Basophils and exercise-induced hypoxemia in extreme athletes

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Mucci, Patrick, Fabienne Durand, Bernard Lebel, Jean Bousquet, and Christian Préaut. Basophils and exercise-induced hypoxemia in extreme athletes. J Appl Physiol 90: 989–996, 2001.—This study examined whether the increase in histamine release (%H, i.e., plasma histamine expressed as a percentage of whole blood histamine) associated with exercise-induced hypoxemia (EIH) is related to high training-induced changes in basophil and osmolarity factors in arterial blood. All parameters were measured in 20 endurance athletes, 11 of whom presented an EIH (HTHyp) and 9 of whom were nonhypoxic (HTnorm), and in 10 untrained control subjects (UT). Measurements were made at rest, at the maximal workload of an incremental exhaustive exercise test, and at the fifth minute of recovery. %H increased during exercise in HTHyp (P < 0.01) but did not increase significantly in HTnorm and UT controls. The results indicated that 1) osmolarity and Na+ and K+ concentrations did not differ between the two trained groups and 2) the basophil count and basophil histamine content did not differ among groups. We concluded that the %H increase associated with EIH was not due to a training effect on these parameters. The relatively low increase in histamine content during exercise in HTHyp in comparison to HTnorm (P < 0.05) and UT (P < 0.01) and the low recovery vs. resting basophil count only in HTHyp (P < 0.01) suggested an accentuated exercise-induced basophil degranulation in the hypoxic athletes.

healthy men; incremental exhaustive exercise; cation concentration

IT IS WELL KNOWN THAT EXERCISE may induce hypoxemia in highly trained endurance athletes (5, 7, 26, 28, 29), who are referred to as “extreme athletes” (2, 29). The mechanisms involved in the development of this exercise-induced hypoxemia (EIH) have long been debated, and two major explanations have been proposed: 1) a lack of compensatory hyperpnea (7, 16, 26) and/or 2) a gas exchange alteration that may result from functionally based mechanisms during exercise (16, 35). This latter may involve ventilation-perfusion (19, 33) and diffusion alterations induced by an incomplete O2 equilibrium between alveolar gas and pulmonary capillary blood as a result of a rapid red blood cell pulmonary transit time (7) and/or a pulmonary interstitial and peribronchial vascular edema during exhaustive exercise (9, 15, 16, 19, 29, 33, 35).

A previous study (2) showed that the drop in arterial partial pressure of O2 (PaO2) during exhaustive exercise was associated with a concomitant increase in histamine release (%H) in extreme athletes, whereas there was no change in either PaO2 or histamine level in the untrained control group. Histamine is widely acknowledged to be an inflammatory mediator that causes increased microvascular permeability to macromolecules (38) and therefore increased transcapillary fluid movement. It has therefore been suggested that this increase in histamine release could be related to a change in pulmonary fluid movement and thus to a gas exchange alteration such as EIH (2, 30). The finding that EIH can be partly inhibited by prior inhalation of nedocromil sodium, a drug thought to act by inhibiting the release of histamine (30), provided evidence that this increase in histamine release during exercise may be involved in the development of EIH. More recently, it was reported that this increase in %H can be suppressed in association with an apparent change in ventilation-perfusion distribution (9). An interesting question concerns the origin of this increase in histamine release. Histamine is a well-known inflammatory mediator and potential contributor to mild hypoxemia (30, 38). The increase in histamine release associated with EIH may thus be the consequence of an inflammatory process in the lung and/or in the peripheral muscles (38) during incremental exercise. This increase may also be the consequence of an elevated basophil number and/or an elevated histamine content of these cells (1, 17, 24) in extreme athletes.

Histamine is essentially contained in mastocytes and basophils, and most of the histamine in whole
blood is derived from basophil polymorphonuclear leukocytes (basophils) (38). It has been reported that 1) a positive relationship exists between histamine release and basophil histamine content in both normal and nonmedicated asthmatic subjects (1) and 2) in both normal and asthmatic subjects, there is a close association between peripheral whole blood histamine concentration after exercise and circulating basophil counts (17, 24).

