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Hemopoietic and angiogenic progenitors in healthy athletes: different responses to endurance and maximal exercise

Maria R. Bonsignore, Giuseppe Morici, Roberta Riccioni, Alice Huertas, Eleonora Petrucci, Mario Veca, Gualtiero Mariani, Anna Bonanno, Laura Chimenti, Maria Gioia, Paolo Palange and Ugo Testa

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Time-dependent mobilization of circulating progenitor cells during strenuous exercise in healthy individuals

Sven Möbius-Winkler, Thomas Hilberg, Kathleen Menzel, Eva Golla, Agnieszka Burman, Gerhard Schuler and Volker Adams

J Appl Physiol, December , 2009; 107 (6): 1943-1950.

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Circulating progenitor cells decrease immediately after marathon race in advanced-age marathon runners

Volker Adams, Axel Linke, Frank Breuckmann, Kirsten Leineweber, Sandra Erbs, Nicole Kränkel, Martina Bröcker-Preuss, Felix Woitek, Raimund Erbel, Gerd Heusch, Rainer Hambrecht, Gerhard Schuler and Stefan Möhlenkamp

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Circulating hematopoietic progenitor cells in runners

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Bonsignore, Maria R., Giuseppe Morici, Alessandra Santoro, Maria Pagano, Lucia Cascio, Anna Bonanno, Pietro Abate, Franco Mirabella, Mirella Profita, Giuseppe Insalaco, Maria Gioia, A. Maurizio Vignola, Ignazio Majolino, Ugo Testa, and James C. Hogg. Circulating hematopoietic progenitor cells in runners. *J Appl Physiol* 93: 1691–1697, 2002. First published July 12, 2002; 10.1152/jappphysiol.00376.2002.—Because endurance exercise causes release of mediators and growth factors active on the bone marrow, we asked whether it might affect circulating hematopoietic progenitor cells (HPCs) in amateur runners [$n = 16$, age: 41.8 ± 13.5 (SD) yr, training: 93.8 ± 31.8 km/wk] compared with sedentary controls ($n = 9$, age: 39.4 ± 10.2 yr). HPCs, plasma cortisol, interleukin (IL)-6, granulocyte colony-stimulating factor (G-CSF), and the growth factor fms-like tyrosine kinase-3 (flt3)-ligand were measured at rest and after a marathon (M; $n = 8$) or half-marathon (HM; $n = 8$). Circulating HPC counts (i.e., CD34⁺ cells and their subpopulations) were three- to fourfold higher in runners than in controls at baseline. They were unaffected by HM or M acutely but decreased the morning postrace. Baseline cortisol, flt3-ligand, IL-6, and G-CSF levels were similar in runners and controls. IL-6 and G-CSF increased to higher levels after M compared with HM, whereas cortisol and flt3-ligand increased similarly postrace. Our data suggest that increased HPCs reflect an adaptation response to recurrent, exercise-associated release of neutrophils and stress and inflammatory mediators, indicating modulation of bone marrow activity by habitual running.

cytokines; growth factors; marathon; endurance training

ALTHOUGH EXERCISE IS CONSIDERED a physiological stimulus for cell release by the bone marrow (5), surprisingly few data are available on circulating hematopoietic precursors in athletes. Erythrocyte production was studied relative to athlete's anemia (25) and to assess the effects of intermittent hypoxic exposure on exercise performance (1). Conversely, little is known of the effects of exercise on myeloid precursors. Over 20 years ago, it was reported that colony-forming cells in periph-

eral blood increased after a short and intense exercise bout in normal subjects (2), but a detailed characterization of hematopoietic precursors in well-trained subjects was never obtained.

The rationale to study myeloid precursors in athletes is that intense and prolonged exercise increases white blood cell (WBC) and neutrophil [polymorphonuclear neutrophil (PMN)] counts (5, 16), partly through mobilization of marginated PMNs (5) associated with increased endogenous plasma glucocorticoids (15). PMN activation also occurs during exercise, as indicated by increased plasma PMN elastase (8). In addition, endurance exercise causes release of hormones, such as cortisol and growth hormone (24), and mediators, such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, and granulocyte colony-stimulating factor (G-CSF) (17), all known to promote the growth and release of hematopoietic progenitor cells (HPCs) (11). Whether exercise may cause release of other hematopoietic growth factors, such as fms-like tyrosine kinase-3 (flt3)-ligand, known to potentially induce the growth and differentiation of HPCs in vivo and in vitro (13), has not been assessed.

