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Original Article

Early changes of the anemia phenomenon in male 100-km ultramarathoners

Yu-Hui Chiu [a,b,c], Jiun-I Lai [d], Shih-Hao Wang [e,f], Chorng-Kuang How [g,h], Li-Hua Li [i], Wei-Fong Kao [c,j,k,*], Chen-Chang Yang [c,l], Ray-Jade Chen [a]

[a] *Department of Emergency Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan, ROC*
[b] *Department of Emergency Medicine, Mackay Memorial Hospital, Taipei, Taiwan, ROC*
[c] *Institute of Environmental and Occupational Health Sciences, National Yang-Ming University School of Medicine, Taipei, Taiwan, ROC*
[d] *National Yang-Ming University School of Medicine, Taipei, Taiwan, ROC*
[e] *Department of Emergency Medicine, National Yang-Ming University Hospital, Yilan, Taiwan, ROC*
[f] *Taiwan Wilderness Medical Association, Taipei, Taiwan, ROC*
[g] *Department of Emergency Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*
[h] *Institute of Clinical Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan, ROC*
[i] *Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*
[j] *Department of Emergency and Critical Care Medicine, Taipei Medical University Hospital, Taipei, Taiwan, ROC*
[k] *Department of Emergency Medicine, Taipei Medical University, Taipei, Taiwan, ROC*
[l] *Division of Clinical Toxicology and Occupational Medicine, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*

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Abstract

Background: Sports anemia is a widely observed phenomenon after prolonged running. There are various factors that contribute to sports anemia, including hemodilution, exercise-induced oxidative stress, iron deficiency, gastrointestinal bleeding, hematuria, and hemolysis resulting from foot-strike and/or from compression of contracting muscles on capillaries. Until now, there has been no published report that describes the overall hematological, urinary, and fecal consequences in Asian male ultramarathoners after a 100-km (62.5-mile) ultramarathon event.
Methods: A total of 25 male runners were recruited into our study. Blood was drawn 1 week before, immediately after, and then 24 hours subsequent to the race. Hematological samples were analyzed for the anemia phenomenon. Additionally, urinary and fecal samples were collected before and after the race for detection of occult blood.
Results: The blood hemoglobin and erythropoietin values of the recruited runners showed a statistically significant rise in the immediate post-race values and a rapid drop in values at 24 hours post-race. Blood concentrations of red blood cells and hematocrit were significantly lower at 24 hours post-race compared with pre-race. The white blood cell count, interleukin-6, tumor necrosis factor-alpha, high-sensitivity C-reactive protein, and ferritin all showed significant increases both immediately after and 24 hours post-race compared with pre-race hematological values. There were immediate decreases of both haptoglobin and iron, as well as an increase of total iron-binding capacity levels in post-race blood tests. For both urinary and fecal samples, there was a statistically significant difference between the pre- and post-race results in occult blood.
Conclusion: Running a 100-km ultramarathon will induce substantial sports anemia, and oxidative stress response, hemolysis, hematuria, and gastrointestinal bleeding are typical factors that contribute to its onset.

Keywords: clinical sports medicine; exercise-induced hemolysis; oxidative stress response; sports anemia; ultramarathon

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Wei-Fong Kao, Department of Emergency and Critical Care Medicine, Taipei Medical University Hospital, 252, Wu Hsing Street, Taipei 110, Taiwan, ROC.
E-mail address: wfkao100a@hotmail.com.tw (W.-F. Kao).

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1. Introduction

The effects of physical exercise on the erythrocyte system have been a topic of continuing interest in sports medicine in the past several decades. Prolonged running is known to modify hematological parameters and promote anemia, which is defined as a condition where one or more of the major red blood cell (RBC) measurements, including RBC count, hemoglobin (Hb), or hematocrit, are below normal.[1–3] In endurance athletes, sports anemia is thought to be mainly related to hemodilution, which can be defined as the increase in plasma volume exceeding the increase of total red cell mass and causing a reduction in RBC count, Hb, or hematocrit.[3,4] Besides hemodilution, various factors contribute to sports anemia, including exercise-induced oxidative stress, iron deficiency, gastrointestinal bleeding, hematuria, and hemolysis resulting from foot-strike and/or from compression of contracting muscles on capillaries.[1,2,5]

In the literature, there are remarkable findings reporting significant changes of Hb, hematocrit, and RBC count in runners who strenuously push themselves during a marathon race, with mean cell volume (MCV) and mean cell Hb (MCH) negligibly affected.[6,7] "Foot strike hemolysis", a condition that develops from RBC destruction in the feet due to frequent striking on hard surfaces, has been proposed to explain the low level of haptoglobin in endurance runners.[5,8] Athletes can also lose considerable quantities of iron due to bleeding from the gastrointestinal and urinary tracts.[9,10] It has been suggested that the lasting high levels of erythropoietin (EPO) may promote an increase in red cell mass to maintain tissue oxygen levels after a marathon run.[11,12]

To our knowledge, there has been no published report that describes the overall hematological, urinary, and fecal changes of the anemia phenomenon in Asian male ultramarathoners after a 100-km (62.5-mile) ultramarathon event. We therefore analyzed these athletes' blood specimens for anemia parameters, oxidative stress cytokines, hemolysis markers, and iron levels. Urinary and fecal samples were also collected to evaluate the hematuria and gastrointestinal bleeding.

