Supramaximal exercise mobilizes hematopoietic progenitors and reticulocytes in athletes

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In this study, we hypothesized that supramaximal ("all-out") exercise may cause reticulocyte turnover. Erythropoietin (Epo) synthesis is stimulated by hypoxia (15), and Epo is released during exercise-induced hypoxemia (30) or when exercise is carried out under hypoxic conditions (31). Little is known about reticulocyte parameters in athletes, although reticulocyte counts and degree of maturity can be easily measured (10). Reticulocyte parameters were found to be steady during a 6-day endurance competition in runners (11).

In this study, we hypothesized that blood cell counts, lymphocyte subpopulations (T, B, and NK cells), and release of progenitors and reticulocytes might be affected by very intense ("all-out") exercise. We tested this hypothesis in young competitive rowers. Our second aim was to gain some insight into the mechanisms involved in progenitor and reticulocyte release by measuring plasma level of cytokines/growth factors known to modulate hematopoiesis. Exercise intensity was assessed by monitoring physiological variables during exercise. Exercise-
induced stress and neutrophil activation were estimated by measuring postexercise levels of muscle enzymes (creatine kinase, lactic dehydrogenase), plasma cortisol, and neutrophil elastase.

SUBJECTS AND METHODS

Subjects. Twenty competitive rowers (13 males, 7 females) were studied in two series of experiments (Table 1). In the first series, 13 subjects were studied (9 males, 5 females); in the second series, 6 subjects (4 males, 2 females). All subjects were nonsmokers and in apparent good health at clinical examination. No one referred infectious episodes in the 3 wk preceding the study or other significant disease. All subjects had been training regularly in the month before the study. Steroidal/nonsteroidal anti-inflammatory agents were not used before or during the study by any subject. All female athletes were eumenorrheic, and only one of them (tested in the second series of experiments) was taking oral contraceptives. Body fat, as percentage of total body weight, was assessed by plicometry in the first series of experiments only (n = 14).

The study protocol was approved by the Ethical Committee of the University of Palermo, and the athletes, or their parents in the case of minors, gave written informed consent.

Experimental protocol. In both series of experiments, the athletes underwent the same exercise protocol during a precompetition period. Besides rowing (training volume 65–95 km/wk), the training program included running and weight lifting, as previously reported (24).

Data were collected at least 24 h after a training session (rest) and shortly after all-out rowing over 1000 m (rowing ergometer Concept II, Morrisville, VT). The rowing test was preceded by 20-min warmup. After 10 min of low-intensity exercise [heart rate: 140 beats per minute (bpm), 22–26 strokes/min], the subject did two to three sprints of five to seven strokes at submaximal intensity (heart rate 160 bpm, 28–32 strokes/min). Active rest (2 min of light rowing, 22–24 strokes/min) was maintained between sprints. The warmup ended with a few start trials at maximal intensity preceded and followed by short active rest periods. After warmup, athletes underwent 10 min of full rest, during which the equipment was mounted and checked (24).

Exercise variables were continuously recorded breath-by-breath by a portable light-weight equipment which did not interfere with rowing movements (K4h, COSMED, Rome, Italy). Full calibration of the system was obtained before each test. During the test, subjects were wearing a neoprene mask holding a support for the gas sampling line and pneumotachograph. Physiological variables recorded during all-out rowing [O₂ consumption, CO₂ production, respiratory exchange ratio (RER), and heart rate] are reported as mean values recorded in the last minute of exercise.

Exercise-induced changes in blood/plasma volume were calculated in all experiments according to the methods described by Dill and Costill (9). The same procedure was applied to correct postexercise concentrations of cytokines/growth factors for plasma volume changes.