As for training-induced adaptations, exercise-induced neutrophilia was shown to become progressively blunted with training (22, 23), but no study ever tested whether circulating HPC counts may differ between trained and sedentary subjects. Circulating immature cells are likely involved in angiogenesis (19) and repair processes (21), both mechanisms being possibly associated with strenuous exercise and progressive training. Given the large use of exercise-based rehabilitation programs in several diseases, knowledge of the physiological effects of training on HPCs might be of potential clinical use.

In this study, we measured circulating CD34-positive (CD34⁺) cells and their subpopulations in healthy amateur runners. CD34⁺ cells are early HPCs with

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undifferentiated morphology, whose maturation steps are indicated by progressive acquisition of CD38, human leukocyte antigen (HLA)-DR, and CD33 markers. Eventually, these cells lose the CD34 marker as they enter the differentiation pathway, thereby developing lineage-specific features (26). Baseline HPC counts in runners, likely to reflect chronic, training-associated changes, were compared with data from sedentary controls. The response of HPCs to exercise was assessed by studying the same runners at the end of the 1999 Palermo International marathon (M) or half-marathon (HM), respectively, and the following morning. This experimental design was chosen to analyze whether amount or duration of endurance exercise could modulate inflammatory and stress mediators, as well as circulating HPC counts.

METHODS

Subjects. Sixteen male amateur runners participating in the 5th Palermo International M ($n = 8$) or HM ($n = 8$) were studied. The entire group had a mean age of 41.3 ± 13.4 yr and a racing experience of 11 ± 9 yr (range 1–35 yr). Mean body weight and height were 70 ± 7 kg and 173 ± 5 cm, respectively. On average, the HM and M groups trained 89 ± 32 and 99 ± 33 km/wk, respectively (not significant). M and HM runners differed significantly for age (M: 50.4 ± 9.5 yr, HM: 33.1 ± 11.5 yr; $P < 0.005$ by unpaired t -test). Nine sedentary healthy men (age 39.4 ± 10.2 yr, body weight 82 ± 9 kg, height 178 ± 6 cm) were studied at baseline as controls. All subjects were nonsmokers, clinically healthy, and with no history of recent infection or other disease. The protocol was approved by the local Ethics Committee, and all subjects gave written informed consent to the study.

M and HM. The 5th Palermo International M and HM were held on December 8, 1999 at sea level. The race began at 9:00 AM. Weather conditions were good (mean hourly data from 9:00 AM to 1:00 PM by the City of Palermo Weather Bureau: barometric pressure: 1,007 mbar; temperature: $12.1 \pm 1.7^\circ\text{C}$; wind: 0.73 ± 0.24 m/s; solar irradiation: 315.6 ± 60.9 W/m²). Runners were allowed free intake of water during the race. Mean race time was 79 ± 7 min (range 69–91 min) in the HM group, and 207 ± 38 min (range 159–254 min) in the M group.

Protocol and measurements. Runners were studied under baseline conditions 9 ± 2 days before the race, shortly (13 ± 7 min) after completion of the race, and the next morning. On the day of the race, blood samples were obtained at the finish line and kept at 4°C during transportation to the V. Cervello Hospital in Palermo, where they were immediately processed. All other samples in runners and controls were collected at the hospital in the morning in fasting conditions, kept at 4°C , and immediately processed.

Blood was drawn from the subject's antecubital vein into sterile tubes containing EDTA (Vacutainer, Becton Dickinson, San Jose, CA) for complete blood cell counts (ADVIA counter, Bayer) and analysis of HPC by cytofluorimetry. For surface marker analysis, peripheral blood samples were analyzed for the expression of the CD34 (human progenitor cell antigen-2 FITC), CD38 (Leu-17 phycoerythrin), CD33 (LeuM9 phycoerythrin), and HLA-DR (HLA-DR peridinin chlorophyll protein) antigens (Becton Dickinson) by using three-color staining. Ig isotype-negative controls were used. The samples were immunofluorescence labeled, and flow cytometry was performed on a FACScan Excalibur with CellQuest software (Becton Dickinson). Analysis was per-

formed by using large contiguous gates on lymphocyte and monocyte regions (7). 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. The number of total cells acquired was 50,000 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 antigens) were analyzed; the mean value was used for analysis. Variability for total CD34⁺ cell counts was estimated by calculating the coefficient of variation in each subject. Circulating reticulocytes were also determined by the microscopic brilliant cresyl blue method.

