Role of lung inflammatory mediators as a cause of exercise-induced arterial hypoxemia in young athletes

THOMAS J. WETTER, ZHUZAI XIANG, DAVID A. SONETTI, HANS C. HAVERKAMP, ANTHONY J. RICE, ADNAN A. ABBASI, KEITH C. MEYER, and JEROME A. DEMPSEY

1John Rankin Laboratory of Pulmonary Medicine, Department of Preventive Medicine, and 2Department of Medicine, Section of Pulmonary and Critical Care Medicine, Clinical Sciences Center, University of Wisconsin, Madison, Wisconsin 53705

Received 1 November 2001; accepted in final form 5 March 2002

Exercise-induced arterial hypoxemia (EIAH), which is defined as an inability to maintain arterial oxygen partial pressure (PaO₂) and arterial oxygen saturation (SaO₂) of hemoglobin (4, 14, 20, 42). Causes of a widened A-aDO₂ include ventilation-perfusion (V̇A/Q̇) mismatch, diffusion limitation, and venoarterial shunts (15). One hypothesis for both mismatch and diffusion limitation is the development of mild interstitial pulmonary edema caused by high pulmonary pressures and/or inflammatory mediator-induced vascular leakage (43). Airway inflammation leading to airway edema, excessive mucus production, and smooth muscle constriction in the small airways (<2 mm in diameter) could affect the distribution of alveolar ventilation during exercise. Alternatively, increased permeability of the pulmonary or bronchial vasculature could lead to cytokine influx into airways and stimulate further mediator release, again contributing to a maldistribution of alveolar ventilation. In fact, administration of the inflammatory mediators histamine and platelet-activating factor can cause V̇A/Q̇ mismatch and a widened A-aDO₂ (9, 22, 45).

How might exercise lead to inflammation in peripheral airways? In well-trained athletes, inflammatory mediators have been found in bronchoalveolar lavage (BAL) fluid both after strenuous exercise (23) and in the basal state (55), indicating that both acute and chronic exercise may lead to increased occurrence of inflammatory processes in the lung. Furthermore, increased incidence of asthma in high-level athletes (30) and indexes of airway inflammation and remodeling in nonasthmatic athletes (29) suggest that in some individuals high levels of exercise training may lead to an asthmalike condition. Potentially, high airflow rates associated with intense exercise may cause mediator release from lung cells because of shear or mechanical stress or by evaporation-induced changes in osmolality (3). Repetitive hyperpnea in a dog model has been shown to cause eosinophilic inflammation and elevated levels of prostaglandins and leukotrienes in BAL fluid (13). Exclusive mouth breathing and dry air could also accentuate the load to humidify incoming air. On the vascular side, high pulmonary arterial pressures that

Address for reprint requests and other correspondence: T. J. Wetter, 808A Franklin St. Stevens Point, WI 54481 (E-mail: tjwetter@yahoo.com).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
accompanied by heavy exercise combined with an exercise-induced rise in circulating cytokines or other mediators may increase microvascular permeability (57). Airway epithelial and mucosal mast cells, as well as circulating cells that are recruited to the lung, release a variety of inflammatory mediators, including histamine, leukotrienes, prostaglandins, and cytokines. These mediators have actions that include increasing microvascular permeability, peripheral airway smooth muscle constriction, mucus secretion, altering vascular tone, and leukocyte activation, all of which may narrow small airways and in part contribute to V/Q mismatch and a widened A-aDO2 during exercise (12, 41, 47).

The best evidence to date for a significant role of inflammatory mediators in EIAH was demonstrated by Prefaut et al. (43). Administration of nedocromil sodium to a small group of master athletes (mean age 63 yr) narrowed the A-aDO2 by ~50% and attenuated the fall in PaO2 during an incremental cycle exercise test. Plasma histamine release was also reduced and the reduction correlated with the improvement in PaO2. In addition to nedocromil sodium, which prevents mediator release by stabilizing the membranes of mast and potentially other cells, histamine-receptor antagonists and leukotriene synthesis inhibitors are important for the management of inflammation in asthma and allergy-related diseases. These types of drugs have been shown to lessen the bronchoconstriction associated with exercise or hyperpnea, reduce inflammatory mediators in BAL, block mediator release from mast and other cells, reduce vascular permeability, and decrease mucus production (1, 16, 34). If airway inflammation is a cause of EIAH, a combination of drugs targeting multiple pathways should be most effective in preventing a worsening of gas exchange in athletes during exercise. Additionally, whether pharmacological treatment will be as effective in reducing the severity of EIAH in younger subjects of both sexes as it was in older male subjects has not been investigated.

The aim of the present study was to investigate the role of inflammation in gas-exchange abnormalities and EIAH in young endurance athletes. To accomplish this, we administered a histamine-receptor antagonist (fexofenadine), a leukotriene-synthesis inhibitor (zileuton), and nedocromil sodium together in a one-time dose before exercise. To document effects on lung inflammation, we examined several mediators in plasma, urine, and sputum as well as changes in lung function, including respiratory resistance. We hypothesized that drug administration would reduce inflammatory mediators, improve gas exchange during exercise, and result in less EIAH compared with placebo.

