Clin Chem Lab Med 2010;48(2):199–203 © 2010 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2010.034

Effect of inflammation induced by prolonged exercise on circulating erythroid progenitors and markers of erythropoiesis

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Abstract

Background: Exercise in humans augments the mobilization of circulating hematopoietic progenitor cells ($CD34^+$) from the bone marrow. We investigated the effect of inflammation on erythroid marrow activity by mobilization of erythroid progenitor cells (EPs) along with soluble markers of erythropoiesis.

Methods: Ten healthy athletes who participated in an ultra-distance foot race participated in the study. Peripheral blood mononuclear cells were isolated, before (phase I), at the end (phase II), and at 48 h post-race (phase III). EPs were detected as burst colony forming units (BFU-e) and colonies were scored at day 14. Markers of inflammation (C-reactive protein, serum amyloid-A, interleukin-6, ferritin and S100B) and bone marrow activity (erythropoietin, soluble transferrin receptor and lipocalin-2) were assessed.

Results: An approximately three-fold decrease in BFU-e number was observed at phase II. sTfR concentrations were also decreased at phase II and remained decreased at phase III. However, EPO and lipocalin-2 concentrations reached a maximum value at phase II, with a tendency to decrease at phase III.

Conclusions: These findings indicate that exercise-induced inflammation modulates bone marrow homeostasis leading to an increase in leukocyte turnover and a decrease in erythroid compartment. It appears that lipocalin-2 is the main factor that regulates the production and mobilization of EPs. Clin Chem Lab Med 2010;48:199–203.

Keywords: erythroid marrow activity; lipocalin-2; ultra-marathon.

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Received July 17, 2009; accepted September 23, 2009; previously published online December 10, 2009

Introduction

A competitive marathon race is a very stressful event and a unique model of exercise-induced acute inflammation (1–6). Acute-phase inflammation is characterized by increased blood flow, in association with the accumulation of fluid, leukocytes and inflammatory mediators known as cytokines and acute-phase proteins (7, 8). Several of these mediators, generated locally and transported systemically during acute inflammation, are critical in the release, trafficking, and differentiation of bone marrow stem cells (9, 10).

Exercise in humans has been shown to augment the mobilization of circulating hematopoietic progenitor cells (HPCs) ($CD34^+$) from the bone marrow (11–13). Since strenuous exercise causes peripheral neutrophilia and mild leukocytosis, exercise-induced changes in $CD34^+$ cell counts could possibly reflect an increase in leukocyte turnover.

However, intense exercise leads to a significant decrease in red blood cells (RBC). The reduction in RBCs is caused by hemolysis due to mechanical trauma and oxidative injury to the cells (14, 15). Whether changes in numbers of erythroid progenitor cells (EPs) induced by exercise are related to the degree of mechanical hemolysis is unclear. There might be other factors that also affect RBC levels, such as lipocalin-2. Lipocalin-2, a secreted member of the lipocalin protein family, has been shown to inhibit the production of RBCs in mice in vitro and in vivo (16). In humans, lipocalin-2 appears to suppress the proliferation of erythroid cells in vitro (17).

Until now, the effect of exercise-induced inflammation on erythroid cells has been examined in terms of hemolysis. In this study, we investigated the effect of inflammation on erythroid marrow activity by means of production of EPs along with soluble markers of erythropoiesis in ultra-marathon runners. Running an ultra-marathon is an extremely strenuous event that might lead to cerebral ischemia. In order to investigate this hypothesis, we also measured protein S100b, a well-known biomarker of brain tissue injury (18).

Materials and methods

Subjects

''Spartathlon'' is an ultra-distance foot race of continuous, moderate intensity exercise over a distance of 246 km, during which runners attempted to cover the distance from Athens to Sparta. Fifty-two runners competing in the 2006 ''Spartathlon'' race were fully informed of the purpose and procedures of the study, and written informed consent was obtained from all. The study protocol was approved by the Bioethics Committee of the Harokopio University, Laboratory of Nutrition and Clinical Dietetics. All experimental procedures conformed to the National Health and Medical Research Council guidelines for experimentation with human subjects. Ten of 52 athletes participated in the study. The median age was 42.8 ± 1.4 years (range 33–53 years) and participants finished the race in <36 h (mean and median running times were 32 h 8 min and 30 h 2 min, respectively; range 25 h 17 min–34 h 43 min). Ten sedentary healthy men (age 42.2 ± 10.4 years) were studied as controls. All subjects were non-smokers, clinically healthy, and with no history of recent infection or other disease. During the run, the ambient daily temperatures were between 5°C and 36°C and the mean daytime relative humidity was 60%–85%.

