of L-selectin correlated with the expression of CD11b/CD18 ($r = 0.77$; $P = 0.01$). Plasma cortisol also inversely correlated to the percentage of sputum PMNs positive for L-selectin ($r = -0.84$; $P < 0.05$) and CD11b/CD18 ($r = -0.75$; $P < 0.05$).

The albumin concentration in induced sputum was 20.0 ± 11.5 mg/100 ml after the race and 7.6 ± 7.6 mg/100 ml at baseline ($P = 0.08$; NS). Corresponding histamine values were 162 ± 228 nmol/ml postrace, and 40.5 ± 43.7 nmol/ml at baseline (NS). PMN elastase concentration was 0.95 ± 0.61 μg/ml after the race and 0.78 ± 0.47 μg/ml at baseline, both values being similar to those of sedentary control subjects [1.2 μg/ml (39)]. α_1-PI and IgA concentrations were normal and unaffected by the race (data not shown). No significant correlation was found between the sputum cell counts and sputum histamine, albumin, or elastase concentration.

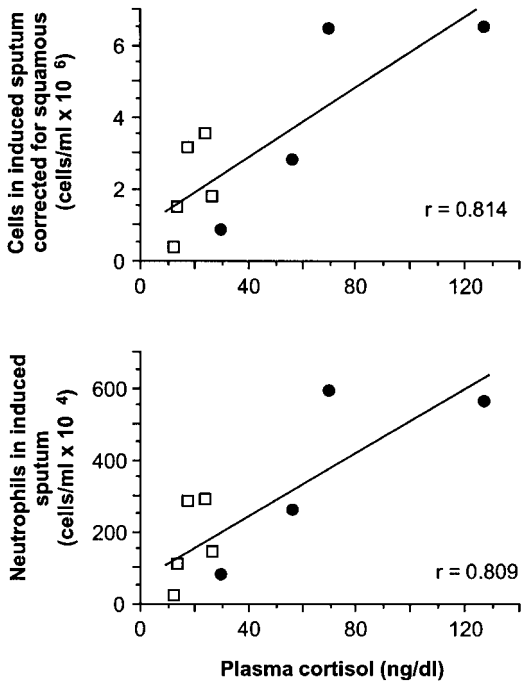

Fig. 4. Correlations between plasma cortisol concentration and total cell (*top*) and neutrophil (*bottom*) counts in induced sputum. ●, Postmarathon; □, baseline conditions. There are only 4 postmarathon points because hemolysis did not allow determination of plasma cortisol in 1 subject.

DISCUSSION

Our study tested the hypothesis that intense and prolonged exercise may affect airway cell number and/or composition in nonasthmatic amateur runners, possibly accounting for the high susceptibility to respiratory infections reported after a marathon race (24, 25). After the race, we found *1*) high exhaled and nasal NO concentrations; *2*) high PMN counts in the induced sputum; *3*) few PMNs positive for L-selectin and CD11b/CD18, possibly related to high exhaled NO and plasma cortisol concentrations; and *4*) normal sputum elastase concentration, suggesting a lack of significant PMN activation in the induced sputum as opposed to the high elastase concentration in serum. Under baseline conditions, the percentage of PMNs in the induced sputum of the runners remained high compared with that in the sedentary control subjects, suggesting a chronic increase in PMNs in the airways, possibly related to habitual training.

The high percentage of PMNs in the induced sputum of the runners confirms and extends the findings obtained in other endurance athletes such as elite cross-country skiers (11) and elite swimmers (7). However, our study differs from the previous ones in two major respects: first, we studied amateur, middle-age runners compared with much younger elite athlete populations in the previous studies (7, 11), and second, our aim was to assess both the acute effects of prolonged endurance exercise (marathon samples) and the possible effects of chronic training (baseline samples), whereas the available studies collected samples only under baseline conditions (7, 11). Indeed, our data show a trend toward different amounts and functional features of PMNs in the induced sputum of the runners after the marathon compared with the baseline conditions. Because our subjects were not asthmatic, our data support the fact that endurance exercise may increase PMNs in the airways irrespective of concomitant asthma or AHR (11).