Exercise and training produce a series of complex physiological changes that may alter circulating leukocytes (8, 12, 14, 20). Given the findings to date, we hypothesized that high endurance training in athletes would induce an increase in the number of basophils and/or the histamine content of these cells, which could explain the increased histamine release observed during exercise. However, there are conflicting data about the effect of exercise on basophil count, with some authors reporting an increase (14, 17) and others reporting no significant change (12, 20, 24) between pre- and postexercise. To test our hypothesis, we measured basophil count and basophil histamine content at rest and during incremental exhaustive exercise in highly trained athletes who have shown EIH, as well as in highly trained athletes who do not exhibit EIH. The first group comprised nine extreme athletes (HT hyp), i.e., highly trained athletes who develop EIH. This was defined as a drop in PaO2 of at least 8 Torr from resting values, corrected for temperature, that lasts for at least the last three steps of an incremental exercise test (2, 9, 29, 30). Three of the HT hyp were triathletes, and the six others were cyclists. They participated in regional or national competition, and all had been training regularly for an average of 4.6 ± 0.5 yr. They trained 16.3 ± 1.1 h/wk. The second trained group was composed of nine control athletes (HT nor), i.e., highly trained athletes who do not exhibit EIH. The maximal decrease in PaO2 in this group was 3 Torr. Three of the HT hyp were triathletes, and the six others were cyclists. They had participated in regional or national competition for an average of 3.6 ± 0.8 yr. All trained regularly for an average of 13.7 ± 1.1 h/wk. The two remaining athletes exhibited PaO2 decreases of 5 and 6 Torr, respectively, i.e., above the definition of resting hypoxemia (31) and under the threshold of 8 Torr. We did not include these subjects in either of the athlete groups to maintain distinct group difference. Nevertheless, these subjects were used in correlational analysis to determine relationships between dependent variables.

Control subjects. Ten untrained men (UT), aged 20–30 yr (26.0 ± 1.2 yr), composed the untrained control group. None trained in endurance sports, although they had active lifestyles with an average of 2.3 ± 0.4 h/wk of physical activity.

**METHODS**

Subjects

Thirty young healthy men participated in this study. The anthropometric characteristics and training regimens are presented in Table 1. None of the subjects reported respiratory or cardiac disease or hypertension or were known to be suffering from any chronic disease. None was on regular medication. None of the subjects had a history of asthma, exercise-induced asthma, or atopic disease or showed signs of allergic disease as detected with the Phadiatop test, a serologic test with a sensitivity of 90% and specificity of 98% (37). All were nonsmoking and presented normal spirometric values. Before admittance to the study, all subjects were evaluated for cardiovascular health. Subjects having an abnormal 12-lead electrocardiogram (ECG) tracing or a supine blood pressure greater than 160/100 Torr at rest were excluded from the study. A preliminary maximal exercise test on a cycle ergometer was then performed. Subjects were excluded from the study if they had ST segment depression in ECG or significant arrhythmias. The study was approved by the institutional ethics committee, and all subjects gave written consent to participate after the design and risks of the study had been described to them.

**Athletes.** Twenty male endurance-trained athletes (HT) were studied. The criteria for selection were as follows: age 19–30 yr and a maximal O2 uptake (V\(\text{O}_2\) max) >60 ml·min\(^{-1}\)·kg\(^{-1}\). They were assigned to one of two groups. The first group comprised nine extreme athletes (HT hyp), i.e., highly trained athletes who develop EIH. This was defined as a drop in PaO2 of at least 8 Torr from resting values, corrected for temperature, that lasts for at least the last three steps of an incremental exercise test (2, 9, 29, 30). Three of the HT hyp were triathletes, and the six others were cyclists. They participated in regional or national competition, and all had been training regularly for an average of 4.6 ± 0.5 yr. They trained 16.3 ± 1.1 h/wk. The second trained group was composed of nine control athletes (HT nor), i.e., highly trained athletes who do not exhibit EIH. The maximal decrease in PaO2 in this group was 3 Torr. Three of the HT hyp were triathletes, and the six others were cyclists. They had participated in regional or national competition for an average of 3.6 ± 0.8 yr. All trained regularly for an average of 13.7 ± 1.1 h/wk. The two remaining athletes exhibited PaO2 decreases of 5 and 6 Torr, respectively, i.e., above the definition of resting hypoxemia (31) and under the threshold of 8 Torr. We did not include these subjects in either of the athlete groups to maintain distinct group difference. Nevertheless, these subjects were used in correlational analysis to determine relationships between dependent variables.