In each experimental condition, aliquots of plasma and serum were collected and stored at -80°C . Muscle enzymes [lactic dehydrogenase (LDH) and creatine kinase (CK)] and serum iron were measured by enzymatic assays (Olympus 640 kits and equipment, Olympus Diagnostica, Hamburg, Germany). Total plasma elastase was measured by a homogeneous enzyme immunoassay specific for human PMN elastase (detection threshold: $4 \mu\text{g/l}$; Ecoline kit, Merck, Darmstadt, Germany). Serum cortisol was measured by radioimmunoassay (sensitivity 0.36 mg/dl; Immunotech, Marseille, France). Plasma levels of TNF- α , IL-6, G-CSF, flt3-ligand, and erythropoietin (EPO) were measured by immunoassay (R&D Systems Europe, Abingdon, UK). High-sensitivity kits were used for TNF- α (sensitivity: 0.18 pg/ml) and G-CSF (sensitivity: 0.8 pg/ml). The lower detection limit of the IL-6 assay was 0.7 pg/ml, and this value was used in statistical analysis for samples with undetectable IL-6 levels. Lower detection limits for the other assays were 7 pg/ml for flt3-ligand and 0.6 mIU/ml for EPO.

Statistics. All data are reported as means \pm SD. Runners and controls at baseline were compared by unpaired t -test (normally distributed variables) and Mann-Whitney test (CD34⁺ cell counts). Data obtained at different time points in runners were analyzed by repeated-measures ANOVA or paired t -test with Bonferroni correction for post hoc comparisons. Relationships between variables were analyzed by simple linear regression. The statistical analysis package used was Statview 4.5 (Abacus Concept, Berkeley, CA). Significance was set at $P < 0.05$.

RESULTS

Baseline conditions. Table 1 reports main data on blood cell counts in runners and controls. Serum iron concentration was in the normal range in both groups, but circulating reticulocytes in runners were about one-half the value of controls ($P < 0.0005$). Total and differential WBC counts did not differ between groups.

Circulating CD34⁺ cells (Fig. 1, Table 2) were three to four times higher in runners compared with controls, without significant differences between M and HM groups for total CD34⁺ cells or any subpopulation. Two HM runners showed very high total CD34⁺ cell counts, accounting for the trend toward higher values in the HM compared with M group (individual total CD34⁺ cell counts at baseline are reported in the x -axis of Fig. 2A). CD34⁺/CD38⁻ cells were very low in runners and controls, whereas total CD34⁺ and all other subpopulations were increased in runners. The coefficient of variation for total CD34⁺ cell counts was $9.5 \pm$

Table 1. Blood cell counts in sedentary controls and runners

| Condition | RBC, cells $\times 10^6/\mu\text{l}$ | Hct, % | Reticulocytes, %RBC | WBC, cells $\times 10^3/\mu\text{l}$ | Neutrophils, %WBC | Lymphocytes, %WBC | Platelets, cells $\times 10^3/\mu\text{l}$ |
|----------------------|---|-----------------|------------------------|---|----------------------|----------------------|---|
| <i>Controls</i> | | | | | | | |
| Baseline | 5.2 \pm 0.4 | 47.7 \pm 2.8 | 1.61 \pm 0.48 | 6.4 \pm 0.8 | 60.8 \pm 4.9 | 27.5 \pm 4.0 | 244 \pm 90 |
| <i>Half-marathon</i> | | | | | | | |
| Baseline | 4.9 \pm 0.2 | 44.6 \pm 2.1* | 0.76 \pm 0.41* | 6.0 \pm 1.1 | 54.1 \pm 8.9 | 31.5 \pm 6.9 | 232 \pm 44 |
| End of race | 4.9 \pm 0.3 | 45.5 \pm 1.4 | 1.84 \pm 0.86† | 10.5 \pm 2.6† | 67.0 \pm 9.7 | 23.6 \pm 8.7 | 311 \pm 51† |
| Morning postrace | 4.7 \pm 0.3 | 43.6 \pm 2.1 | 0.89 \pm 0.25 | 6.1 \pm 1.1 | 55.5 \pm 10.0 | 30.0 \pm 8.9 | 234 \pm 44 |
| <i>Marathon</i> | | | | | | | |
| Baseline | 4.8 \pm 0.2* | 43.9 \pm 1.8* | 0.80 \pm 0.59* | 6.6 \pm 1.4 | 53.7 \pm 7.8 | 32.5 \pm 5.4 | 224 \pm 34 |
| End of race | 4.8 \pm 0.3 | 43.8 \pm 1.7 | 1.59 \pm 0.25† | 15.2 \pm 3.2† | 82.3 \pm 5.0† | 10.0 \pm 3.0† | 299 \pm 43† |
| Morning postrace | 4.7 \pm 0.2 | 43.4 \pm 1.9 | 0.65 \pm 0.26 | 7.5 \pm 0.9 | 57.4 \pm 6.9 | 29.9 \pm 4.5 | 230 \pm 45 |

Values are means \pm SD. RBC, red blood cells; WBC, white blood cells. Significant difference vs. *controls and †baseline, $P < 0.05$.

