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 (HT hyp) and 9 of whom were nonhypoxemic (HT nor), 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 HT hyp (P < 0.01) but did not increase significantly in HT nor 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 HT hyp in comparison to HT nor (P < 0.05) and UT (P < 0.01) and the low recovery vs. resting basophil count only in HT hyp (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 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 present EIH and in untrained control subjects. In addition, we examined blood osmolarity and the concentrations of Na⁺ and K⁺, noninflammatory parameters known to increase with exercise (11) and to influence histamine release (4, 10).

**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 (VO₂ 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 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.

**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 VO₂ max. Minute ventilation (Ve), O₂ uptake (VO₂), and CO₂ output (VCO₂) 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 VO₂ max was attained, at least three of the following criteria had to be met: 1) a plateau of VO₂ 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 1. Physical and physiological characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Age, yr ± SE</th>
<th>Height, cm ± SE</th>
<th>Weight, kg ± SE</th>
<th>FEV₁, % ± SE</th>
<th>VO₂ max, ml·min⁻¹·kg⁻¹</th>
<th>P max, W</th>
<th>HR max, beats/min</th>
</tr>
</thead>
<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>185.5 ± 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. FEV₁, forced expiratory volume in 1 s expressed as a percentage of predicted value; VO₂ max, maximal oxygen uptake; P max, maximal load achieved during incremental exhaustive exercise; HR max, maximal heart rate achieve 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.

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BASOPHILS AND HIGH TRAINING

despite maximal effort and verbal encouragement. A three-
lead ECG (DII, V2, V5) (Quinton Q 3000, Seattle, WA) was
monitored continuously during testing and recorded at the
end of every minute of the entire test to determine heart rate.
It was possible to monitor and record the nine other leads at
any time.

Blood Analysis

Blood samples were drawn from the brachial artery of the
nondominant arm with a 1-mm-diameter catheter (Seldicath;
Plastimed, Paris, France).

Blood gases. Arterial blood gases were immediately ana-
alyzed for \( \text{PaO}_2 \), arterial partial pressure of \( \text{CO}_2 \) (\( \text{PaCO}_2 \)), and
pH at 37°C using the appropriate electrode (IL Meter 1306,
Milan, Italy). Because of the rise in central temperature
during exercise testing, there was a risk of overestimating
the \( \text{PaO}_2 \) decrease. Blood gases therefore needed to be cor-
rected by core temperature estimated with a tympanic probe
(York VT 150, York Medical Equipment, Marietta, GA) at each
testing level. We observed an average change of 0.7 ± 0.1°C
during the incremental test with a maximal value at the fifth
minute of recovery. Because we corrected blood gases by the
appropriate temperature increase, we used the following rationale
to define EIH: given the variability in \( \text{PaO}_2 \) measurement at rest, hypoxemia
in the resting condition should be defined as a significant re-
duction of 5 Torr compared with normal individual values
(31). Because the tympanic probe is accurate to estimate changes
in central blood temperature, i.e., ~0.1 or 0.2°C, and can thus be used
to correct values in blood gases (32), and also because we needed to detect mild hypoxemia, we added 3 Torr to the 5-Torr
resting drop of \( \text{PaO}_2 \) for a final value of 8 Torr to ensure a rigorous definition of EIH. Therefore, a drop in
\( \text{PaO}_2 \) of at least 8 Torr that lasts for at least the last three steps
of an incremental exercise test (9) is necessary to establish
the presence of hypoxemia during exercise.

Osmolarity analysis. Blood osmolarity was immediately measured
by an advanced micro-osmometer (3Mo+, Advanced Instruments,
Needham Heights, MA). K\(^+\) and Na\(^+\) blood concentrations were deter-
mined by use of an appropriate automatic analyzer (Vitros
700 XRC, Johnson and Johnson Clinical Diagnostic).