Blood samples. In the first series of experiments (n = 14), 20 ml of blood were drawn, with the subject in the sitting position, at rest, and after exercise from the antecubital vein into sterile tubes containing EDTA (Vacutainer, Becton Dickinson, San Jose, CA) for complete blood cell counts (ADVIA 120 counter, Bayer Diagnostics) and analysis of CD34+ cells by flow cytometry on whole blood, as in our previous study (3). Postexercise samples were obtained 14 ± 4 min after the end of the test. In the second series of experiments (n = 6), 30 ml of venous blood were similarly obtained in sitting subjects at rest and 8 ± 2 min after the end of exercise for complete blood cell counts. Separation of peripheral blood mononuclear cells (PBMC) was performed by density gradient (see Clonogenic assays of peripheral blood hematopoietic progenitors) and CD34+ and AC133+ progenitors, T-, B-, and natural killer (NK) lymphocytes were analyzed by flow cytometry. In both series of experiments, plasma and serum samples were collected for biochemical analyses.

Flow cytometry. In the first series of experiments, we analyzed the expression of the CD34 (HPCA-2 FITC), CD38 (Leu-17 PE), CD33 (LeuM9 PE), and HLA-DR (HLA-DR PERCP) antigens (Becton Dickinson) on whole blood using three-color staining. Ig isotype negative controls were used. The samples were immunofluorescence-labeled, and flow cytometry was performed on a FACSscan Excalibur using CellQuest software (Becton Dickinson). Analysis was performed using large contiguous gates on lymphocyte and monocyte regions (5). The percentage of CD34+ cells was calculated by adding the percentage of CD34+ cells on the lymphocyte and monocyte gates and subtracting the percentage of cells stained with the control reagents. Acquisition of 100,000 cells was necessary to ensure adequate sensitivity of the analysis. Total CD34+ cells were determined in triplicate in each subject, because three samples (for CD34/CD38, CD34/HLA-DR, and CD34/CD33, respectively) were analyzed; the mean value was used for analysis.

Clonogenic assays of peripheral blood hematopoietic progenitors. In the second series of experiments, venous blood samples were collected in preservative-free heparin (15 ml) and separated over a Ficoll-Hypaque density gradient (density 1.077) (Pharmacia Fine Chemicals, Piscataway, NJ) at 400 g for 40 min at 20°C. The interface peripheral blood mononuclear cells (PBMC) were collected, washed twice, and resuspended in Iscove’s modified Dulbecco’s medium (IMDM). Flow cytometry was used to analyze hematopoietic and angiogenetic progenitors (CD34, AC133) lymphocytes (CD3, CD19) and natural killer cells (CD56) (all from Becton Dickinson with the exception of the anti-AC133, purchased from Miltenyi Biotech, Germany). Venous blood was also collected in EDTA for complete blood cell and reticulocyte counts.
PBMCs were cultured at a concentration of 3 x 10^5 cells/ml for each dish (four plates per point) in 0.9% methycellulose, 40% fetal calf serum (Flow Laboratories, Glasgow, UK) in IMDM supplemented with α-thioglycerol (10^-4 mol/l; Sigma, St. Louis, MO) at 37°C in a 5% CO2 humidified atmosphere. For burst forming units-erythrocyte (BFU-E) colony assay, 3 U of recombinant human Epo (Amgen, Thousand Oaks, CA) and 50 ng/ml of recombinant human stem cell factor (R&D Systems, Minneapolis, MN) were added; for colony forming units-granulocyte and monocyte (CFU-GM) colony assay, 10 ng/ml of recombinant human granulocyte-macrophage CSF and 10 ng/ml of recombinant human granulocyte CSF were added; for colony forming units-granulocyte, erythrocyte, monocyte, megacaryocyte colonies assay (CFU-GEMM), all the growth factors used for BFU-E and CFU-GM colony assays were added together.

Colonies were scored at days 14 through 20 of culture under an inverted microscope. Data are expressed as the number of colonies per million of blood.