7.0% in runners and $13.2 \pm 10.6\%$ in controls (difference not significant).

In runners, red blood cell counts were inversely correlated to training volume, estimated as kilometers per week ($r = -0.51$, $P < 0.05$). Neither circulating CD34⁺ cell or reticulocyte counts at baseline correlated with age or training volume. When the analysis of CD34⁺ cell counts was repeated after excluding two HM runners showing high baseline CD34⁺ counts (see above), results of regression analyses did not change.

M and HM. Total WBC and PMN counts increased after the race, more after M than after HM (Table 1). Reticulocyte counts doubled after both M and HM (Table 1). All values had returned to baseline value by the morning after the race.

Total circulating CD34⁺ cells or subpopulations at the end of the M or HM were unchanged compared with baseline (Table 2). The coefficients of variation for total CD34⁺ counts at the end of M and HM were 12.8 ± 9.1 and $11.7 \pm 11.2\%$, respectively (not different from baseline or control values).

In individual runners, the change in total CD34⁺ count at the end of the race was smallest in subjects with the highest baseline counts ($r = -0.862$, $P < 0.0001$ for pooled HM and M data; Fig. 2A). Absolute

CD34⁺ counts after the race did not correlate with training volume, but their change at the end of the race was smallest in M runners with the highest training volume ($r = -0.88$, $P < 0.005$ in the M group; Fig. 2B).

Markers of muscle damage, stress, and inflammation. Muscle enzymes at baseline were higher in runners (LDH: 352 ± 90 U/l; CK: 290 ± 173 U/l) than in controls (LDH: 288 ± 36 U/l; CK: 110 ± 69 U/l; $P = 0.05$ and <0.01 , respectively, vs. runners). LDH increased immediately postrace (M: 523 ± 133 U/l; HM: 438 ± 84 U/l; $P < 0.001$ vs. baseline), whereas CK increased markedly on the day after the race (M: $1,279 \pm 1,049$ U/l; HM: 944 ± 856 U/l; $P < 0.005$ vs. baseline), without significant differences between M and HM runners.

Figure 3 summarizes the plasma levels of total neutrophil elastase, TNF- α , IL-6, G-CSF, cortisol, and flt3-ligand in all groups and experimental time points. At baseline, runners and controls were similar, but flt3-ligand was lower in HM than M runners, possibly due to an age effect (flt3-ligand vs. age: controls, $r = 0.76$; runners, $r = 0.63$; $P < 0.05$ for both). TNF- α , PMN elastase, cortisol, and flt3-ligand increased after the race, irrespective of M and HM distance. Conversely, IL-6 and G-CSF increased more after M (40-fold and 3-fold, respectively) than after HM (10-fold and by 47%, respectively). EPO was unchanged after the race.

In runners, plasma cortisol was positively associated with G-CSF ($r = 0.76$, $P < 0.0001$) and flt3-ligand ($r = 0.71$, $P < 0.001$), whereas its association with IL-6 was less consistent ($r = 0.76$, $P < 0.05$ in post-M samples only). Weaker associations were found between cortisol and markers of inflammation (TNF- α : $r = 0.56$, elastase: $r = 0.72$, $P < 0.001$ for both). Plasma cortisol correlated with WBC ($r = 0.82$, $P < 0.0001$), neutrophil ($r = 0.83$, $P < 0.0001$), and reticulocyte counts ($r = 0.61$, $P < 0.0001$) but not with CD34⁺ cell counts.

Both TNF- α and PMN elastase correlated with flt3-ligand ($r = 0.76$ and 0.66 , respectively; $P < 0.0001$) and reticulocyte counts ($r = 0.47$ and 0.66 , respectively; $P < 0.001$) but not with CD34⁺ cell counts.

Morning postrace. In both HM and M groups, total CD34⁺ cell counts were lower on the morning postrace (Table 2) compared with end of the race (pooled data:

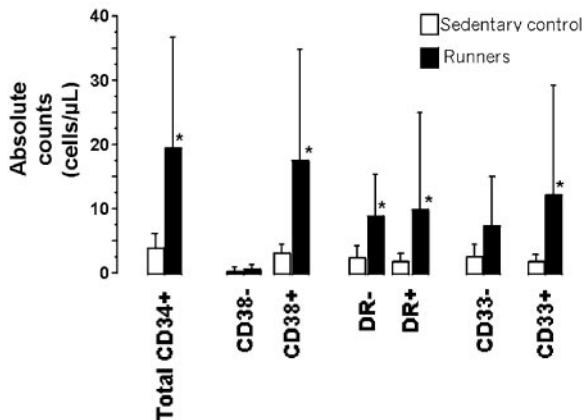


Fig. 1. Total circulating CD34⁺ cells and their subpopulations in controls and runners at baseline. Values are means \pm SD. *Significant difference between groups by Mann-Whitney test, $P < 0.005$.