Plasma histamine assay. Blood samples for plasma histo-
mine determination were obtained in Vacutainer tubes (Bect-
ton Dickinson, Rutherford, NJ) containing EDTA and were
immediately placed on ice. Plasma was obtained via centrif-
ugation at 900 \( \times \) g for 10 min at 4°C in a model PR-J refrigerat-
er 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 per-
formed 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 \( \mu \)l
of distilled water, and aliquots were frozen at ~80°C until
histamine measurement. For whole blood histamine quanti-
fication, the diluted blood was then frozen and thawed twice
for cell lysis. After final thawing and the removal of mem-
branes by centrifugation at 900 g for 10 min at 4°C, quanti-
fication of histamine was performed in supernatant as de-
cscribed 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 deter-
mined as \%H = 100 × \( \text{PH/TH} \), where \( \text{PH} \) is the plasma
histamine for a given testing level in each subject and \( \text{TH} \) is
the corresponding total histamine.

Basophil count. Basophil counts were made in anticoagu-
lated blood samples (EDTA) taken at the same time as those
for histamine assay. The blood was stained with Alcian blue,
which is highly specific, accurate, and reproducible method
for basophil counting based on the staining of basophil gran-
ules 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 = (\( \text{TH} \) – \( \text{PH} \))/\( \text{BC} \), where \( \text{BC} \) 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 envi-
ronment, to detect any exercise-related cardiac anomaly and
to determine \( \text{Vo}_{2\max} \). 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 20 s of the third and fifth
minutes of rest, of the last load corresponding to \( \text{V}\dot{O}_2\max \),
and of the fifth minute of recovery. During the third exercise test,
tympanic temperature was measured at the times that corre-
sponded to the blood sampling; i.e., we used the mean value
in temperature obtained during the last 20 s of the third and
fifth minutes of rest, of the last load corresponding to \( \text{V}\dot{O}_2\max \),
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. Ventilatory variables (\( \text{V}\dot{O}_2 \), \( \text{V}\dot{C}_O_2 \), and \( \text{Ve} \))
were averaged over an integral number of breaths during the last 20 s of each
minute of rest, exercise, and recovery. This allowed the calculation of \( \Delta\text{AlaDO}_2 \)
using the standard formula \( \text{PaCO}_2 = \text{PaCO}_2 - \text{PaCO}_2 - \text{PaCO}_2[F\text{O}_2 + (1 - \text{FIO}_2/R)] \),
where \( \Delta\text{AlaDO}_2 \) is ideal alveolararterial difference in \( \text{Po}_2 \), \( \text{PaCO}_2 \) is ideal alveolar \( \text{Po}_2 \), \( \text{PaCO}_2 \) is
inspired \( \text{Po}_2 \), \( \text{FiO}_2 \) is the inspired \( \text{O}_2 \) fraction, \( \text{R} \) is respiratory
exchange ratio, \( \text{PaCO}_2 \) is alveolar \( \text{PcO}_2 \), and \( \text{PaCO}_2 = \text{PaCO}_2 + (1 - \text{FIO}_2/R) \).

Statistical Analysis

The values are means ± SE. Data concerning subject
characteristics were compared for homogeneity among UT,
HTmax and HTboy, by using a one-way ANOVA after verifica-
tion of a normal distribution. The means of difference (\( \Delta \))
between each testing level for a given histamine parameter
were calculated and then compared between the three groups
with this test. A two-way ANOVA with repeated measure-
ments 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 sig-
nificance was tested by using correlation coefficients. Sta-
tistical significance for all tests was set at \( P < 0.05 \), two-tailed.
Blood Gases

 maximum of the incremental exercise; Rec, 5th min of recovery. Significantly different in trained groups from untrained group: * †

mal workload (P histamine at any testing level. All showed a significant three groups in plasma histamine and whole blood