Blood collection

Blood samples were obtained from the subject's antecubital vein with the subject in a sitting position. Samples were collected prior to the start of the race, immediately following the race and at the recovery period, 48 h after the end of the race. The same preanalytical conditions were used for all blood collections and storage of samples for the 3 time intervals examined. The subjects consumed water, electrolytes and carbohydrates ad libitum before, during and after running. Peripheral venous blood samples were drawn into plastic syringes under sterile conditions and transferred immediately to tubes containing the appropriate anti-coagulant (citrate, EDTA, heparin). In each experimental condition, aliquots of plasma and serum were collected and stored frozen at −80°C until analysis. Samples for determination of burst colony forming units (BFU-e) were processed within 24 h of collection.

Hematology and blood chemistry

Hematologic parameters were measured using the Siemens-ADVIA 120 whole blood analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Serum erythropoietin (EPO), soluble transferrin receptor (sTfR) and ferritin were measured in duplicate using a two site chemiluminescence immunoassay (Nichols Institute Diagnostics, CA, USA). Concentrations of serum amyloid-A (SAA) and serum C-reactive protein (CRP) were measured using particle-enhanced immunonephelometry with the Dade Behring BN ProSpec nephelometer (Siemens Healthcare Diagnostics, Liederbach, Germany).

Serum lipocalin-2 levels were determined by a solid phase ELISA technique (R&D Systems, Minneapolis, MN, USA).

Serum S100B protein was analyzed with the electrochemiluminescence immunoassay (ECLIA) on the ROCHE ELECSYS 2010 immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Cell culture assay for circulating EP

Mononuclear cells from peripheral blood samples were separated using Ficoll ($d = 1.077$ g/mL, Histopaque, Sigma, Gaithersburg, MD, USA) and light-density cells were suspended in RPMI medium (GIBCO Laboratories, Grand Island, NY, USA). Mononuclear cells were plated at a concentration of 1×10^5 cells/mL in 0.8% methylcellulose in Isscove's modified Dulbeco's medium supplemented with 30% fetal calf serum, 1% de-ionized bovine serum albumin, 10^{-4} M 2-α-mercaptoethanol (Terry Fox Laboratory, Vancouver, Canada), 2 units/mL recombinant human rhu-EPO (Amgen, Thousand Oaks, CA, USA) and 100 ng/mL recombinant human stem cell factor (R&D Systems, Minneapolis, MN, USA). Cells were cultured in a humidified incubator containing 5% CO_2 at 37°C. Colonies were scored at day 14 using previously described criteria (19). Data are expressed as the number of colonies per milliliter of blood. All experiments were performed in duplicate. The total count of the plates was performed in duplicate and the mean values were used in data analysis.

Statistical analysis

Data are presented as mean ± SE, and statistical significance was set at $p < 0.05$. Analysis of variance (ANOVA) repeated measures test was used to analyze time-course changes. All the statistical procedures were performed using the STAT-GRAPHICS PLUS version 5.1 for Windows program (StatPoint Technologies Inc., Warrenton, VA, USA).

Results

Inflammation and bone marrow activity markers

Markers of inflammation and bone marrow activity examined before (phase I), at the end (phase II), and 48 h post-race (phase III) are shown in Table 1. Biomarkers of inflammation including interlenkin-6 (IL-6), CRP, SAA and ferritin were markedly increased by more than 6000-, 100-, 150- and 3-fold, respectively, over baseline at the end of the race. However, IL-6 concentrations returned to normal values by 48 h post-race (phase III), while CRP, SAA and ferritin remained increased.

Hemoglobin and sTfR concentrations decreased progressively from 143.4 ± 2.9 g/L and 20.9 ± 1.5 nmol/L, respectively, at phase I to 124.3 ± 2.2 g/L and 17.0 ± 1.1 nmol/L at phase III. Erythropoietin and lipocalin-2 concentrations reached a maximum value at phase II, with a tendency to decrease by phase III. Serum ferritin concentrations increased significantly by phase II and remained increased at phase III.

For the same time period, S100b protein followed the same pattern (phase I: 0.13 ± 0.01 mg/L, phase II: 0.29 ± 0.01 and phase III: 0.13 ± 0.01 mg/L). A moderately strong positive correlation between EPO and S-100b protein was found in phase II ($r = 0.521$, $p < 0.03$), while this correlation was absent in the other two phases ($p > 0.40$ and $p > 0.08$, respectively).