METHODS

Subjects

Seventeen healthy endurance-trained athletes (9 men, 8 women) were selected from a total of 42 recruited to participate in this study. Informed consent was obtained, and procedures were approved by the Institutional Review Board of the University of Wisconsin-Madison. Subject characteristics are shown in Table 1. All subjects engaged in endurance exercise at least three times a week, which included running 15–85 miles/wk. Many also incorporated other forms of exercise (biking, swimming, weight training) into their weekly regimens. At the time of the study, none was taking any medication except birth control pills (5 women).

Resting Pulmonary Function Tests

Baseline pulmonary function was determined (Pulmonizer model PFT 3000, Med Science, St. Louis, MO) as previously described (49). Lung function tests performed pre- and post-exercise (~5 and 30 min) include total respiratory resistance (Rrs), maximal flow-volume loops (FVL), and exhaled nitric oxide concentration measured at a constant expiratory airflow rate of 46 ml/s (NOCF). Rrs was measured using the forced oscillation technique (Jaeger, MS- IOS, Germany). Subjects were encouraged to breathe normally, and they followed a metronome set to a breathing frequency of 12 breaths/min and the ratio of inspiratory time to total time of 0.35. A total of seven to eight breaths were analyzed. Frequency dependence of resistance was calculated as Rrs at 5 Hz − Rrs at 25 Hz. Resonant frequency was the frequency at which reactance = 0. Measurements obtained from FVL were peak expiratory and inspiratory flows, forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and expiratory flows at selected lung volumes, including mean forced expiratory flow in the middle 50% of expiration. NOCF was measured as previously described (54).

Study Design

Day 1: subject screening. All subjects completed medical, allergy, and activity questionnaires and performed baseline pulmonary function tests. Each completed a maximal incremental exercise test on a treadmill with a finger pulse oximeter (model 3900, Datex-Ohmeda, Madison, WI) in place to estimate the percentage of SaO2 followed by postexercise pulmonary function tests. Subjects were selected for the study if estimated SaO2 fell below 92% during heavy exercise, were of relatively high fitness, and appeared comfortable completing all tests. Twenty subjects were selected, and, of these, three women withdrew from the study (primarily because of difficulty in placement of the arterial catheter) before completion of all tests. None of their data are included in analysis.

Day 2: induced sputum. On a subsequent day, induced sputum was collected. Subjects were asked not to engage in
strenuous exercise for at least 24 h preceding the collection. Sputum was induced by inhalation of a 3% saline mist generated from an ultrasonic nebulizer (Ultra-Neb99, DeVilbiss, Somerset, PA). Wearing noseclips, subjects inhaled the saline mist with tidal breaths and with an inspiration to total lung capacity once every minute. Every 4 min, subjects were instructed to blow their noses and rinse their mouths with water before expectoration to minimize nasal contamination of the sample. This procedure continued for 12–24 min until an adequate volume of sputum was produced. Sputum was stored in a sterile container on ice and processed immediately (see Sputum Analysis).

Days 3 and 4: drug or placebo trials. The final two study days involved administration of either placebo or drug cocktail, incremental exercise tests, and arterial blood sampling. Administration of interventions was done in a randomized double-blind manner. Each subject was studied at approximately the same time of day for the placebo and drug trial. Men completed the two trials ≥2 wk apart, and women were studied ≥4 wk apart. We did not control for phase of menstrual cycle between subjects, although for each woman, the two final trials were scheduled at approximately the same point of the menstrual cycle (self-reported). Subjects were asked to refrain from hard exercise on the day before each trial and to avoid nonsteroidal anti-inflammatory drugs for the week before each trial (no subject indicated using nonsteroidal anti-inflammatory drugs).

On the day of the drug trial, subjects arrived at the laboratory, emptied their bladders, and were administered one capsule containing 60 mg fexofenadine hydrochloride (Allegra, Hoechst Marion Roussel Pharmaceuticals, Kansas City, MO) and one tablet containing 600 mg zileuton (ZyFlamadyl, Abbott, North Chicago, IL). Dosing was done by a technician who was not involved in data collection or analysis. An arterial catheter and nasopharyngeal temperature probe were placed as previously described (58). Three inhalations (1.75 mg/actuation for a total of 5.25 mg) of nedocromil sodium (Tilade inhaler, Rhone-Poulenc Rorer Pharmaceuticals, Collegeville, PA) were taken after actuation into an AeroChamber (Monaghan Medical, Plattsburgh, NY). Subjects wore a noseclip and rinsed their mouths with mouthwash after the inhalations to mask any taste of the drug. After ~10 min, preexercise FVL, Rrs, and NOCF measurements were made, and subjects emptied their bladders and provided a urine sample. Subjects rested quietly for 5 min while resting expired gases and arterial blood were collected. The incremental exercise test began 132 ± 30 min after administration of oral pills and 40 ± 4 min after nedocromil sodium administration; this timing was designed to result in near-peak circulating drug levels at time of exercise. Exercise tests consisted of six to seven exercise levels. Initial speed was ~5 miles/h for women and ~6 miles/h for men [54 ± 4% of maximal oxygen consumption (VO_{2\text{max}})], which was increased by 1–1.5 miles/h every 3 min up to a comfortable running speed (stage 5) and thereafter the grade of the treadmill was increased by 2% every 3 min. This protocol kept the stride turnover at a reasonable rate and was deemed safe for sampling arterial blood. Maximal speeds were 8.7 ± 0.6 miles/h for women and 10.2 ± 0.3 miles/h for men. Final grades were 5 ± 1% for both men and women. During the last 45 s of each exercise stage, arterial blood samples were collected. Additional blood for histamine analysis was collected at rest, at stages 2 and 4, and at the final stage. During the final stage, blood samples were collected every 1 min to ensure collection near exhaustion. Ratings of perceived exertion of legs and breathing were collected at the end of stage 4 and at the end of the test. Lung function tests were repeated at ~5 and 30 min postexercise. Urine was collected 15 min postexercise, and induced sputum collection was initiated 40 min postexercise.