Table 2. Total CD34⁺ cells and their subpopulations in controls and runners

| | CD34 ⁺ Total | CD34 ⁺ / CD38 ⁻ | CD34 ⁺ / CD38 ⁺ | CD34 ⁺ / HLA-DR ⁻ | CD34 ⁺ / HLA-DR ⁺ | CD34 ⁺ / CD33 ⁻ | CD34 ⁺ / CD33 ⁺ |
|----------------------|-------------------------|--|--|--|--|--|--|
| <i>Controls</i> | | | | | | | |
| Baseline | 4.0 ± 2.0 | 0.4 ± 0.7 | 3.0 ± 1.4 | 2.3 ± 1.9 | 1.8 ± 1.4 | 2.4 ± 2.1 | 1.6 ± 1.1 |
| <i>Half-marathon</i> | | | | | | | |
| Baseline | 23.4 ± 23.3* | 0.9 ± 1.1 | 22.0 ± 23.3* | 7.3 ± 4.0* | 14.6 ± 20.1* | 4.9 ± 4.7 | 18.5 ± 22.5* |
| End of race | 17.5 ± 13.1 | 1.3 ± 2.1 | 15.6 ± 14.8 | 10.7 ± 11.5 | 6.5 ± 3.3 | 12.3 ± 10.8 | 4.4 ± 3.2 |
| Morning postrace | 9.8 ± 6.6† | 1.2 ± 1.2 | 7.9 ± 6.0 | 6.2 ± 6.9 | 3.9 ± 1.7 | 7.1 ± 6.2 | 2.7 ± 1.1 |
| <i>Marathon</i> | | | | | | | |
| Baseline | 15.5 ± 7.6* | 0.2 ± 0.6 | 13.2 ± 6.4* | 10.7 ± 8.2* | 4.2 ± 2.8 | 9.5 ± 9.7 | 6.0 ± 5.8* |
| End of race | 18.6 ± 10.5 | 1.3 ± 1.6 | 14.7 ± 9.7 | 10.6 ± 6.1 | 5.0 ± 4.6 | 14.4 ± 8.8 | 4.2 ± 4.1 |
| Morning postrace | 9.5 ± 3.6† | 0.5 ± 0.6 | 8.1 ± 3.2 | 5.4 ± 2.9† | 3.8 ± 2.2 | 7.3 ± 3.8† | 2.2 ± 1.7 |

Values are means ± SD in cells/μl. HLA, human leukocyte antigen. *Significant difference between controls and HM or M runners at baseline ($P < 0.05$ by Mann-Whitney test). †Significant difference between end of race and morning postrace ($P \leq 0.05$ by paired t -test and Bonferroni correction).

from 18.0 ± 11.4 to 9.7 ± 5.1 cells/μl; $P < 0.001$). A similar trend was observed in CD34⁺/HLA-DR⁻ and CD34⁺/CD33⁻ subpopulations ($P < 0.05$ for both) only in M runners.

We asked whether changes in HPC counts during recovery after the race (i.e., the change in cell counts between the morning postrace minus end of race) correlated with exercise-associated release of cytokines and growth factors. This hypothesis was not confirmed for plasma cortisol, IL-6, and G-CSF, as their levels at end of race did not correlate with total CD34⁺ cells or any subpopulation on the morning postrace. Conversely, the higher the level of flt3-ligand or TNF-α at the end of the race, the lower CD34⁺/HLA-DR⁺ and CD34⁺/CD33⁺ cell counts the morning postrace (Fig. 4).