Effects of the prolonged brisk exercise on erythroid progenitors

EP decreased in peripheral blood nearly 3-fold at phase II from 480.0 ± 86.0 BFUe/mL in phase I to 177.0 ± 29.5

Table 1 Serum biochemical parameters (mean ± SE) measured in athletes that successfully completed the 2006 ''Spartathlon'' foot-race (ANOVA repeated measures).

	Phase I	Phase II	Phase III	Difference
Hemoglobin, g/L	143.0 ± 3.0	140.4 ± 3.0	124.2 ± 2.3	$p < 0.0001$
White blood cells, $\times 10^9$ L	6.5 ± 0.3	13.5 ± 0.6	6.0 ± 0.2	$p < 0.0001$
Platelets, $\times 10^9$ L	276 ± 13	272 ± 17	230 ± 11	$p < 0.05$
Ferritin, μg/L	49.7 ± 7.4	121.7 ± 13.3	150.0 ± 13.9	$p < 0.0001$
EPO, IU/L	13.5 ± 1.5	51.9 ± 12.2	21.1 ± 3.4	$p < 0.001$
sTfR, nmol/L	20.9 ± 1.5	20.1 ± 1.4	17.1 ± 1.0[b]	$p > 0.09$
S100B, μg/L	0.14 ± 0.01	0.29 ± 0.03	0.13 ± 0.02	$p < 0.0001$
IL-6, ng/L[a]	0.8 ± 0.1	8376.0 ± 1819.8	0.7 ± 0.1	$p < 0.00001$

[a]From ref. (20). [b]Significant lower than phase I (pair-observation t-test $p < 0.01$).

BFUe/mL, and increased at phase III to 693.0 ± 113.0 BFUe/mL (Figure 1).

Discussion

Running a marathon is a model for exercise-induced inflammation (21, 22). We have previously reported that prolonged strenuous exercise induces an increase in plasma IL-6, CRP, and SAA, reaching concentrations seen in major trauma, septic shock, systemic inflammation, or near-death (5). Consistent with our previous study, we found that SAA and CRP were extremely elevated, up to 150- and 116-fold, respectively, supporting the concept of increased inflammation (20). The concentration of serum ferritin was increased significantly immediately after the race and remained increased 48 h post-race due to the acute-phase response. In addition, a dramatic increase in the inflammation-responsive cytokine IL-6 was observed at the end of the race. IL-6 also modulates hematopoietic cell growth and differentiation (23). In marathon runners, large increases in reticulocytes and leukocytes have been observed (2, 11, 12, 24). Therefore, release of IL-6 might be linked to increased turnover of leukocytes and reticulocytes. We cannot make the same hypothesis as far as it concerns erythroid precursor mobilization, since the BFU-e number declined, despite the increased concentrations of IL-6 ($r = -0.735$, $p < 0.0001$).

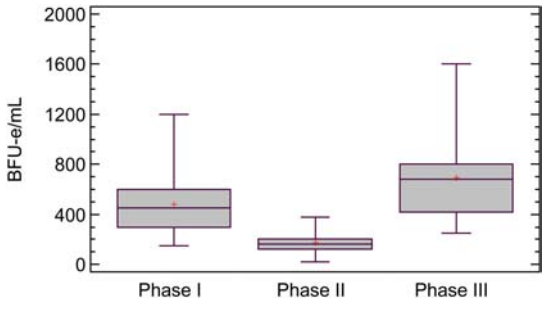

Figure 1 Numbers of erythroid progenitor cells.
Numbers of erythroid progenitor cells are depicted as box-plots before (phase I), at the end (phase II) of and 48 h after (phase III) the end of the ''Spartathlon'' race (boxes represent the interquartile range; lines inside boxes represent the median value; whiskers represent 5th and 95th percentiles).

The same pattern was also observed between the BFU-e number and lipocalin-2. During the recovery process, lipocalin-2 concentrations tend to decrease, whereas the number of EPs increases (Figures 1 and 2), ($r = -0.672$, $p < 0.001$). According to our results, it seems that during the course of acute inflammation induced by prolonged strenuous exercise, lipocalin-2 acts to suppress the production of EPs from the bone marrow.