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**Exercise Testing**

An incremental exhaustive test was performed on a calibrated cycle ergometer (Monark 860, Varberg, Sweden). The initial power setting was 30 W for UT and 60 W for HT for 3 min, with successive increases of 30 W every minute except at the end of the test, when the increase was smaller to be as close as possible to V\(\text{O}_2\) max. Minute ventilation (Ve), O2 uptake (V\(\text{O}_2\)), and CO2 output (V\(\text{CO}_2\)) were measured continuously by use of a breath-by-breath automatic exercise metabolic system (CPX, Medical Graphics, St. Paul, MN). The data were averaged during the last 20 s of each load. To ensure that V\(\text{O}_2\) max was attained, at least three of the following criteria had to be met: 1) a plateau of V\(\text{O}_2\) with the last increase in work rate (“leveling-off” criterion); 2) attainment of age-predicted maximal heart rate [210 – (0.65 × age) ± 10%]; 3) a respiratory exchange ratio > 1.1; and 4) an inability to maintain the required pedaling frequency (60 rpm)

<table>
<thead>
<tr>
<th></th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>FEV(_1), %</th>
<th>V(\text{O}_2) max, ml·min(^{-1})·kg(^{-1})</th>
<th>P(\text{max}), W</th>
<th>HR(\text{max}), beats/min</th>
</tr>
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<tbody>
<tr>
<td>HT hyp</td>
<td>23.4 ± 1.6</td>
<td>182.3 ± 1.9</td>
<td>72.9 ± 2.2</td>
<td>110.5 ± 2.6</td>
<td>65.6 ± 1.3</td>
<td>365.0 ± 10.0</td>
<td>187.7 ± 3.2</td>
</tr>
<tr>
<td>HT nor</td>
<td>22.8 ± 0.9</td>
<td>180.5 ± 1.6</td>
<td>71.0 ± 3.1</td>
<td>110.0 ± 3.3</td>
<td>65.8 ± 1.9</td>
<td>365.0 ± 8.7</td>
<td>188.5 ± 2.7</td>
</tr>
<tr>
<td>UT</td>
<td>26.0 ± 1.2</td>
<td>176.5 ± 2.5</td>
<td>73.8 ± 3.0</td>
<td>100.6 ± 2.6</td>
<td>44.2 ± 1.6</td>
<td>267.0 ± 8.3</td>
<td>188.6 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. FEV\(_1\), forced expiratory volume in 1 s expressed as a percentage of predicted value; V\(\text{O}_2\) max, maximal oxygen uptake; P\(\text{max}\), maximal load achieved during incremental exhaustive exercise; HR\(\text{max}\), maximal heart rate achieved during incremental exhaustive exercise; HT hyp, highly trained hypoxemic group; HT nor, highly trained nonhypoxemic group; UT, untrained control group. Significantly different in trained groups from untrained group: *P < 0.05; †P < 0.001.

Table 1. Physical and physiological characteristics of subjects
histamine release was expressed as a percentage of the total histamine measured in heparinized Vacutainer tubes by centrifugation at 900g for 10 min at 4°C in a model PR-J refrigerated centrifuge (Beckman Instruments, Irvine, CA). Aliquots were separated 0.5–1 cm above the cells to avoid picking up any leukocytes, especially basophils, and were frozen at −80°C until assay. Quantification of histamine was performed by use of an enzyme immunoassay kit (Immunotech, Marseille, France).