After the race, the elastase level was very high in the serum (threefold increase over baseline conditions) (4) and normal in the induced sputum, indicating that the high number of PMNs in the induced sputum was not associated with a significant degree of PMN activation. The pattern of adhesion molecules in the induced sputum also suggested a low grade of activation (1) in airway PMNs after the marathon. Few PMNs in the induced sputum were positive for CD11b/CD18 after the race, in contrast to the increased percentage of PMNs expressing CD11b/CD18 found in peripheral blood after incremental exercise (37) or a marathon race (9). Conversely, expression of L-selectin was low in

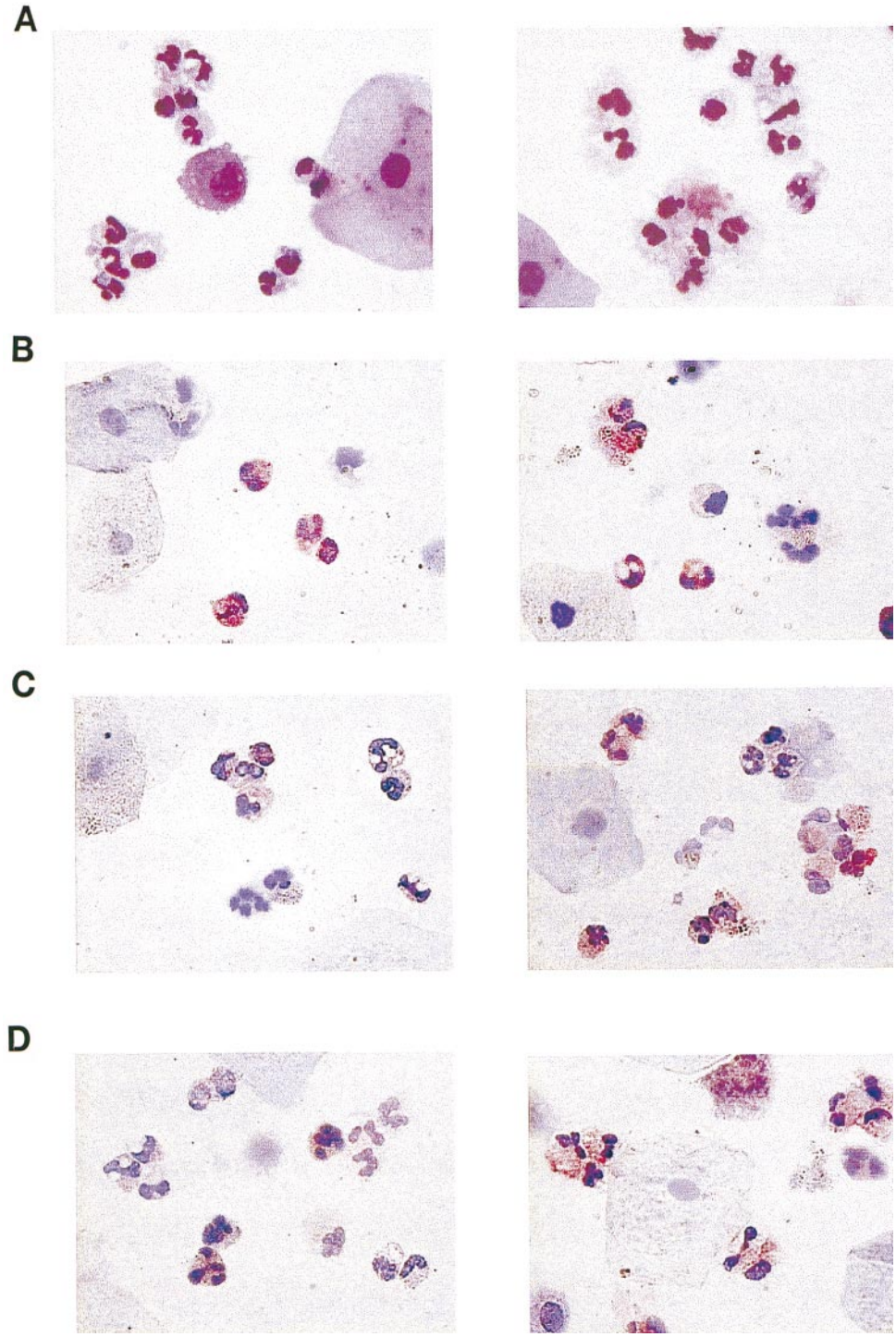

Fig. 5. Samples obtained during recovery after the marathon race (*left*) and at baseline (*right*). *A*: Diff-Quik staining. *B–D*: immunostaining for lymphocyte function-associated antigen-1, L-selectin, and CD11b/CD18, respectively, in induced sputum samples. Original magnification, ×40.

the induced sputum after the marathon, similar to the findings in peripheral blood PMNs after incremental (34) or moderate-intensity (15) exercise. As a possible explanation, corticosteroid-induced downregulation of both L-selectin and CD11b/CD18 (2) is supported by the correlation found between plasma cortisol and expression of both adhesion molecules in induced sputum PMNs. Alternatively, the time lag between the end of the race and data collection may have allowed a progressive loss of CD11b/CD18 by PMNs, in agreement with recent findings in infarcted myocardium and in vitro (40), suggesting involvement of PMNs not expressing CD11b/CD18 in the healing processes. Further investigation is needed to define this point.

Exhaled NO was measured in our runners as a noninvasive marker of airway inflammation (14). During exercise, the volume of expired NO followed the changes in minute ventilation, with a rapid fall toward baseline in early recovery (32). About 3 h after the marathon, however, exhaled NO was high, suggesting

late changes in the time course of exhaled NO after exercise. Nasal NO was also high after the marathon in nonrhinitic runners, possibly in relation to marathon-induced nasal alterations (21, 22). Although exhaled NO is traditionally considered in the asthma literature as a marker of airway inflammation, recent data suggest some role for NO and derived products in modulating PMN chemotactic activity (29) and