0.001) and HTnor (P 0.001) of Pa O2

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 for 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 HThyp than in UT for osmolarity (P < 0.001), K+ (P < 0.005), and Na+ (P < 0.005). There were no significant difference between HThyp and HTnor. 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></th>
<th>Rest</th>
<th>Max</th>
<th>Rec</th>
<th></th>
<th>Rest</th>
<th>Max</th>
<th>Rec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Histamine, nM</td>
<td></td>
<td></td>
<td></td>
<td>Whole Blood Histamine, nM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HThyp</td>
<td>1.57±0.13</td>
<td>2.50±0.27†</td>
<td>1.55±0.20</td>
<td>HThyp</td>
<td>779.0±67.0</td>
<td>840.0±60.6*</td>
<td>948.0±66.4*</td>
</tr>
<tr>
<td>HTnor</td>
<td>1.70±0.10</td>
<td>2.39±0.20†</td>
<td>1.91±0.39</td>
<td>HTnor</td>
<td>732.0±78.5</td>
<td>978.5±87.3†</td>
<td>994.3±74.6†</td>
</tr>
<tr>
<td>UT</td>
<td>1.66±0.19</td>
<td>2.50±0.39*</td>
<td>2.26±0.30</td>
<td>UT</td>
<td>707.5±90.8</td>
<td>963.9±87.4†</td>
<td>1,012.2±121.8‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Significantly different from resting values: *P < 0.05; †P < 0.01; ‡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), VO2 max (P < 0.001), and maximal load (P < 0.001) (Table 1).

Blood Gases

PaO2 and Ai-aDo2 are reported in Table 2. At rest, there were no significant differences in the mean PaO2 and Ai-aDo2 values between the groups. However, during exercise testing, HThyp showed lower values of PaO2 and higher values of Ai-aDo2 than UT (P < 0.001) and HTnor (P < 0.01) at the maximal workload. During recovery, the data for PaO2 were significantly different between HThyp and HTnor (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 for osmolarity (P < 0.001) but no difference between rest and recovery. Whole blood histamine was significantly higher at maximal workload and recovery in HThyp (P < 0.05), HTnor (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 HThyp (P < 0.01) but not in the nonhyposmic groups (Fig. 1A). During recovery, the %H values did not differ from the resting values in any group. There were significant correlations in HThyp (r) between the increase in %H and the drop in PaO2 between rest and maximal exercise (n = 9, r = 0.74, P < 0.05) (Fig. 2) but not in HTnor (n = 9, r = 0.09, P = 0.82) or UT (n = 10, r = 0.16, P = 0.65), and 2) between the changes in %H and the increase in Ai-aDo2 between rest and maximal exercise (n = 9, r = 0.68, P < 0.05) but not in HTnor (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 HTnor returned to resting values, and not HThyp (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 HThyp, HTnor, 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 HThyp than in HTnor or UT (P < 0.05) (Fig. 3).
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 PaO2 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 0.02%. Also, we did not find significant differences in %H at maximal exercise between HThyp and the nonhypoxemic groups. Despite this, we found the same significant correlation between changes...
in %H and the drop in PaO₂ during exercise (2, 30) in HTₜₜₜₜ.

**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 HTₜₜₜₜ than in UT or HTₙₙ. 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 nonhypoxemic highly trained athletes and show that HTₙₙ 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 HTₙₙ was associated with a maximal Ai-aDO₂ that was lower than in HTₜₜₜₜ. 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 nonhypoxemic 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 nonhypoxemic subjects, whereas the increase in plasma histamine was relatively more accentuated (mean ~50%) than the increase in total histamine in the hypoxemic athletes. Histamine release may be related to a peripheral inflammatory process (38) during exercise (14) in highly trained athletes. However, HTₙₙ did not present an increase in %H, although they reached the same high absolute level of exercise as HTₜₜₜₜ. 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-yr-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.

### Table 4. Changes in blood osmolarity and K⁺ and Na⁺ blood concentrations during incremental exhaustive exercise testing

<table>
<thead>
<tr>
<th>Osmolarity, osmol/kgH₂O</th>
<th>K⁺ concentration, g/l</th>
<th>Na⁺ concentration, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest Max Rec</td>
<td>Rest Max Rec</td>
</tr>
<tr>
<td>HTₜₜₜₜ</td>
<td>287.2 ± 1.0</td>
<td>303.0 ± 2.9*</td>
</tr>
<tr>
<td>HTₙₙ</td>
<td>288.1 ± 1.6</td>
<td>303.6 ± 2.3*</td>
</tr>
<tr>
<td>UT</td>
<td>286.2 ± 0.8</td>
<td>311.3 ± 1.5†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Significantly different from resting values: *P < 0.01; †P < 0.001.
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 nonhypoxemic 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 hypoxemic and nonhypoxemic 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 nonhypoxemic 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 research, 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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