Whole blood histamine. Blood samples for whole blood histamine were obtained in heparinized Vacutainer tubes exclusively and were placed on ice immediately after being drawn. Fifty microliters of whole blood were added to 950 µl of distilled water, and aliquots were frozen at −80°C until histamine measurement. For whole blood histamine quantification, the diluted blood was then frozen and thawed twice for cell lysis. After final thawing and the removal of membranes by centrifugation at 900 g for 10 min at 4°C, quantification of histamine was performed in supernatant as described above.

Histamine release. As previously described (2, 9, 23, 30), histamine release was expressed as a percentage of the total histamine (%H). The specific release of histamine was determined as %H = 100 × PH/TH, where PH is the plasma histamine for a given testing level in each subject and TH is the corresponding total histamine.

Basophil count. Basophil counts were made in anticoagulated blood samples (EDTA) taken at the same time as those for histamine assay. The blood was stained with Alcian blue, which is a highly specific, accurate, and reproducible method for basophil counting based on the staining of basophil granules as described by Gilbert and Ornstein (13). Counts were made in a Fuchs-Rosenthal counting chamber.

Histamine content. Because most histamine is derived from basophils (38), the histamine content of the basophils was determined as HC = (TH − PH)/BC, where BC is the basophil count corresponding at the testing level of the plasma histamine and the total histamine assays (1).

Protocol

The subjects performed three exercise tests at intervals of a few weeks, all in the morning between 9 and 11 AM to avoid diurnal variations. On the first day, clinical interviews and examinations were conducted, and height, body mass, blood pressure, resting ECG, and resting lung spirometry were recorded. The first exercise test was then performed to allow the subject to adapt to the ergosystem and laboratory environment to detect any exercise-related cardiac anomaly and to determine V̇O₂max. During the second exercise test, blood samples were drawn from the brachial artery for blood gas, basophil, and histamine measurements. Arterial samples were drawn during the last 30 s of the third and fifth minutes of rest, of the last load corresponding to V̇O₂max, and of the fifth minute of recovery. During the third exercise test, tympanic temperature was measured at the times that corresponded to the blood sampling; i.e., we used the mean value in temperature obtained during the last 30 s of the third and fifth minutes of rest, of the last load corresponding to V̇O₂max, and of the fifth minute of recovery. Similar physiological maximal values were achieved during the last two exercise tests in each subject. During every exercise test, subjects were monitored for ventilatory variables and three-lead ECG for the 5 min preceding the exercise test, from the beginning to the end of testing, and recovery. Ventiłatory variables (V̇O₂, V̇CO₂, and V̇E) were averaged over an integer number of breaths during the last 20 s of each minute of rest, exercise, and recovery. This allowed the calculation of Ai-dAO₂ using the standard formula PaO₂ = P O₂ − PaCO₂[F O₂ + (1 − F O₂/R)]. where Ai-dAO₂ is ideal alveolar-arterial difference in P O₂, PaO₂ is ideal alveolar PaO₂, P O₂ is inspired P O₂, F O₂ is the inspired O₂ fraction, R is respiratory exchange ratio, PaCO₂ is alveolar P CO₂, and P ACO₂ = PaCO₂ (3).

Statistical Analysis

The values are means ± SE. Data concerning subject characteristics were compared for homogeneity among UT, HTelev, and HThyp by using a one-way ANOVA after verification of a normal distribution. The means of difference (Δ) between each testing level for a given histamine parameter were calculated and then compared between the three groups within each test. A two-way ANOVA with repeated measurements was used to compare mean values across testing levels between the three subject groups, and a one-way ANOVA with repeated measurements was used to test for within-group changes. Linear regression analysis was used to define the relationship between two variables, and statistical significance was tested by using correlation coefficients. Statistical significance for all tests was set at P < 0.05, two-tailed.
Table 2. \(PaO_2\) and \(D(ai-a)O_2\) during incremental exhaustive exercise testing