The placebo day was identical to the drug day with the following exceptions: instead of administration of drugs, two cornstarch capsules were administered and a placebo inhalation aerosol (GlaxoWellcome, Research Triangle Park, NC) was actuated three times into an AeroChamber. Ventilatory measurements of pressure, gas, flow, and volume during the exercise tests and blood-gas, body temperature, blood lactate, and plasma histamine measurements and analyses were conducted as previously described (58).

Sputum Analysis

Whole sputum samples were weighed, and an equal volume of 0.1% dithiothreitol (Sputolytin; Calbiochem, La Jolla, CA) was added. Samples were mixed by using a transfer pipette and incubated at 37°C in a shaking water bath for 15 min. Cell counts of total, white blood, bronchial, and squamous epithelial cells were done. Cytospin slides were prepared after centrifugation at 500 rpm for 5 min. An investigator blinded to the subject treatment read at least 200 nonsquamous cells on each sputum slide. The remainder of the homogenized sputum was centrifuged at 2,000 rpm and 20°C for 5 min, and supernatant was analyzed by commercial enzyme immunoassay for histamine (Immunotech) and myeloperoxidase (Oxis, Portland, OR). Tryptase in sputum supernatant was analyzed in the laboratory of Dr. Lawrence Schwartz at Virginia Commonwealth University (Richmond, VA) with a noncommercial enzyme-linked immunosorbent assay.

Urine Analysis

Urinary leukotriene E4 (LTE4) and 11β-prostaglandin F2α (11β-PGF2α) were determined in duplicate by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI), and creatinine was determined in duplicate (Sigma Chemical, St. Louis, MO). Values for LTE4 and 11β-PGF2α, are reported as nanograms per millimole of creatinine.

Statistical Analysis

Data were analyzed with the use of two-way repeated-measures analysis of variance with time (level of exercise test or pre- and postexercise time points) and treatment (drug or placebo) as within-subject variables, and sex (male or female) as a between-subject factor. When data were not normally distributed, nonparametric tests were used and median values reported. Linear regression was used to establish correlations. Significance was set at P < 0.05.

RESULTS

Resting Lung Function

Data from impulse oscillometry measurements are listed in Table 2. Mean Rrs at 5- and 25-Hz oscillation was unchanged after exercise (placebo trial); however, 5 of 17 subjects had a >10% increase in Rrs at 5 Hz after exercise. After drug treatment preexercise Rrs at 5 and 25 Hz was slightly higher (5 Hz: 8%, not significant; 25 Hz: 9%, P = 0.021) compared with placebo, but then it decreased significantly (5 Hz: −14%, P = 0.004; 25 Hz: −14%; P = 0.003) after exercise. Figure 1 shows individual data for Rrs at 5 Hz. In addition, Rrs...

J Appl Physiol • VOL 93 • JULY 2002 • www.jap.org
Table 2. Total respiratory resistance measures

<table>
<thead>
<tr>
<th></th>
<th>Trial</th>
<th>Preexercise</th>
<th>6 ± 2 min Postexercise</th>
<th>30 ± 4 min Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rrs at 5 Hz</td>
<td>P</td>
<td>3.89 ± 0.55</td>
<td>3.83 ± 0.71</td>
<td>3.73 ± 0.54</td>
</tr>
<tr>
<td>cmH2O 1-1/s</td>
<td>D</td>
<td>4.19 ± 0.91</td>
<td>3.62 ± 0.96†</td>
<td>3.52 ± 0.80†</td>
</tr>
<tr>
<td>cmH2O 25 Hz</td>
<td>P</td>
<td>3.35 ± 0.68</td>
<td>3.20 ± 0.51</td>
<td>3.34 ± 0.55</td>
</tr>
<tr>
<td>cmH2O 25 Hz</td>
<td>D</td>
<td>3.65 ± 0.71</td>
<td>3.13 ± 0.72†</td>
<td>3.17 ± 0.55†</td>
</tr>
<tr>
<td>ΔRrs 5-25 Hz</td>
<td>P</td>
<td>0.53 ± 0.55</td>
<td>0.62 ± 0.59</td>
<td>0.39 ± 0.62</td>
</tr>
<tr>
<td>cmH2O 1-1/s</td>
<td>P</td>
<td>0.54 ± 0.60</td>
<td>0.48 ± 0.58</td>
<td>0.35 ± 0.58</td>
</tr>
<tr>
<td>Resonant frequency, Hz</td>
<td>P</td>
<td>11.7 ± 3.3</td>
<td>12.0 ± 3.8</td>
<td>10.8 ± 3.3</td>
</tr>
</tbody>
</table>
| Total respiratory resistance measures across all measured frequencies is shown in Fig. 2. There was no correlation between baseline respiratory resistance or changes in resistance and improvements in A-aDO2 or EIAH with drug administration. In addition, there was no relationship between change in Rrs after exercise and the nadir PaO2 during exercise either on the drug or placebo days.