DISCUSSION

The main result of this study is that chronic hematopoietic adaptations occur in well-trained athletes, as indicated by higher baseline circulating HPCs in runners than in sedentary controls. The second result is that HPCs did not change immediately after the race but were decreased on the following morning. Plasma cytokines and growth factors increased after both M and HM races and correlated with some changes in HPC counts observed the morning postrace, supporting the hypothesis that daily bouts of exercise, with release of hormones and other mediators, may act as

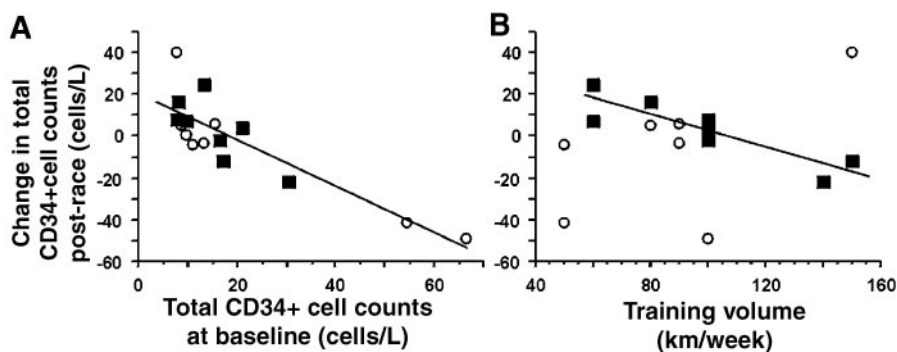
chronic intermittent stimuli modulating HPCs in peripheral blood.

A marathon is an established model of exercise-induced inflammation (6, 8, 16, 17, 23, 24). Exercise-induced neutrophilia partly results from mobilization of the marginated pool (5), secondary to increased cardiac output and plasma cortisol (15). Running a marathon increases plasma PMN elastase (8) and induces a complex pattern of pro- and anti-inflammatory mediators (17, 24), including cytokines and growth factors known to affect hematopoiesis, supporting the rationale of our study.

Circulating HPCs were unaffected acutely by the M or HM. However, in both HM and M groups, subjects with the highest CD34⁺ counts at rest showed the smallest change in CD34⁺ cells at the end of the race (Fig. 2A). Furthermore, the change in CD34⁺ counts at the end of the race in M runners correlated inversely with training volume, strongly supporting a training effect. Sedentary subjects undergoing a week of daily exercise showed progressively blunted exercise-induced neutrophilia and band cell release (22, 23). Our observations on more immature blood cells support exercise-dependent modulation of the early HPC pool. However, no significant relationship could be demonstrated between training volume and CD34⁺ cell counts at baseline.

Exercise-induced release of stress and inflammatory mediators could be the link between exercise and mod-

Fig. 2. Change in total CD34⁺ cell counts at the end of race vs. baseline total CD34⁺ counts (A) and training volume (B). ○, Half-marathon (HM) runners; ■, marathon (M) runners. A: the regression line refers to pooled HM and M points ($r = -0.86$, $P < 0.001$; regression on HM and M points only: $r = -0.89$, $P < 0.005$ and $r = -0.76$, $P < 0.05$, respectively). B: the regression line refers to M points ($r = -0.88$, $P < 0.005$), as it was not significant in the HM group.