Reticulocytes. Reticulocytes were automatically counted (ADVIA 120, Bayer Diagnostics), based on the measurement of scatter and absorption of laser light. RNA content was analyzed by the oxazine 750 method (10); reticulocyte fractions with low (L), medium (M), and high (H) RNA content were assessed, the M and H fraction being immature reticulocytes.

Inflammatory markers, cytokines, and growth factors. Aliquots of plasma and serum were collected at rest and after exercise in all athletes, and stored at −80°C. The muscle enzymes lactate dehydrogenase (LDH) and creatine kinase (CK) and resting serum iron and ferritin were measured by enzymatic assays (Olympus 640 kits and equipment, Olympus Diagnostica, GmbH Hamburg, Germany). Total plasma neutrophil elastase was measured by a homogeneous enzyme immunoassay specific for human PMN elastase (detection threshold: 4 µg/l, Ecoline, Kit Merck, Darmstadt, Germany). Serum cortisol was measured by a homogeneous enzyme immunoassay specific for human cortisol (detection threshold: 0.36 mg/dl; Immunotech, Marseille). Plasma levels of IL-6, G-CSF, flt3-ligand, kit-ligand, ferritin were measured by enzymatic assays (Olympus 640 kits and equipment, Olympus Diagnostica, GmbH Hamburg, Germany), based on the measurement of scatter and absorption of laser light. RNA content was analyzed by the oxazine 750 method (10). Monocytes, % of WBC 5.4

Statistics. Data are reported as means ± SD. Paired t-test was used to compare normally distributed variables (by z score) at rest and postexercise. Differences between genders were tested by unpaired t-test. Changes in circulating CD34^+ cell populations after exercise were tested by the Wilcoxon test. Relationships between variables were analyzed by simple linear regression. As statistical analysis package, Statview 5.0.1 was used (SAS Institute Inc, Cary, NC). Significance was set at P < 0.05 for all tests.

RESULTS

Subjects. The athlete groups tested in the two series of experiments did not differ for gender distribution, anthropometric variables, or exercise performance (Table 1). Compared with males, females were slightly older, showed a higher fat mass (males, n = 9: 12.5 ± 3.5% of body weight; females, n = 5: 21.1 ± 7.7% of body weight, P < 0.0001) and lower performance indices (Table 1).

Rowing test. In the entire group, mean test duration was 208 ± 16 s; average workload was 322 ± 74 W, with significant differences between genders (Table 1), but not between the first and second series of experiments. In the last minute of exercise, mean heart rate was 176 ± 8 bpm (or 87 ± 4% of maximal heart rate), oxygen consumption (V\textsubscript{O2}) was 4.03 ± 0.93 l/min or 56.4 ± 11.4 ml·min\(^{-1}\)·kg\(^{-1}\), production of carbon dioxide (V\textsubscript{CO2}) was 4.53 ± 1.09 l/min, and the RER, that is, the ratio V\textsubscript{CO2}/V\textsubscript{O2}, was 1.13 ± 0.12, indicating ventilatory compensation for increased lactate levels (34).

Muscle enzymes did not change significantly postexercise [CK baseline: 176 ± 119, CK postexercise: 266 ± 199 IU/l, P = 0.09; LDH baseline: 379 ± 174, LDH postexercise: 380 ± 130 IU/l, nonsignificant (NS)].

Blood and plasma volume changes. Blood volume tended to fall postexercise (−2.4 ± 3.4%), while plasma volume decreased (−7.1 ± 5.3%) and cell volume increased (+4.6 ± 2.1%) compared with resting values.