downregulating nuclear factor-κB activation in the airways (35). Our data in runners suggest that NO may indeed be involved in the control of inflammatory processes in the airways. High exhaled NO after the marathon race was associated with a low expression of L-selectin and CD11b/CD18 in induced sputum PMNs, the opposite pattern being found at baseline. Because NO donors may inhibit the upregulation of CD18 by zymosan-activated plasma without affecting L-selectin shedding of PMNs (30), we speculate that a similar mechanism may explain the inverse correlation between exhaled NO and CD11b/CD18 expression in sputum PMNs of runners. Although our sample was too small to draw definitive conclusions, the data suggest a possible role for NO in controlling or decreasing airway inflammation after intense and prolonged endurance exercise. However, a simple association of independent changes in PMN adhesion molecules and exhaled NO cannot be ruled out at present, and further studies are needed to confirm our hypothesis.

A trend toward increased albumin concentration in induced sputum after the race suggests ongoing inflammation and increased vascular permeability in the airway compartment. The lack of significance in comparing albumin concentration between postrace and baseline measurements is likely due to the small sample size: type II error analysis showed that 14 observations/group would be necessary to show a significant difference between postrace and baseline by unpaired *t*-test, assuming that mean ± SD values remained the same after the sample size was increased. Histamine concentrations in induced sputum also tended to be higher after the marathon compared with baseline (sample size required to show a significant difference, 27 observations/group). However, several findings do not support the finding that histamine is a key mediator of exercise-induced airway changes: *1*) histamine is known to inhibit PMN chemotaxis (3); *2*) the normal pulmonary function tests (PFTs) in our runners argue against a bronchoconstrictor effect of histamine after exercise; and *3*) although histamine exerts immunoregulatory functions by increasing expression of ICAM-1 and human leukocyte antigen-DR and by promoting release of fibronectin by bronchial epithelial cells (38), ICAM-1 expression in sputum was absent after the marathon. Therefore, our data do not indicate a clear-cut role for histamine in the context of exercise-induced airway inflammation.