<table>
<thead>
<tr>
<th>(PaO_2), Torr</th>
<th>(D(ai-a)O_2), Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rest</strong></td>
<td><strong>Max</strong></td>
</tr>
<tr>
<td>HT(_{hyp})</td>
<td>99.2 ± 2.0</td>
</tr>
<tr>
<td>HT(_{nor})</td>
<td>97.7 ± 3.0</td>
</tr>
<tr>
<td>UT</td>
<td>99.7 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. \(PaO_2\), arterial partial pressure of \(O_2\); \(D(ai-a)O_2\), ideal alveolar-arterial difference in partial pressure of \(O_2\); Max, maximum of the incremental exercise; Rec, 5th min of recovery. Significantly different in trained groups from untrained group: *\(P < 0.01\); †\(P < 0.001\). Significantly different in HT\(_{hyp}\) from HT\(_{nor}\); ‡\(P < 0.05\), §\(P < 0.001\).

RESULTS

Physical and Maximal Data

No significant differences were found among the three groups for age, height, body mass, or maximal heart rate. The highly trained groups showed significantly higher values than the untrained subjects for forced expiratory volume in 1 s (\(P < 0.05\)), \(V_{O_2} max\) (\(P < 0.001\)), and maximal load (\(P < 0.001\)) (Table 1).

Blood Gases

\(PaO_2\) and \(ai-aD0_2\) are reported in Table 2. At rest, there were no significant differences in the mean \(PaO_2\) and \(ai-aD0_2\) values between the groups. However, during exercise testing, HT\(_{hyp}\) showed lower values of \(PaO_2\) and higher values of \(ai-aD0_2\) than UT (\(P < 0.001\)) and HT\(_{nor}\) (\(P < 0.01\)) at the maximal workload. During recovery, the data for \(PaO_2\) were significantly different between HT\(_{hyp}\) and HT\(_{nor}\) (\(P < 0.05\)).

Histamine

There were no significant differences among the three groups in plasma histamine and whole blood histamine at any testing level. All showed a significant increase in plasma histamine between rest and maximal workload (\(P < 0.01\) and \(P < 0.05\), respectively) but no difference between rest and recovery. Whole blood histamine was significantly higher at maximal workload and recovery in HT\(_{hyp}\) (\(P < 0.05\)), HT\(_{nor}\) (\(P < 0.01\)) and UT (\(P < 0.001\)) than at rest (Table 3). Baseline %H values did not differ significantly among the three groups. However, at maximal exercise, there was a significant rise in HT\(_{hyp}\) (\(P < 0.01\)) but not in the nonhypoxic groups (Fig. 1A). During recovery, the %H values did not differ from the resting values in any group. There were significant correlations in HT\(_{hyp}\) (\(r = 0.82\)) or UT (\(n = 10, r = 0.16, P = 0.65\), and 2) between the changes in %H and the increase in \(ai-aDO_2\) between rest and maximal exercise (\(n = 9, r = 0.68, P < 0.05\) but not in HT\(_{nor}\) (\(n = 9, r = 0.16, P = 0.73\)) or UT (\(n = 10, r = 0.25, P = 0.49\)).

Basophils

The basophil counts did not differ significantly between the groups at any testing level. The HT groups and UT showed a significant decrease during exercise (\(P < 0.01\)), but during recovery only UT and HT\(_{nor}\) returned to resting values, and not HT\(_{hyp}\) (\(P < 0.05\)) (Fig. 1B).

Histamine Content

Basophil histamine content was not significantly different among the three groups at any testing level. There was a significant increase in histamine content in HT\(_{hyp}\), HT\(_{nor}\), and UT between rest and maximal exercise (\(P < 0.05\), \(P < 0.01\), and \(P < 0.001\), respectively) and between rest and postexercise recovery (\(P < 0.05\), \(P < 0.05\), and \(P < 0.001\), respectively) (Fig. 1C). However, the increase in basophil histamine content between rest and maximal exercise was significantly lower in HT\(_{hyp}\) than in HT\(_{nor}\) or UT (\(P < 0.05\)) (Fig. 3).