Hematopoietic progenitors. In the entire sample, absolute circulating CD34^+ cells were 7.6 ± 3.0 cells/µl at rest and increased to 16.3 ± 9.1 cells/µl after exercise (P < 0.001 vs. baseline, Fig. 1). Circulating CD34^+ cell counts increased postexercise in both series of experiments (series 1, n = 14: baseline 7.1 ± 3.3, postexercise 12.0 ± 6.5 cells/µl, P < 0.05; series 2, n = 6: baseline 8.7 ± 2.2, postexercise 25.6 ± 6.4 cells/µl, P < 0.005; difference between baseline samples: NS; difference between postexercise samples: P = 0.0005). The higher cell counts in the second series of experiments were likely accounted for by a shorter time between end of exercise and blood sampling, since CD34^+ cell counts decreased as time of blood sampling after exercise increased (r = −0.65, P < 0.005). No difference was found between males and females.

Table 2. White blood cells and platelets

<table>
<thead>
<tr>
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<th>Rest</th>
<th>All-Out Rowing</th>
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<tbody>
<tr>
<td>WBC, cells/10^9/µl</td>
<td>5.96 ± 1.32</td>
<td>9.83 ± 2.19*</td>
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<tr>
<td>Neutrophils, % of WBC</td>
<td>51.2 ± 5.8</td>
<td>48.3 ± 5.7</td>
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<tr>
<td>Lymphocytes, % of WBC</td>
<td>35.5 ± 8.8</td>
<td>38.5 ± 10.1</td>
</tr>
<tr>
<td>Eosinophils, % of WBC</td>
<td>4.3 ± 7.7</td>
<td>3.6 ± 7.9</td>
</tr>
<tr>
<td>Basophils, % of WBC</td>
<td>1.1 ± 0.5</td>
<td>1.6 ± 1.3</td>
</tr>
<tr>
<td>Monocytes, % of WBC</td>
<td>5.4 ± 1.7</td>
<td>4.6 ± 1.3</td>
</tr>
<tr>
<td>Neutrophils, cells/10^9/µl</td>
<td>3.08 ± 0.90</td>
<td>4.73 ± 1.13*</td>
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<tr>
<td>Lymphocytes, cells/10^9/µl</td>
<td>2.18 ± 0.50</td>
<td>3.78 ± 1.43*</td>
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<tr>
<td>Eosinophils, cells/µl</td>
<td>161 ± 95</td>
<td>370 ± 905</td>
</tr>
<tr>
<td>Basophils, cells/µl</td>
<td>63 ± 35</td>
<td>156 ± 141*</td>
</tr>
<tr>
<td>Monocytes, cells/µl</td>
<td>318 ± 97</td>
<td>447 ± 143*</td>
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<tr>
<td>Platelets, cells/10^9/µl</td>
<td>275 ± 74</td>
<td>354 ± 97*</td>
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Values are presented as means ± SD. *Significant difference vs. baseline; n = 20. WBC, white blood cells.
CFU-GM, and a 4.8-fold increase in CFU-GEMM (P < 0.01 vs. rest for all). Furthermore, it is of interest to note that, as shown in Fig. 2, some BFu-Es and CFU-GEMM observed in blood samples collected after exercise were larger than those observed in the corresponding samples drawn at baseline.

**Lymphocyte subpopulations.** After exercise, T-lymphocytes (CD3+ cells) increased by 70%, while NK cells (CD56+ cells) showed a four-fold rise over baseline (Fig. 3). The increase of the CD56+/CD3− and CD56+/CD3+ fractions was 4.37-and 3.37-fold, respectively. All-out exercise did not affect B lymphocytes (CD19+) (baseline: 126 ± 50·10⁵ cells/ml; post-exercise: 162 ± 90·10⁵ cells/ml).

**Red blood cells and reticulocytes.** At rest, red blood cell (RBC) and reticulocyte counts were within normal range (Table 4). Compared with males, females showed significantly lower RBC counts (4.55 ± 0.30 vs. 4.98 ± 0.23·10⁶ cells/μl, P < 0.001), Hb (12.6 ± 1.2 vs. 14.6 ± 0.8 g/dl, P < 0.0002), and Hct (37.8 ± 3.1 vs. 42.6 ± 1.7%, P < 0.0005). Baseline serum iron (n = 18) tended to be higher in males (92.5 ± 35.3 μg/dl) than in females (63.6 ± 26.5 μg/dl, P = 0.15, NS). Serum ferritin (n = 13) also showed a similar trend (males: 40.5 ± 23.9; females: 19.8 ± 9.9; P = 0.10, NS).