The results from pre- and postexercise maximal FVL on the placebo and drug days are shown in Table 3. After exercise in the placebo trial, FEV1 was slightly (2.6%, *P* = 0.006) increased whereas FVC, other flow rates, and NOCF were unchanged. Drug treatment did not affect preexercise or postexercise resting lung function.

Exercise Data

There were no significant effects of drug treatment on ventilatory or metabolic parameters at rest or during submaximal or maximal exercise compared with placebo. Exercise performance, in terms of VO2max or the final exercise workload (speed and grade) did not differ between trials. The average time at the final workload was 2.0 ± 1.0 min for placebo trial and 2.0 ± 0.6 min for the drug trial, and total exercise times were 17.80 ± 1.36 min and 17.75 ± 1.07 min for placebo and drug trials, respectively. Breathing frequency at maximal exercise was 56 ± 6 and 56 ± 7 breaths/min for placebo and drug trials, respectively. Similarly, values for the ratio of minute ventilation to carbon dioxide production (31.8 ± 2.8 and 31.5 ± 3.0), heart rate (190 ± 8 and 191 ± 7), blood lactate (10.2 ± 2.2 and 10.4 ± 2.7), and arterial pH (7.25 ± 0.06 and 7.24 ± 0.07) were all nearly identical between trials.

There were no significant differences in ratings of perceived exertion (RPE) between trials. At exhaustion, RPE for leg effort was 8.1 ± 1.7 (placebo trial) and 8.3 ± 1.8 (drug trial) and RPE for breathing effort was 8.7 ± 1.4 (placebo trial) and 8.4 ± 1.4 (drug trial) out of a scale from 0 to 10. After the final exercise test, subjects were asked which day they thought they had received the drug treatment, six reported that they could not guess, six identified the wrong day, and five guessed correctly. Of these five who guessed correctly, two reported that breathing felt easier and one reported greater than usual saliva production on the drug day.

During the placebo trial, mean SaO2 fell from 97.5 ± 0.6% at rest to 91.9 ± 2.1% at maximal exercise. The fall in SaO2 was partly a result of a decreasing PaO2 as the A-aDO2 widened throughout exercise and of metabolic acidosis and temperature-induced shifts in the oxyhemoglobin-dissociation curve. Arterial carbon dioxide partial pressure (PaCO2) was relatively stable during mild and moderate exercise before decreasing due to hyperventilation during heavy exercise. Drug treatment had no significant effects on blood-gas variables at rest or during exercise (Figs. 3 and 4).

Nadir PaO2, the widest A-aDO2, and PaCO2 at maximal exercise for drug and placebo trials are shown in Fig. 5, A–C. These identity plots show the observed degree of individual variation in exercise-induced changes and drug vs. placebo treatment effects for these variables. They demonstrate that subjects who had a greater fall in PaO2 or widening of A-aDO2 during the placebo trial did not have greater improvements in these variables with drug treatment. They also reveal a slight trend for higher PaCO2 during the drug trial. Mean nadir PaO2 for men was 78.4 ± 6.8 Torr (placebo) and 78.5 ± 5.9 Torr (drug) and for women was 81.8 ± 9.9 Torr (placebo) and 79.2 ± 9.6 Torr (drug). Widest A-aDO2 was 31.3 ± 4.7 Torr and 30.0 ± 5.7 Torr (men) and 30.7 ± 7.4 and 30.0 ± 8.0 Torr (women) for placebo and drug trials, respectively. These values were not significantly different either between the sexes or between drug and placebo treatment.

Inflammatory Indexes in Plasma, Urine, and Sputum

Plasma histamine at maximal exercise increased 128 ± 98% above resting values during the placebo trial. Drug treatment did not alter resting (preexercise)
histamine levels, and the exercise-induced increase (101 ± 79%), while tending to be lower, was not significantly different (P = 0.118) during the drug compared with placebo trials (Fig. 6).

There was no significant increase in mean urinary levels of LTE4 and 11β-PGF2α (expressed ng/mmol creatinine) pre- to postexercise during the placebo trial, and drug treatment had no effect at rest or after exercise (Fig. 6).

Induced sputum cell counts and sputum supernatant inflammatory mediator levels at baseline and after exercise on the drug and placebo treatment days are shown in Table 4. Although total cell counts did not change significantly after exercise, the white blood cell count (combined count of macrophages, lymphocytes, and neutrophils) decreased after exercise (P = 0.002, placebo trial), and there was a slight increase in the number of bronchial and squamous cells found in the sputum. The proportion of macrophages, lymphocytes, and neutrophils did not change with exercise relative to baseline, and eosinophils were generally absent from the sputum samples. In the supernatant, myeloperoxidase did not change after exercise, whereas histamine was significantly elevated postexercise compared with baseline (P < 0.05). In contrast, tryptase, which was below the limit of detection in two-thirds of the samples, did not change after exercise. The only effect of drug treatment was to reduce neutrophil counts in sputum after exercise; however, as a percentage of the white blood cell count, they did not differ from baseline or placebo.