As for the mechanism(s) of exercise-induced airway changes in runners, we favor the explanation that they may be at least partly linked to hyperventilation for prolonged periods of time. Mechanical ventilation at high tidal volumes was shown to cause pulmonary inflammation and cytokine release (27, 36). A similar mechanism may operate in runners during intense and prolonged acute exercise and chronic training. Both elite cross-country skiers (11) and swimmers (8) show increased PMNs in endobronchial biopsies or induced sputum under baseline conditions, supporting the fact that exercise may cause low-grade airway cell changes by repeated, training-associated episodes of ventilation at high tidal volumes. Again, the small sample size of our study may be responsible for the lack of significance found between the runners and sedentary control subjects in the cellularity of the induced sputum (sample size required to show a significant difference between runners postmarathon and control subjects, 18 observations/group). Another possible explanation for the high PMN counts in the induced sputum of the runners is that air pollutants were low during the marathon due to closure of the circuit to motor vehicles. In addition, our subjects usually trained in an extraurban environment, making a chronic exposure to high levels of pollutants an unlikely explanation of our findings. The occurrence of cold air-induced bronchoconstriction is not supported by normal PFTs, and there was no report of respiratory symptoms after the race; in addition, cross-country skiers with "ski asthma" show a high percentage of lymphocytes in bronchoalveolar lavage fluid at baseline (33), suggesting at least some biological differences between exercise at moderate temperature and exercise in the cold. Finally, the very high percentage of PMNs in the induced sputum of the runners was unlikely to result from the sputum induction procedure itself (20). As for a possible relationship between high PMN counts in induced sputum and exercise-induced systemic changes, a definitive conclusion is hard to draw: induced sputum total cell and PMN counts increased with plasma cortisol, but such a correlation may simply indicate that the sputum cell content reflected the stress of a marathon race in a dose-response fashion. A larger sample size is needed to extend studies on this point.

Some features of our study need comment. First, the absence of AHR or atopy was not objectively documented in our sample. However, the clinical history was negative for symptoms and/or signs of asthma, and induced sputum cell composition or baseline exhaled NO did not support that our runners were asthmatic. Second, the small sample size was imposed by technical limitations linked to sputum processing on the day of the marathon. Third, we are aware that normal spirometric and diffusion measurements ~2 h after the end of the race cannot exclude the possibility that we may have missed transient changes occurring immediately after the race. After a marathon, forced expiratory volume in 1 s was increased (19) or unchanged (18), forced vital capacity was decreased (18), and D_{LCO} was decreased (19) or unaffected (18), all changes returning to baseline by the following morning (18, 19). Because no study ever reported PFTs during late recovery after a marathon, we felt it was important to measure PFTs together with exhaled NO and the col-

lection of induced sputum samples. The finding of normal PFTs at a time when markers of airway inflammation were increased suggests a light impact of exercise-induced airway changes on airway function, at least in nonelite nonasthmatic runners during late recovery after a marathon. Finally, our measurements provide a "snapshot" of a relatively late phase of recovery after the race, the complex protocol not allowing data collection at the site of race arrival. Additional studies are necessary to fully describe the time course of NO and airway cells after prolonged and intense endurance exercise. Despite these limitations and difficulties, we feel that data obtained after a real competition are valuable because they take into account aspects of a marathon (i.e., weather conditions or psychological stress) that cannot be easily mimicked in the laboratory.

In summary, in nonasthmatic middle-aged amateur runners, a marathon race increased the markers of inflammation in the airways without symptoms or late changes in the PFTs. The absence of clear signs of PMN activation in induced sputum suggests a tight control of exercise-induced airway changes, extending to the respiratory compartment the view that exercise-induced changes in biological markers of inflammation appear altogether quite small (31). Further studies are needed to fully characterize the pathogenesis of exercise-induced airway inflammation in both asthmatic and nonasthmatic athletes, the possible long-term consequences of prolonged training, and the effects on athlete's performance.

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