All-out exercise increased Hct and reticulocytes, especially the immature M and H fractions, with a relative decrease in L-reticulocytes (Fig. 4, top).

Plasma Epo concentration at rest (n = 14) correlated inversely with RBC counts (r = −0.56, P < 0.05). After all-out rowing, Epo decreased (Fig. 4, bottom), and correlated inversely with Hct (r = −0.77, P < 0.01). No correlation was found between Epo and reticulocyte counts or L, M, and H fractions after all-out rowing.

**Growth factors/cytokines.** Plasma cortisol, neutrophil elastase, flt3-ligand, and serum TGF-β1 increased after all-out exercise, but IL-6 and G-CSF were not affected (Fig. 5). All data in text and figures are actually measured plasma levels; correcting for plasma volume changes slightly decreased plasma concentrations of all these mediators postexercise but did not modify the significance of the reported changes.

Closer analysis of plasma G-CSF data (Fig. 5) revealed that it increased by about 3.5 ng/ml in most subjects (n = 14, P < 0.001) but showed an opposite trend in subjects with high G-CSF resting levels (n = 3). Plasma IL-6 (n = 20, Fig. 5), K-ligand (n = 6, not shown) and SDF-1 concentrations (n = 6, not shown) did not change.

Plasma VEGF and HGF concentration increased slightly (+28% and +41%, respectively) after all-out exercise, but

**Table 3. Flow cytometry analysis of CD34+ cell subpopulations in whole blood**

<table>
<thead>
<tr>
<th></th>
<th>CD38− % of WBC, cells/μl</th>
<th>CD38+ % of WBC, cells/μl</th>
<th>HLA-DRneg % of WBC, cells/μl</th>
<th>HLA-DR+ % of WBC, cells/μl</th>
<th>CD33− % of WBC, cells/μl</th>
<th>CD33+ % of WBC, cells/μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>0.011 ± 0.015</td>
<td>0.097 ± 0.060</td>
<td>0.057 ± 0.055</td>
<td>0.053 ± 0.042</td>
<td>0.079 ± 0.062</td>
<td>0.043 ± 0.034</td>
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<td></td>
<td>(0.66 ± 0.89)</td>
<td>(5.61 ± 2.75)</td>
<td>(3.44 ± 2.22)</td>
<td>(2.95 ± 2.08)</td>
<td>(4.66 ± 3.22)</td>
<td>(2.43 ± 1.82)</td>
</tr>
<tr>
<td>Postexercise</td>
<td>0.003 ± 0.011</td>
<td>0.108 ± 0.056</td>
<td>0.066 ± 0.060</td>
<td>0.046 ± 0.024</td>
<td>0.088 ± 0.065</td>
<td>0.036 ± 0.024</td>
</tr>
<tr>
<td></td>
<td>(0.31 ± 1.12)</td>
<td>(10.40 ± 4.99*)</td>
<td>(6.15 ± 5.43)</td>
<td>(4.70 ± 6.23)</td>
<td>(8.32 ± 6.12*)</td>
<td>(3.65 ± 2.76)*</td>
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Values are presented as means ± SD; n = 14. All comparisons between rest and postexercise values were by Wilcoxon rank test. *Significant difference (P < 0.05) vs. the corresponding value at rest.
Fig. 2. **Top:** clonogenic assays from representative experiments, showing 4 BFU-Es and 2 CFU-GEMM colonies. **Bottom:** Mean (thick line) and individual results for BFU-E, CFU-GM, and CFU-GEMM. *Significant difference between baseline and postexercise conditions. Solid circles indicate female subjects.