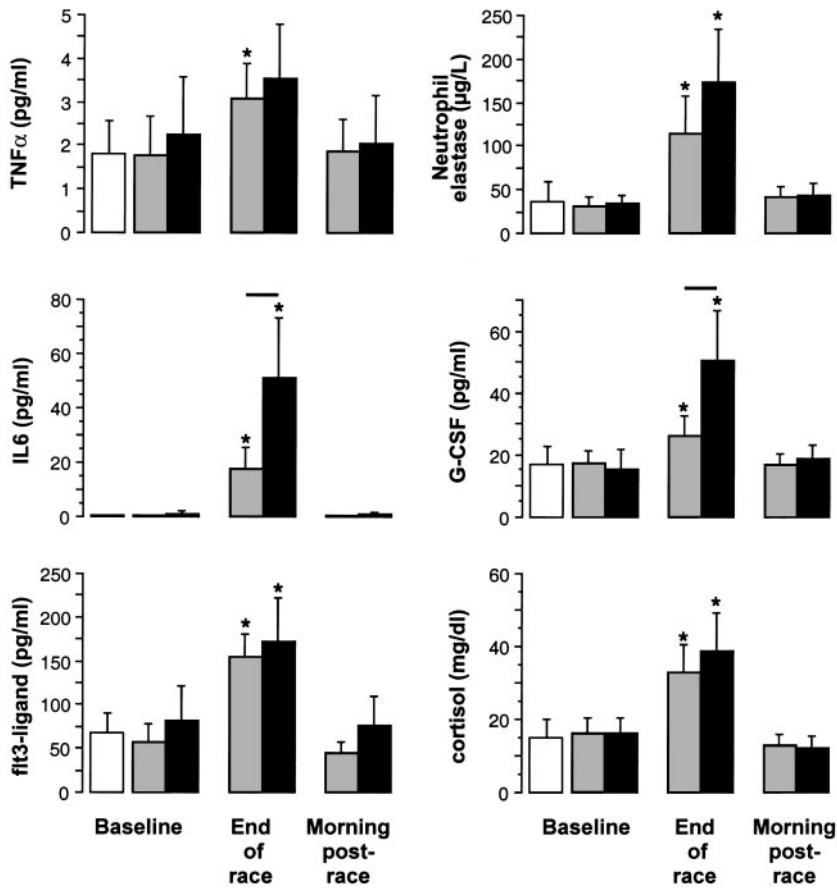


Fig. 3. Inflammatory markers, cytokines, and hormone and growth factors in controls (open bars) and in runners (shaded bars, HM; solid bars, M) at baseline, end of race, and morning post-race. TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; flt3-ligand, fms-like tyrosine kinase-3-ligand; G-CSF, granulocyte colony-stimulating factor. Values are means \pm SD. *Significant changes compared with baseline, $P < 0.01$. Horizontal bars, significant differences between HM and M groups.

ulation of hematopoiesis. Changes in circulating HPC counts appeared delayed compared with exercise-induced release of mediators. Total CD34⁺ cells decreased the morning post-race, together with CD34⁺/

HLA-DR⁻ and CD34⁺/CD33⁻ subpopulation counts in M runners. Plasma cortisol increased similarly after M and HM and correlated with plasma levels of growth factors known to act on the bone marrow. Our study is

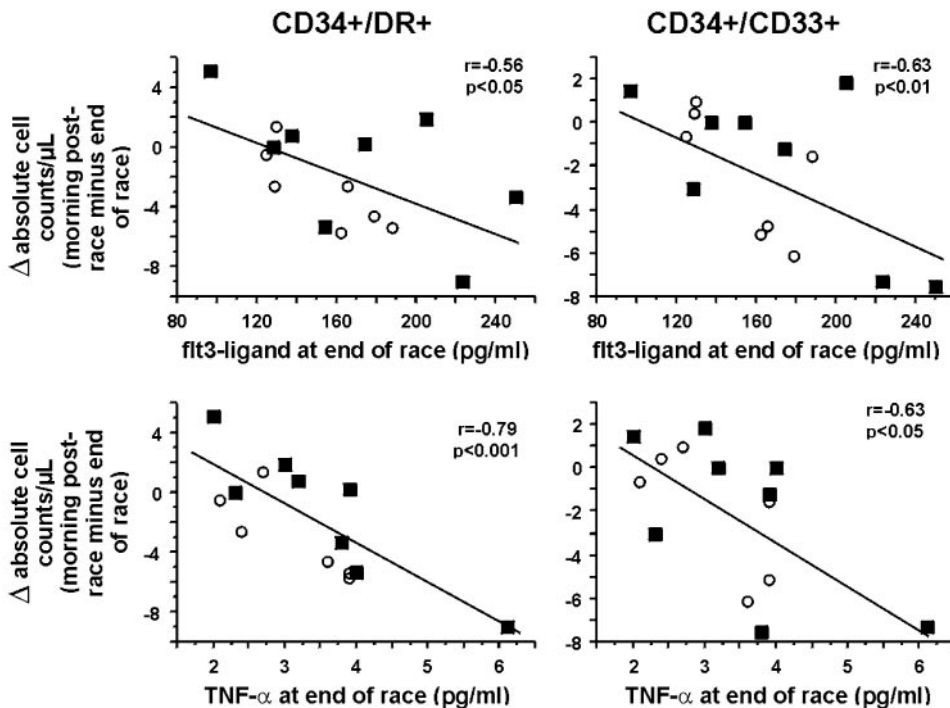


Fig. 4. flt3-Ligand (top) and TNF- α (bottom) plasma levels at end of race correlated inversely with CD34⁺/human leukocyte antigen-DR⁺ (left) and CD34⁺/CD33⁺ (right) cell counts on the morning post-race. Δ , Change. Symbols are as described in Fig. 2 legend. Regression lines refer to pooled HM and M points.