Osmolarity

There were increases in blood osmolarity (\(P < 0.001\)), \(K^+\) (\(P < 0.001\)), and \(Na^+\) (\(P < 0.001\)) between rest and maximal exercise in all groups. The values of all these parameters returned to resting level at the fifth minute postexercise, except for the \(Na^+\) concentration in UT (Table 4). The resting values were not different in the three groups; however, the maximal values were lower in HT\(_{hyp}\) than in UT for osmolarity (\(P < 0.01\), \(K^+\) (\(P < 0.05\), and \(Na^+\) (\(P < 0.005\)). There were no significant difference between HT\(_{hyp}\) and HT\(_{nor}\). There were no significant correlations between the changes in these parameters and the change in %H.

Table 3. Changes in plasma and whole blood histamine during incremental exhaustive exercise testing

<table>
<thead>
<tr>
<th>Plasma Histamine, nM</th>
<th>Whole Blood Histamine, nM</th>
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</thead>
<tbody>
<tr>
<td><strong>Rest</strong></td>
<td><strong>Max</strong></td>
</tr>
<tr>
<td>HT(_{hyp})</td>
<td>1.57 ± 0.13</td>
</tr>
<tr>
<td>HT(_{nor})</td>
<td>1.70 ± 0.10</td>
</tr>
<tr>
<td>UT</td>
<td>1.66 ± 0.19</td>
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Values are means ± SE. Significantly different from resting values: *\(P < 0.05\); †\(P < 0.01\); ‡\(P < 0.001\).
DISCUSSION

The results of the present investigation showed that the increase in histamine release during exercise was not associated with elevated basophil count, basophil histamine content, or blood osmolarity in extreme athletes. However, we found a lower increase in histamine content in these athletes during exercise than in control athletes and untrained subjects and a decrease in basophil count in each group at maximal exercise but only in extreme athletes at 5 min postexercise.

Methodology

Because Anselme et al. (2) and Préfaut et al. (30) found a strong correlation between %H and the drop in \( \text{PaO}_2 \) in extreme athletes, we used %H as the histamine release index, rather than plasma histamine or whole blood histamine. Nevertheless, the %H index takes into account variations in both plasmatic and total histamine (2, 20, 27).

We found an increase in %H during incremental exhaustive exercise in extreme athletes but not in untrained subjects, as did Anselme et al. (2). However, in our extreme athletes the mean increase in %H was below the 0.1–0.2% found by these authors; e.g., one of our subjects showed a decrease in %H and two showed an increase of \( \pm 0.02\% \). Also, we did not find significant differences in %H at maximal exercise between HT\texthyp and the nonhypoxic groups. Despite this, we found the same significant correlation between changes

![Fig. 1. Histamine release (%H; A), basophil count (basophils; B), and histamine content (HC; C) in untrained control subjects (UT; open bars), in nonhypoxic highly trained subjects (HT\textnor; hatched bars), and in highly trained subjects with exercise-induced hypoxemia (HT\texthyp; solid bars) at rest (Rest), at maximal exercise (Max), and at the 5th min of recovery (Rec). Significantly higher than resting values within groups: *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).

![Fig. 2. Relationship between increase in histamine release (\( \Delta \text{%H} = \%H \text{ at maximal exercise} - \%H \text{ at rest} \)) and drop in \( \text{PaO}_2 \) (\( \Delta \text{PaO}_2 = \text{PaO}_2 \text{ at maximal exercise} - \text{PaO}_2 \text{ at rest} \)) during exercise in HT\texthyp (\( n = 9 \) subjects).](http://jap.physiology.org/)

![Fig. 3. Comparison among UT, HT\textnor, and HT\texthyp of changes in basophil histamine content (\( \Delta \text{HC} \)) between maximal and resting levels (Max-Rest) and between recovery and resting level (Rec-Rest). Significantly higher than HT\texthyp group, *\( P < 0.05 \).

\( r = 0.74 \)
\( P < 0.05 \)
\( (n = 9) \)
in %H and the drop in PaO₂ during exercise (2, 30) in HTₜₚₑᵦᵦ.