Plasma histamine concentration at maximal exercise and postexercise sputum histamine and urinary LTE4 or 11β-PGF2α levels did not correlate with A-aDO2 at maximal exercise (data not shown). This was the case whether drug and placebo trial data were separated, combined, or expressed as a difference. Changes in plasma histamine, LTE4, and 11β-PGF2α from rest (or preexercise) to maximal exercise (or postexercise) also did not correlate with magnitude of change in A-aDO2 from rest to maximal exercise. Subgroup analysis of the seven subjects with the lowest nadir PaO2 during exercise on the placebo day (71.9 ± 3.3 Torr, all subjects <80 Torr) compared with those subjects with the seven highest nadir PaO2 (87.5 ± 5.5 Torr) revealed the following comparisons: plasma histamine at maximal exercise was 4.5 ± 2.5 vs. 4.1 ± 4.2 nmol/l, postexercise urine 11β-PGF2α levels were 38 ± 22 vs. 57 ± 55 ng/mmol creatinine, and urine LTE4 levels were 38 ± 9 vs. 43 ± 17 ng/mmol creatinine, and median sputum histamine was 1.5 vs. 4.2 ng/ml, for the low-PaO2 group compared with the high-PaO2 group, respectively. None of the differences in mediators were statistically significant.

**DISCUSSION**

We tested whether lung inflammation was an important contributor to EIAH by pharmacological blockade of inflammatory mediators and comparing blood-gas values during exercise with placebo administration. This study demonstrated that gas exchange and \( \text{SaO}_2 \) changes were minimal during exercise with placebo administration.

**Table 3. Maximal flow-volume loop and expired nitric oxide data**

<table>
<thead>
<tr>
<th></th>
<th>Trial</th>
<th>Preexercise</th>
<th>8 ± 2 min Postexercise</th>
<th>32 ± 4 min Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1, liters</td>
<td>P</td>
<td>4.20 ± 0.73</td>
<td>4.31 ± 0.74 †</td>
<td>4.31 ± 0.77 †</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>4.22 ± 0.88</td>
<td>4.25 ± 0.75</td>
<td>4.33 ± 0.79</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>P</td>
<td>5.18 ± 1.02</td>
<td>5.19 ± 1.05</td>
<td>5.25 ± 1.07</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>5.19 ± 1.07</td>
<td>5.14 ± 1.04</td>
<td>5.25 ± 1.09</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>P</td>
<td>81.6 ± 6.8</td>
<td>83.7 ± 6.6 †</td>
<td>82.7 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>81.6 ± 5.6</td>
<td>83.5 ± 8.0</td>
<td>83.1 ± 5.5</td>
</tr>
<tr>
<td>FEF25–75%, l/s</td>
<td>P</td>
<td>4.05 ± 1.04</td>
<td>4.23 ± 1.15</td>
<td>4.16 ± 1.05</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>4.00 ± 1.15</td>
<td>4.15 ± 1.17</td>
<td>4.28 ± 0.89 †</td>
</tr>
<tr>
<td>PIF, l/s</td>
<td>P</td>
<td>7.3 ± 2.1</td>
<td>7.8 ± 2.3</td>
<td>7.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>7.4 ± 2.1</td>
<td>8.2 ± 2.3 †</td>
<td>8.0 ± 2.2 †</td>
</tr>
<tr>
<td>PEF, l/s</td>
<td>P</td>
<td>8.3 ± 1.7</td>
<td>8.5 ± 1.5</td>
<td>8.3 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>8.2 ± 1.9</td>
<td>8.6 ± 2.0</td>
<td>8.6 ± 2.0</td>
</tr>
<tr>
<td>NOCF, ppb</td>
<td>P</td>
<td>24.3 ± 28.6</td>
<td>25.5 ± 26.2</td>
<td>27.0 ± 27.9</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>29.7 ± 27.9</td>
<td>30.0 ± 25.2</td>
<td>32.5 ± 27.4</td>
</tr>
</tbody>
</table>

Values are means ± SD for 17 subjects. FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF25–75%, forced expiratory flow of midexpiratory volume; PIF, peak inspiratory flow; PEF, peak expiratory flow; NOCF, exhaled nitric oxide concentration measured at a constant flow rate. Times that measures were made after exercise did not differ between drug and placebo trials. *Significantly different from placebo, P < 0.05. †Significantly different from preexercise value, P < 0.05.
during exercise in young healthy endurance-trained athletes are unaffected by administration of drugs known to reduce or prevent inflammatory responses in the lungs. These findings applied to all subjects, including either those subjects who showed mild EIAH or those with relatively severe hypoxemia. Markers of inflammation were also unchanged by either drug administration or, in most cases, by exercise itself. Our findings, in young athletes, differ from previous findings in older athletes (43) (see the introduction).

**Blockade of Inflammatory Mediators**

The inflammatory mediators targeted in this study were histamine and leukotrienes along with others released from mast cells (e.g., prostaglandins). These mediators were targeted because their effects might cause peripheral airway narrowing and lead to abnormal gas exchange. The drug doses selected were similar to those used clinically for relief of asthma and allergic rhinitis symptoms. Zileuton reduces leukotriene synthesis by selective inhibition of 5-lipoxygenase-mediated conversion of arachidonate to leukotriene A₄, and a single administration of 600 mg has been shown to produce rapid bronchodilation in individuals with mild to moderate asthma (26). Acute administration of 4 mg nedocromil sodium (lower than our dose) has been shown to provide protection against exercise-induced asthma in a large number of studies (52). Fexofenadine is a nonsedating histamine H₁-receptor antagonist that is often prescribed for seasonal allergic rhinitis. We administered a dose of 60 mg, and we cannot be sure that this totally blocked histamine action in the airways; however, we do know that an acute dose of 60 mg suppresses histamine skin prick-induced flares and reduces symptom scores after exposure to allergens (33). It is possible that higher doses or a prolonged period of drug administration may result in greater attenuation of effects of inflammatory mediators.