the first to report that plasma flt3-ligand, a known activator of HPCs (13), doubled after exercise, irrespective of running distance. TNF- α , G-CSF, and IL-6 are all known to affect hematopoiesis (11, 18, 27). In humans, G-CSF is used to mobilize and apheretically collect HPCs for transplantation and acts synergistically with IL-1, IL-3, and IL-6 on the bone marrow (11, 27). G-CSF-induced mobilization of HPCs was shown to be mediated by release of elastase by PMNs in the bone marrow (12). A similar mechanism may operate in runners through exercise-induced PMN activation and elastase release. IL-6 also modulates hematopoietic cell growth and differentiation (11, 18) and is released by exercising muscles (6). However, because G-CSF and IL-6 increased less after HM than after M, their role on HPCs might be relatively minor compared with that of flt3-ligand. Indeed, the increase in flt3-ligand during exercise correlated inversely with decreased CD34⁺/HLA-DR⁺ and CD34⁺/CD33⁺ counts on the morning postrace. Conversely, we found no correlation between increased plasma IL-6 or G-CSF after the race and changes in CD34⁺ cells on the morning postrace. However, because several significant associations were found between plasma cortisol and inflammatory markers, it is likely that both stress and inflammation may come into play to modulate HPC subpopulations in runners. Only circulating CD34⁺/CD38⁻ cells were low in both runners and controls, suggesting no effect of exercise on the very early CD34⁺ cell pool.

Data from M and HM runners were similar for many variables. This is not surprising, because data after a real competition reflect the best possible performance in each runner, accounting for similar levels of markers of stress (cortisol) and muscle damage (enzymes) in both groups. Conversely, peripheral blood neutrophilia, IL-6, and G-CSF were higher after the M race (16), supporting the fact that they were influenced by the duration of exercise.

Because M runners were slightly older than HM runners or controls, our conclusions may be affected at least partly by an age effect. We consider this hypothesis unlikely. CD34⁺ cells tend to decrease with age, but M runners showed higher, not lower, counts compared with controls. In addition, CD34⁺ cell counts did not differ significantly between M and HM runners, despite the significant age difference between groups. An effect of age, instead, was apparent for flt3-ligand at baseline in both runners and controls. The slight increase in flt3-ligand levels could reflect decreasing bone marrow sensitivity to this factor with age.

We are aware that circulating HPC subpopulations reflect hematopoiesis only indirectly, and the precise mechanism of increased HPCs in runners or the effects of M or HM races on maturation of HPC cannot be inferred by our data. Ethical limitations prevent obtaining bone marrow samples in healthy subjects. On the other hand, our runners were not elite athletes, and the results of this study represent physiological responses in normal, healthy individuals.

The data prompt the question on the physiological role of circulating HPCs in runners. It is possible that increased "turnover" of polymorphonuclear cells associated with exercise and the consequent inflammatory state may stimulate HPC production and mobilization; in this case, the decrease in HPC counts documented after the race might reflect maturation and replenishment of the peripheral PMN compartment after prolonged and intense exercise. Alternatively, we speculate on a possible HPC involvement in angiogenesis and repair processes associated with exercise and training. Circulating HPCs were involved in neovascularization after myocardial damage in humans (20) and include cells with an angioblastic potential (19). Exercise is a potent cause of angiogenesis in skeletal muscle (9), and circulating HPCs might contribute to this process. Further studies with measurements of specific markers of early endothelial or muscle cell differentiation, however, are necessary to support this hypothesis.

As for the possible HPC involvement in tissue repair, it could apply to several tissues, including the lung, as bone marrow stem cells can differentiate into alveolar epithelium (10). In induced sputum of runners, we found very high PMN counts after a marathon and a pattern in PMN adhesion molecules, suggesting ongoing repair postrace (4). Should our findings be confirmed, they may be relevant in exercise-based cardiac and/or respiratory rehabilitation programs, as well as in other diseases. A study in well-trained subjects, however, cannot provide information on the exercise threshold necessary to trigger hematopoietic adaptations. This point deserves further study, together with the molecular and functional characterization of HPCs before and after training.

Opposite to the behavior of HPCs, runners appeared to "spare" reticulocytes at rest and released them transiently after exercise. Our data confirm the low-reticulocyte counts at rest in runners studied during a training period (14). The increase in reticulocytes after the race correlated with plasma cortisol but was not associated with changes in EPO, as already reported by Bodary and coworkers (3). Besides stress, hemolysis secondary to the mechanical impact of running (25) could also modulate the reticulocyte response to exercise.

In conclusion, HPC counts were increased in peripheral blood of runners at baseline and did not change immediately after a M or HM race. Circulating CD34⁺ cells decreased on the morning postrace, suggesting modulation of hematopoiesis during recovery after intense and prolonged exercise. Exercise-induced release of inflammatory and stress mediators, together with increased levels of hematopoietically active growth factors such as flt3-ligand, IL-6, and G-CSF, may be the pathophysiological link between exercise and chronically increased circulating HPCs in runners. The potential application of these findings to competitive training and exercise-based rehabilitation programs deserves further study.

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