**Exercise-Induced Hypoxemia and Histamine Release**

The development of EIH in our highly trained athletes is consistent with reports in the literature. Seven athletes maintained PaO₂ within 8–15 Torr of the resting values, another two showed 15- to 20-Torr reductions in PaO₂. Moreover, this drop in PaO₂ was accompanied by a greater maximal Ai-aDO₂ in HTₜₚₑᵦᵦ than in UT or HTₜₒᵦᵦ. This last result for extreme athletes has previously been suggested, with a significant correlation between the changes in PaO₂ and the rise in Ai-aDO₂ (16, 35). In the present study, we studied for the first time %H during exercise in nonhypoxic highly trained athletes and show that HTₜₒᵦᵦ did not present a significant change in %H during incremental exercise, as was the case for the untrained subjects. This result strengthens the hypothesis of a relationship between EIH and %H. The lack of increased %H in HTₜₒᵦᵦ was associated with a maximal Ai-aDO₂ that was lower than in HTₜₚₑᵦᵦ. This is consistent with the hypothesis of the contribution of histamine release to the impaired gas exchange in EIH. Nonetheless, our results cannot determine whether %H increase is an effect or a cause of EIH. The finding of Préfaut et al. (30) (a partial inhibition in EIH with antihistamine inhalation) provided evidence that this increase in histamine release during exercise may be involved in the development of EIH, but it is probable that histamine alone cannot induce gas exchange alteration and that another phenomenon must occur (30).

**Histamine and Incremental Exhaustive Exercise**

We noted that the incremental exhaustive exercise induced the same relative increase in total and plasma histamine in the nonhypoxic subjects, as has been previously described after exercise in normal subjects (8, 17, 24). These subjects thus showed no change in mean %H. In contrast, %H increased in the extreme athletes because of an accentuated augmentation in plasma histamine (2, 23); i.e., during exercise, the plasma and the total histamine levels increased in about the same proportion in the nonhypoxic subjects, whereas the increase in plasma histamine was relatively more accentuated (mean ~50%) than the increase in total histamine in the hypoxic athletes. Histamine release may be related to a peripheral inflammatory process (38) during exercise (14) in highly trained athletes. However, HTₜₒᵦᵦ did not present an increase in %H, although they reached the same high absolute level of exercise as HTₜₚₑᵦᵦ. Furthermore, the gas exchange impairment in these extreme athletes was significantly correlated with %H. This suggests that an alternative explanation for the increased histamine release during exercise may be that part of the histamine released comes from the activation of basophils or mastocytes located in the lung after lung inflammation (38). This hypothesis is supported by two facts: 1) we drew blood from the artery and not the vein (17) and 2) recent research with bronchoalveolar lavages has suggested that intense exercise may impair the integrity of the pulmonary blood-gas barrier in elite athletes and induce the release of inflammatory mediators (18).

**Basophil Count**

This study demonstrated no significant difference in basophil count in the highly trained endurance groups compared with the untrained group. Nieman et al. (27) originally reported this result for athletes in general at rest, and we have enlarged upon this result with similar data under exercise conditions and in a group of highly trained athletes exhibiting EIH. We may thus conclude that high training does not induce a high basophil count and that therefore basophil number cannot explain the increase in %H associated with EIH. Other factors must therefore intervene.

We describe, for the first time to our knowledge, a decrease in basophil count during an incremental exhaustive exercise in healthy subjects. This is the first study that has specifically investigated changes in basophil count during incremental exercise, with the sole exception of a 40-year-old study using a much longer exercise (8). Our data suggest that incremental exhaustive testing was accompanied by a “basopenia” in all groups. This exercise-induced basopenia may have been due to the counting method, which is based on specific staining of basophil granules and the detection of the stained cells. It also may have been due to a partial degranulation of the basophils during exercise and the consequent undercounting that occurs in the presence of lightly stained basophils that are difficult to distinguish from nonbasophil leukocytes with nuclear staining. These indeterminate cells are normally omitted from the basophil count because of this uncertainty (13). This hypothesis is supported by the fact that this basopenia disappeared during the recovery
period in the nonhypoxemic men. Indeed, as with most investigations (12, 20, 22, 24), we report no change in basophil number between pre- and postexercise basophil counts in normal subjects. Therefore, we prefer the term pseudobasopenia. The drop in basophil count likely reflects a basophil degranulation, which suggests an inflammatory process induced by incremental exercise. The basophil count in the extreme athletes, however, had not returned to resting level at the fifth minute of recovery. This result may be related to the fact that this population presents an increase in %H during exercise, and we hypothesized that this low basophil count after exercise may have been due to a complete degranulation of basophils. Again, this result suggests an inflammatory response induced by incremental exercise that is enhanced in extreme athletes.