**Exercise-induced Changes in Lung Function**

We found no evidence of exercise-induced changes in lung function. In fact, the majority of subjects demonstrated slight bronchodilation when measured by FEV₁ at 8 and 32 min after exercise. Because postexercise lung function was measured at only two time points, it
is possible we may have missed the time of greatest bronchoconstriction (or bronchodilation) for individual subjects. However, nadir FEV\textsubscript{1} in exercise-induced bronchospasm is typically recorded within 5–10 min of cessation of exercise (11). Changes in FVC have been suggested to indicate small-airway closure (32); this measure was unchanged in the present study. Rrs values at 5 and 25 Hz after exercise were unchanged in

Fig. 5. Identity plots for the comparison of nadir Pa\textsubscript{O\textsubscript{2}} (A), widest A-aDo\textsubscript{2} (B), and Pa\textsubscript{CO\textsubscript{2}} at maximal exercise (C) during the drug and placebo trials. Each symbol represents the data from a different individual: ▲, men; ☉, women. Line of identity is displayed. For comparison, data from Prefaut et al. (43) are presented. Squares are means ± SE for data from maximal exercise in master athletes given nedocromil sodium or placebo; no SE was available for Pa\textsubscript{CO\textsubscript{2}} data.

Fig. 6. Plasma histamine concentration in arterial blood at rest and during incremental exercise after drug and placebo administration. Values are means ± SD.

Fig. 7. Urinary leukotriene E\textsubscript{4} (LTE\textsubscript{4}; A) and 11β-prostaglandin F\textsubscript{2α} (11β-PGF\textsubscript{2α}; B) excretion in athletes with placebo (open bars) and drug (solid bars) treatments shown preexercise and 15 min postexercise. Values are means ± SD.
the placebo trial and were significantly but modestly (-0.6 ± 0.7 cmH2O·s at 5 Hz) decreased in the drug trial. Moreover, the absence of change in the frequency dependence of resistance after exercise is consistent with the absence of an effect on small airways (5). Our group found no statistically significant correlation between an increase in Rrs after exercise and a wide A-aDO2 or low PaO2 during high-intensity endurance exercise in women runners (58) or in the present study. The nadir PaO2 during exercise for five subjects who had a >10% increase in Rrs after exercise was 80 Torr, which was identical to the mean nadir PaO2 of the remaining subjects, whose Rrs decreased by 9 ± 11% after exercise. Although these negative data reveal that exercise did not compromise postexercise resting lung function, one must keep in mind that these tests may not be sensitive enough to detect mild changes in small-airway caliber and/or disturbances during exercise that may have resolved by the time measurements were made after exercise.

Inflammatory Mediators in Plasma, Urine, and Sputum

Because postexercise lung function may not be an accurate indicator of the degree of lung inflammation, we also measured inflammatory mediators in plasma, urine, and sputum. Similar to other studies in athletes with EIAH, plasma histamine was significantly elevated from rest to moderate and maximal exercise (4, 43). However, in contrast to Prefaut et al. (43), who showed at maximal exercise a 63% decrease in plasma histamine with nedocromil sodium compared with placebo, administration of the drugs used in the present study had no significant effect on histamine levels. Perhaps the higher absolute histamine levels seen in older subjects compared with our younger subjects reflects greater release of histamine from airway mast cells. However, exercise-induced asthma is not associated with increased levels of histamine in the airways (27), and therefore elevations in plasma histamine may simply be a result of increased basophil degranulation in the circulation (24). Recently, differences in blood osmolarity, basophil number, and histamine content per basophil were ruled out as causes of histamine release in subjects with both increased histamine release and EIAH (38). These investigators suggested an exercise-induced increase in inflammatory cytokines as a cause of basophil degranulation.

In addition to plasma analysis, we also measured the concentration of metabolites of leukotrienes and prostanoids in the urine before and after exercise. Elevated levels of LTE4 in the urine are thought to reflect increased production of cysteinyl leukotrienes within the lung (10). Urinary LTE4 is elevated in mildly asthmatic individuals after exercise (44), although this is not a consistent finding (48, 56), and 5-lipoxygenase inhibitors reduce urinary LTE4 after exercise or allergen challenge (25, 31). Because allergen appears to be a greater stimulus for cysteinyl leukotriene release, it may not be surprising that we found no increase in LTE4 after exercise in our nonasthmatic subjects. Our subjects were, however, exercising at much higher intensities than is typical of an exercise-provocation test and therefore may provide a greater stimulus for leukotriene production. Prostaglandin D2 is the major cyclooxygenase metabolite of arachidonic acid released after stimulation of mast cells, and 11β-PGF2α, but not metabolites of histamine or leukotrienes, was found to be increased in individuals with mild asthma, who experienced an episode of exercise-induced bronchoconstriction (40), indicating that 11β-PGF2α may be a more sensitive measure of mast cell activation. Our athletes showed no increase in urinary 11β-PGF2α after exercise, suggesting a lack of mast cell activation. This is consistent with the previous data (40) because bronchoconstriction was absent in all but one of our subjects. Urine was collected shortly (~15 min) after the exercise bout ended because we wanted to capture inflammatory processes occurring during the exercise bout and avoid those occurring during the sputum induction procedure. Possibly, collections taken later after exercise may have provided different results, although both urinary LTE4 and 11β-PGF2α have