A similar profile was observed for EPO. An increase in serum EPO at the end of the race with a tendency to decrease by 48 h post-race was observed. It appears that during acute inflammation, erythroid progenitors fail to respond appropriately to EPO stimulation. This hypothesis is further supported by studies in dialysis patients (25–27) where maintaining a stable hemoglobin concentration with EPO supplementation often shows increased requirements for EPO during inflammation or surgery (25–27). It is thus possible that failure of the bone marrow to respond to increased EPO leads to a further incremental response. This in turn results in the concentrations observed in ultra-marathon runners. In addition, the increasing concentrations of EPO may reflect a feedback mechanism that helps maintain erythroid marrow homeostasis. It is also possible that the increase in EPO concentrations in phase II reflects its neuroprotective properties (28), since a positive correlation was found between EPO and S100B concentrations. We can speculate that EPO is released in order to avoid a probable episode of brain ischemia. Thus, EPO may have a dual role in cases of acute inflammation.

sTfR, another marker of overall erythropoietic activity, decreased progressively reaching a minimum by 48 h post-race. sTfR concentrations are decreased in situations characterized by diminished erythropoietic activity, and are increased when erythropoiesis is stimulated by hemolysis or ineffective erythropoiesis (29). Consistent with our results, the decline in sTfR concentrations reflects the diminished activity of the erythroid marrow. BFU-e numbers decreased markedly by the end of the race, and returned to baseline by 48 h post-race. On the contrary, sTfR concentrations did not return to baseline but decreased further 48 h post-race. TfRs exist on colony-forming unit-erythroid rather than on burst-

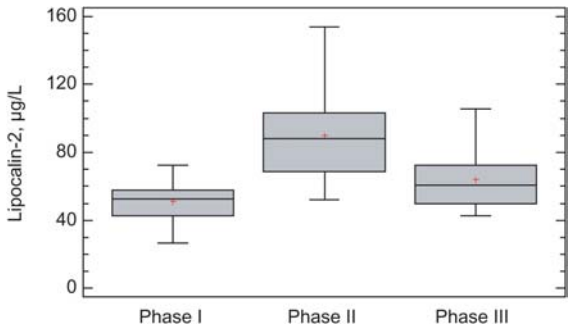

Figure 2 Plasma concentrations of lipocalin-2.
Plasma concentrations of lipocalin-2 are depicted as box-plots before (phase I), at the end (phase II) of and 48 h after (phase III) the end of the ''Spartathlon'' race (boxes represent the interquartile range; lines inside boxes represent the median value; whiskers represent 5th and 95th percentiles).

forming unit erythroid. Their numbers increase to about 300,000 on early normoblasts and up to 800,000 on intermediate normoblasts, before declining to about 100,000 on reticulocytes and none on mature red cells (30). Thus, the lower sTfR concentrations observed 48 h post-race are a result of the reduced number of BFU-e at the end of the race. Unfortunately, we were not able to measure sTfR concentrations at a later time post-race where normalization of sTfR values would be expected.

To date, only a few studies are available in the literature that describe the impact of supra-maximal exercise on erythropoietic marrow activity. Most of the studies have focused on the impact of exercise on the concentration of circulating $CD34^+$ cells. The results of these studies differ. In 2002, Bonsignore and colleagues demonstrated that the amount of $CD34^+$ cells is not affected by a half-marathon or marathon, but decreased significantly the morning following the race (11). In a second report, the same group analyzed the impact of maximal intensity rowing on the concentration of progenitor cells in 20 young rowers (12). Using fluorescence activated cell sorting (FACS) analysis or a clonogenic assay, a significant increase in $CD34^+$ cells and BFU-es was evident at the end of the race when compared to baseline. In 2008, Wardyn and colleagues investigated the effects of exercise on circulating $CD34^+$ and side population (SP) cell numbers using flow cytometry (13). The study demonstrated that circulating SP cells increased in number with exercise. On the contrary, the levels of circulating $CD34^+$ cells did not change significantly. In another report in 2008, Adam and colleagues observed down regulation in circulating $CD34^+$ cells after a marathon race (31).

Differences between these studies may in part be explained by the different intensity and duration of exercise. Therefore, one might speculate that the increase in progenitor cells may be a consequence of mobilization processes occurring in the bone marrow after short supra-maximal exercise, whereas a longer time period leads to a reduction in the amount of cells due to inflammation. The hypothesis that inflammation influences the amount of circulating cells is further supported by the negative correlation between inflammatory markers and circulating progenitor cells (31). In addition, completing a marathon or an ultra-marathon, as in our study, leads to a significant rise of inflammatory markers, such as IL-6, ferritin, SAA and CRP.

It seems that exercise-induced inflammation modulates bone marrow homeostasis leading to increased leukocyte turnover and a decreased erythroid compartment. According to our results, it appears that lipocalin-2 is a factor that regulates the production and mobilization of erythroid progenitors.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. The funding sources played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
Research funding: Funding was received from Athens University (to I.P.) and from University of Peloponnesus (to P.B.).
Employment or leadership: None declared.
Honorarium: None declared.

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