This high basophil degranulation may also be caused by an increased stimulation due to several factors, such as hyperosmolarity (10). In agreement with the literature (11), we found an increase in osmolarity and cations during exercise. Nevertheless, the extreme athletes did not present a higher mean maximal value in osmolarity than the untrained or nonhypoxic trained subjects. This observation is not consistent with the hypothesis of a hyperosmolar activation of histamine release in these athletes. Moreover, the untrained controls showed a higher mean maximal value than the extreme athletes in Na\(^+\) blood concentration, which supports the hypothesis of an inhibitory effect of external Na\(^+\) on histamine release (4). We did not, however, find a significant difference between hypoxic and nonhypoxic groups nor a significant relationship between change in %H and Na\(^+\) concentration. Our data thus do not support the hypothesis of an influence of noninflammatory factors such as hyperosmolarity or blood concentration in cations on the increase in histamine release in extreme athletes. Other factors could induce an elevated histamine release, such as cytokines. For example, interleukin-1 (IL-1) is known to be an inflammatory mediator and a histamine-releasing factor (21) and to increase with exercise (6, 34). Recently, our laboratory showed that histamine releasability was elevated in highly trained athletes compared with untrained subjects (25). Obviously, further studies are needed to elucidate the mechanisms involved to confirm or eliminate inflammatory and cytokine hypotheses.

**Histamine Content**

The results of this study showed no difference in histamine content between nonhypoxic subjects and extreme athletes. We thus cannot explain the increase in %H in the extreme athletes by an elevated histamine content induced by high training. No other search, to our knowledge, has been conducted on the cellular content in histamine during exercise since the investigation of Duner and Pernow in 1958 (8). We found, as did they, an increase in cellular histamine in the untrained control subjects; we also found this increase in the highly trained athletes. This increase in both groups could be due to an increase in histamine synthesis during exercise. The literature is consistent with this hypothesis: 1) exercise leads to elevated plasma IL-1 activity (6, 34); and 2) IL-1 can induce an increase in histamine synthesis from basophils (36).

The increase in basophil histamine content observed in the extreme athletes during exercise was lower than in the untrained or nonhypoxemic highly trained subjects. One explanation of a low basophil histamine content associated with a decreased basophil count and an increased %H in highly trained athletes during exercise is an increased degranulation of the basophil cells. As we showed above, the augmented basophil %H in extreme athletes cannot be due to an effect of high training on basophils or noninflammatory hyperosmolar stimulation. It may, however, be explained by a systemic (14) and/or pulmonary (18) inflammatory process induced by the high workload achieved by extreme athletes.

In conclusion, we cannot explain the increase in histamine release associated with EIH by elevated basophil number or histamine content in extreme athletes. This suggests that another cell type may intervene: mast cells. However, the observation of a low recovery basophil count and a relatively low increase in basophil content at maximal exercise associated with the increase in %H suggested increased basophil degranulation during exercise in extreme athletes. This degranulation was not related to a hyperosmolar stimulation during exercise in these athletes, but it could be related to an inflammatory process. We hypothesize that the increase in histamine release in extreme athletes is at least partly due to an elevated degranulation of circulating basophils in response to stimulation by inflammatory factors such as inflammatory cytokines during exercise. This speculation obviously requires further investigation.

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**REFERENCES**

6. Cannon JG, Evans WJ, Hughes VA, Meredith CN, and Dinarello CA. Physiological mechanisms contributing to in-