### Table 4. Sputum cell counts and supernatant analyses

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Placebo</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells × 10⁶/ml</td>
<td>1.4 (0.81–2.71)</td>
<td>1.1 (0.99–2.39)</td>
<td>1.2 (0.97–1.95)</td>
</tr>
<tr>
<td>Macrophages × 10⁶/ml</td>
<td>756 (415–1,251)</td>
<td>523 (270–827)</td>
<td>357 (187–735)</td>
</tr>
<tr>
<td>Lymphocytes × 10⁶/ml</td>
<td>10 (3–38)</td>
<td>6 (3–25)</td>
<td>6 (1–22)</td>
</tr>
<tr>
<td>Neutrophils × 10⁶/ml</td>
<td>530 (102–936)</td>
<td>285 (104–412)</td>
<td>144 (83–244)†</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>62 (48–85)</td>
<td>73 (32–82)</td>
<td>72 (58–85)</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>1.0 (0.5–2.0)</td>
<td>1.0 (0.5–1.7)</td>
<td>1.0 (0.5–2.5)</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>37 (13–49)</td>
<td>26 (17–44)</td>
<td>28 (15–40)</td>
</tr>
<tr>
<td>Squamous epithelium, %</td>
<td>12 (7–19)</td>
<td>23 (15–39)</td>
<td>28 (18–41)</td>
</tr>
<tr>
<td>Histamine, ng/ml</td>
<td>0.3 (0.3–1.9)</td>
<td>0.3 (0.3–1.3)</td>
<td>0.8 (0.3–1.1)</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>1.0 (0.5–2.0)</td>
<td>1.0 (0.5–1.7)</td>
<td>1.0 (0.5–2.5)</td>
</tr>
<tr>
<td>Squamous epithelium, %</td>
<td>12 (7–19)</td>
<td>23 (15–39)</td>
<td>28 (18–41)</td>
</tr>
<tr>
<td>Histamine, ng/ml</td>
<td>0.3 (0.3–1.9)</td>
<td>0.3 (0.3–1.3)</td>
<td>0.8 (0.3–1.1)</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>37 (13–49)</td>
<td>26 (17–44)</td>
<td>28 (15–40)</td>
</tr>
<tr>
<td>Squamous epithelium, %</td>
<td>12 (7–19)</td>
<td>23 (15–39)</td>
<td>28 (18–41)</td>
</tr>
<tr>
<td>Histamine, ng/ml</td>
<td>0.3 (0.3–1.9)</td>
<td>0.3 (0.3–1.3)</td>
<td>0.8 (0.3–1.1)</td>
</tr>
</tbody>
</table>

Values are medians with the interquartile range in parentheses except for myeloperoxidase, which is means ± SD, for 17 subjects at each collection. Eosinophils were found in only 3 of the 51 samples; in these cases, counts were always ≤0.5% of total cells. For tryptase samples, the limit of detection was 0.4 ng/ml; samples below this limit were assigned a value of 0.3 ng/ml. †Significantly different from baseline, P < 0.05.
been found to be elevated at the first collection time point (1 h) after allergen challenge in asthmatic individuals (39).

Cellular and biochemical analysis of induced sputum have also been used as a noninvasive method for evaluating airway inflammation in asthma. Sputum measures correlate with BAL and bronchial biopsy measures (18). The effect of exercise on changes in sputum constituents was measured in asthmatic individuals and showed exercise that produced bronchoconstriction had no effect on inflammatory cells in sputum when measured 2–24 h after challenge; this differed from results after allergen challenge (19). Sputum collected ~3 h after long-term exercise in nonasthmatic individuals showed slight increases in the percentage of cells that were neutrophils compared with baseline (6); however, we did not find this in sputum collected 40 min after high-intensity exercise in our athletes. The relative absence of eosinophils in our samples is consistent with a lack of chronic lung inflammation.

One limitation of our sputum induction procedure is that we did not standardize the sputum induction time, which ranged from 12 to 24 min. The time was increased to obtain an adequate sputum sample from each subject. Potentially, this may have increased variability in the cell counts. Analysis of myeloperoxidase, histamine, and tryptase in sputum supernatant revealed that drug administration had no effect on their levels. However, sputum histamine was elevated after exercise on both placebo and drug days relative to baseline (nonexercise day). The significance of this finding is unclear, because tryptase, a specific marker of mast cell degranulation, was not similarly affected by exercise. The histamine found in sputum is potentially derived from a number of sources, including mast cells, eosinophils, epithelial cells, and sensory neurons (2). Sputum histamine tended to be higher after a marathon race in healthy subjects (6) and was elevated in eosinophilic bronchitis but not asthma compared with normals, perhaps because of differences in the location of mast cells (7). In our subjects, histamine release by airway cells may have occurred because of mechanical stress of high airflow rates. It is unclear why nedocromil sodium administration did not prevent this increase. Even so, was the small increase in sputum histamine sufficient to cause any gas-exchange disturbances? We consider it unlikely not only because we administered an H1-receptor antagonist but also because there was no correlation between sputum histamine and exercise A-aDO2 or PaO2.