Fig. 3. Mean (thick line) and individual results for T-lymphocyte (top, left), NK cells (top, right) and their subpopulations (CD56+/CD3- and CD56+/CD3+, bottom) in subjects from the second series of experiments (n = 6).
showed no relationship with the increase in circulating CD34+ cells.

Postexercise plasma Flt3-ligand concentration correlated with the change in CD34+ cell counts \((r = 0.59, P < 0.05)\) and with performance indices such as \(\dot{V}O_2/kg \) \((r = -0.48, P = 0.05)\), mean workload in W \((r = -0.51, P < 0.05)\), and exercise time \((r = 0.56, P < 0.05)\).

**DISCUSSION**

The result of our study confirmed the hypothesis that in young well-trained athletes supramaximal (“all-out”) exercise causes release of hematopoietic and angiogenetic progenitors and of immature reticulocytes; mobilization of T- and NK lymphocytes was also remarkable. The pattern of cytokine/growth factor release differed from the one previously found in marathon runners (3), in particular, plasma IL-6 and G-CSF did not show major changes after all-out exercise. Activation of angiogenesis was suggested by the rise in AC133+ cells, HGF, and VEGF, supporting a possible role of tissue hypoxia in modulating the response observed after supramaximal exercise.

Similar to findings in runners, the most immature precursors (CD34+/CD38− cells) were few in peripheral blood of rowers at rest and did not change after exercise. However, clonogenic assays showed intense hematopoietic stimulation after all-out rowing, which involved all lineages. To our knowledge, this is the first study in humans demonstrating such a powerful effect of intense exercise on hematopoietic progenitors.

In marathon runners, the intense neutrophilia after the race suggested that mobilization of CD34+ cells might be causally linked to increased leukocyte turnover and to the release of IL-6 and G-CSF, both factors being known, and the latter being clinically used, to induce precursor mobilization. After all-out rowing, however, precursor mobilization occurred at a time when peripheral blood neutrophils were only slightly increased, and G-CSF and IL-6 were unchanged, suggesting a different mechanism operating in the two types of exercise.

Progenitor mobilization occurred together with large increases in T/NK lymphocytes, as expected at the end of intense exercise of short duration (27). It is worth noting that hematopoietic growth factors can be released by T/NK lymphocytes and/or monocytes/macrophages, suggesting the possibility of direct interactions between different cell types, as previously shown in patients undergoing IL-2 administration (33). However, unlike in the IL-2 model, exercise-induced changes occurred in the absence of major increases in plasma G-CSF or IL-6. Therefore, other mediators are likely involved in the all-out exercise model. Similar to data obtained in long-dis-
tance runners (3) or after intermittent rowing (Bonsignore, MR, unpublished observations), we found that Flt3-ligand concentration increased after all-out rowing. A specific role of Flt3-ligand is suggested by unchanged concentration of K-ligand, another important hematopoietic growth factor (21), after supramaximal exercise. Finally, Flt3-ligand concentration correlated with postexercise circulating CD34+ cell counts, supporting the interpretation that the level of fitness may modulate precursor release. The postexercise change in Flt3-ligand was smaller in highly trained athletes compared with less fit individuals. Overall, our data confirm a likely important modulation of Flt3-ligand by intense exercise in healthy subjects.