**Exercise-Induced Airway Inflammation and EIAH**

Based on the lung function data and the mediator levels in the plasma, urine, and sputum, we find little evidence for exercise-induced lung inflammation. However, it is certainly possible that lung inflammation occurred during exercise but was not detected by our indirect measures. Nonetheless, the conclusion we consider more likely is that inflammation in the lungs of young healthy athletes is absent or is of insufficient magnitude to markedly impact A-aDO2 during exercise. Other findings also speak against a significant role for exercise-induced inflammation in EIAH. A study examining the time course of A-aDO2 and EIAH during exhaustive endurance exercise found that gas exchange worsened within 1–3 min of high-intensity exercise but did not worsen further over time throughout the 14-min exercise bout (58). In addition, if a maximal exercise bout is repeated ~20 min after an initial maximal exercise bout, there is a narrowing of A-aDO2 and a lessening of EIAH during the second bout (53). Both these findings are opposed to inflammatory factors, which would likely worsen over time or persist for a length of time after exercise is completed.

One primary reason for conducting this study was to see whether previous results of pharmacological blockade of inflammatory mediators in older athletes (age 61–65 yr) with EIAH (43) would hold true for younger athletes (age 20–30 yr). There are a number of important differences between the present study and that of Prefaut et al. (43). First, we used two drugs (fexofenadine and zileuton) in addition to nedocromil sodium. The nedocromil sodium dose was slightly higher in our study (5.25 vs. 4 mg), and we used a chamber to maximize delivery of the dose to smaller airways and prevent deposition in the mouth. One might expect that subjects with more severe EIAH may respond to drug treatment more positively. However, differences in the severity of hypoxemia cannot account for the differences between the two studies because, in the present study, seven subjects had A-aDO2 above 30 Torr and PaO2 below 80 Torr at maximal exercise (similar values to those of Prefaut et al.); their responses to drug administration were not different from our other subjects (see Fig. 5). We also caution that because Prefaut et al. did not temperature correct arterial blood gases, falls in PaO2 are overestimated. Finally, Prefaut et al. reported higher histamine values at maximal exercise and greater increases above baseline, from 1.2 to 6.0 nmol/l (400% increase), whereas we report 1.9 to 4.1 nmol/l (128% increase). These findings suggest that, in younger subjects, less histamine may be released during exercise, a finding supported by previous research (4, 21), although, again, subjects in the present study with the largest exercise-induced increases in histamine were equally nonresponsive to drug administration compared with other subjects with more moderate increases in histamine. Furthermore, the widening of the A-aDO2 during exercise was also not correlated with the increase in histamine from rest to maximal exercise.

The large age difference of the subjects between the studies and greater histamine levels in the older subjects raises the possibility that an aging lung is more prone to inflammation. Advancing age is associated with morphological and physiological changes in lung function that include a decline in the number of capillaries per alveolus, total alveolar tissue, total gas exchange surface area, and diffusing capacity (46), and loss of elastic recoil (28). Cross-sectional studies have revealed altered inflammatory cell profiles and low-
grade inflammation in the lower respiratory tracts of many asymptomatic, clinically normal older individuals (35–37). Increased basophil counts are associated with the development of increased nonspecific airway responsiveness during aging, suggesting that inflammatory mechanisms may be involved (51). Although resting $P_{aO_2}$ is often lower and $A-aDO_2$ wider in older individuals (50), it is unclear whether the prevalence of hypoxemia during exercise is higher, especially in athletes. Prefaut et al. (42) found lower $P_{aO_2}$ and wider $A-aDO_2$ throughout exercise in older athletes compared with both age-matched controls and training-matched young athletes. However, Johnson et al. (28) concluded that alveolar ventilation is adequate for carbon dioxide elimination even during maximal exercise and that arterial oxygen homeostasis is generally maintained; only one-third of 30 very fit (VO$_2$ max 43 ml·kg$^{-1}$·min$^{-1}$) healthy subjects age 70 ± 5 yr had significant EIAH during maximal exercise (28). If EIAH were indeed caused by inflammatory processes that worsen with age, because of repeated exposure to environmental allergens, toxins, physical stimuli (temperature and humidity), and high airflow rates, a young athlete who currently exhibits moderate-to-severe EIAH would be faced with even greater respiratory limitation with aging. We are unaware of any longitudinal studies that have followed young athletes with EIAH over time.

**Summary**

In contrast to a previous study, drugs known to inhibit inflammatory mediator release and action in the lungs had no effect on EIAH. Two possibilities may account for this: either these drugs did not reduce lung inflammation sufficiently to alter gas exchange in our subjects, or in fit young male and female athletes, lung inflammation is not a significant contributor to the widened $A-aDO_2$ during exercise. The lack of an increase in mediators with exercise, whether or not anti-inflammatory drugs were administered, suggests that the latter explanation is more likely.

We thank our subjects for enthusiastic participation in this study. Special thanks to A. W. Sheel, P. A. Derchak, and D. F. Pegelow for technical help during the experiments. We are indebted to Dr. Nizar Jarjour for helpful advice. Support for this project was provided by National Heart, Lung, and Blood Institute (NHLBI) Grant HL-15469. T. J. Wetter was supported by a NHLBI training grant.

**REFERENCES**