We also looked at the possible involvement of other mediators. Plasma TGF-β1 increased after all-out rowing, confirming previous reports (16). Skeletal muscle and bone tissue are known sources of TGF-β1 (13, 16). In addition, precursors bearing the TGF-β1 receptor (CD105) circulate in peripheral blood (28), and TGF-β1 preserves early precursors in the bone marrow by regulating the cell cycle (12). As for stem cell mobilization and homing, they are known to be regulated by the expression of SDF-1 (8). After all-out rowing, plasma SDF-1 did not change, but this finding does not rule out a possible involvement in progenitor homing, as tissue levels of SDF-1 were not measured in our study. Studies in animal models will be necessary to improve our understanding of the role of these two growth factors in exercise-induced progenitor modulation in the bone marrow and peripheral tissues. Given the lack of change in plasma IL-6 or G-CSF level after all-out rowing, we asked whether tissue hypoxia might be involved in the modulation of precursor and reticulocyte release after supramaximal exercise. This issue is hard to address directly, because of the difficulty in measuring tissue oxygen levels. Some indirect information, however, may be obtained by analysis of angiogenic precursors, VEGF, HGF, and reticulocyte release in our rowers. As for mobilization of angiogenic precursors, exercise training (20) and incremental exercise (29) were found to increase circulating endothelial progenitor cells (EPCs) in subjects with cardiovascular disease or risk factors. In the study by Rehman and coworkers, EPCs increased four-fold in middle-aged subjects after exhaustive exercise (about 10 metabolic equivalents, or METS); in our rowers, instead, AC133+ cells, considered to reflect the angiogenic potential (14), only doubled after strenuous exercise (about 15 METS). Therefore, everyday competitive training may blunt circulating EPC responses. This interpretation appears supported by the relatively modest increase in plasma levels of proangiogenic growth factors like HGF and VEGF after the all-out test.

VEGF is a marker of hypoxic activation (15), and its expression is closely correlated with the expression of the hypoxia-inducible factor-1α in skeletal muscle of trained humans (26). Besides skeletal muscle (13, 17), VEGF can also be released by hematopoietic cells like erythroid and megakaryocytic precursors (7). HGF, a potent proangiogenic factor (6), also increased after all-out exercise, and our findings suggest a possible role of HGF in exercise physiology. We speculate that increased plasma VEGF and HGF levels after all-out rowing may be secondary to tissue hypoxia. This interpretation is supported by the concomitant release of immature reticulocytes in rowers. Conversely, reticulocyte counts and degree of maturity had been found to be stable in runners during an endurance race (11).

Together with reticulocyte release, we measured Epo concentration after exercise and found it decreased, similar to previous data by Schmidt and coworkers (31); supramaximal exercise for 3 min under hypoxic conditions, instead, increased Epo (30). Our data confirm a feedback regulation of Epo by RBC mass not only at rest, but also after intense exercise.

The increase in circulating CD34+ cells and reticulocytes was unlikely to depend on changes in blood or plasma volume, since these were much smaller than changes in cell counts. As for the effects of catecholamines, the involvement of neural regulation in hematopoiesis is highly controversial (2, 22). The marrow is richly innervated (1), but norepinephrine exerts inhibitory effects (4), whereas we found the opposite after exercise.

Some limitations of our study need comment. First, blood samples were not collected after the warmup, to avoid an invasive procedure possibly interfering with subsequent rowing; although we cannot exclude that the warmup might have affected the results, its intensity was far lower than that attained during the test. Second, the time course of progenitor mobilization was not assessed and deserves further investigation. Third, the results of the second series of experiments cannot be automatically transferred to the first sample; however, results of both series of experiments were consistent, and the two groups of athletes studied were very similar. Our work also shows some points of strength: to our knowledge, this is the first study indicating that very intense exercise might be used as a physiological model of progenitor mobilization in human athletes. In addition, we monitored exercise variables (V˙O2, V˙CO2, RER, and heart rate) during the test, whereas studies on hematopoietic stem cells in animals have used uncontrolled exercise training without physiological measurements (19).

In conclusion, all-out exercise in well-trained rowers acutely mobilized hematopoietic progenitors, T and NK lymphocytes, and immature reticulocytes. The type of exercise and fitness level appear to modulate cytokine/growth factor release. We speculate that progenitor mobilization may occur in response to tissue hypoxia, or signals originating in skeletal muscle at a very high workload. From a clinical standpoint, the intensity of the response was similar to that found after pharmacologic (i.e., IL-2) stimulation.

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