2. Methods

2.1. Study design and population

Twenty-seven experienced male ultramarathon runners participating in the event known as the 2011 Flexpower Cup National 100-km ultramarathon, in Taipei, Taiwan volunteered for this study. Approval was obtained from the Institutional Review Board of Taipei Veterans General Hospital, Taipei, Taiwan. All participants provided written consent to participate in the study. The competition began at 7:00 AM and ended at 9:00 PM on October 10, 2011. Ultimately, the data of 25 male runners were included in the analysis; two male runners who did not finish the 100-km race were excluded. All runners ran around a 400-m oval track, and they were permitted to rest and to ingest water and food freely. Before the competition, all runners were required to complete a questionnaire for demographic data and information on medical and training history. The body weight of each of the 25 participants was measured 30 minutes before, immediately after, and 24 hours after the race.

2.2. Laboratory assessment

Using sterile techniques, blood (20 mL) was drawn from an antecubital vein from each individual 1 week before, immediately after, and 24 hours after the race. All specimens were refrigerated and transported to the laboratory within 4 hours of sampling. Complete blood cell (CBC) counts were performed on a Coulter LH 750 Hematology Analyzer (Beckman Coulter, Miami, FL, USA), which was based on impedance detection for counting and sizing the blood cells. The CBC parameters included white blood cell (WBC) count, hematocrit, Hb, RBC count, platelet count, MCV, MCH, mean corpuscular Hb concentration (MCHC) and red cell distribution with width (RDW).

Free plasma Hb was tested with a HemoCue Plasma/Low Hb System (HemoCue, Lake Forest, CA, USA) utilizing the azide-methemoglobin method. The evaluated plasma haptoglobin was assayed by the rate nephelometry on an Immage 800 analyzer (Beckman Coulter, Fullerton, CA, USA).

Plasma interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Europe, Ltd). IL-6 and TNF-α levels were established in a sandwich assay by comparison with standard curves according to the recommendations of the manufacturer. High-sensitivity C-reactive protein (hs-CRP) was assayed on the Siemens Dimension RXL Max Integrated Chemistry System (Erlangen, Germany) using reagents supplied by the manufacturer, and determined using the bichromatic rate method.

Plasma EPO and ferritin levels were determined by an Architect I-2000 analyzer (Abbott Diagnostics, Abbott Park, IL, USA), which used a chemiluminescent microparticle immunoassay. Iron and total iron-binding capacity (TIBC) were measured on a Modular E170 Analyzer (Roche Diagnostics, Mannheim, Germany) using an electrochemiluminescence immunoassay.

The urinary samples of the runners were collected 30 minutes before, immediately after, and 24 hours after the race and analyzed using the Siemens Cliniteck Atlas Automated Urine Chemistry analyzer (Siemens Healthcare, Erlangen, Germany). The Atlas combines the reflectance spectroscopy with a reagent format to analyze the color and intensity of light reflected from a reacted reagent area for detection of occult blood.

We used the orthotolidine test to detect the presence of occult blood in fecal samples gathered within 24 hours before and after the race. This test depends on the peroxidase activity of the red cell and its iron-containing derivatives. Oxygen freed from hydrogen peroxide by this action is utilized by the

orthotolidine to produce a blue compound. The color-designated results are indicated by strong positive (dark blue), moderate positive (medium blue), weak positive (pale blue), and negative (no color change).

2.3. Statistical analysis

Descriptive results were reported as mean ± standard deviation (SD). The Spearman's rank correlation coefficient was used to evaluate the association of hematological/cytokine changes and the patients' demographics. The plasma hematological and biochemical values immediately following and 24 hours post-race were compared to the pre-race values using the Wilcoxon signed-ranks test. The results immediately following and 24 hours post-race of occult blood levels in urine were compared to the pre-race results by McNemar's Chi-square test. The results of fecal occult blood within 24 hours before and after the race were also compared by McNemar's Chi-square test. The one-way analysis of variance was applied for evaluating the association between plasma haptoglobin and urine occult blood in immediately following species. Commercially available statistical software (SPSS version 21.0; IBM Corp., Armonk, NY, USA) was used for statistical analysis. Differences were considered to be statistically significant when two-tailed $p < 0.05$.

3. Results

During the competition, the ambient temperature ranged from 24.9°C to 28.7°C, the relative humidity was 66–87%, and the wind speed ranged from 0 m/second to 6.5 m/second (data provided by the Taiwan Central Weather Bureau, Taipei, Taiwan). There were 25 male runner participants (mean ± SD, 47.0 ± 9.2 years) who finished the 100-km ultramarathon race. The mean ± SD finishing time of the 25 participants was 11.2 ± 1.4 hours. Their demographic data are summarized in Table 1. We collected samples from the runners at two time points: D1 indicates the difference between levels measured immediately after the race (within 1 hour of finishing) and before the race, while D2 indicates the difference between levels measured 24 hours post-race and before the race. In other words, D1 and D2 indicate the change in levels before and after the race, with D1 being immediately after the race and D2 being 24 hours later. After data analyses were completed using Spearman's rank correlation coefficient (*rs*), we found that the changes in TIBC levels were negatively correlated with the runners' age and running experience (years of running marathons) in both D1 (age: $rs = -0.548$, $p = 0.005$; experience: $rs = -0.547$, $p = 0.005$) and D2 (age: $rs = -0.582$, $p = 0.002$; experience: $rs = -0.489$, $p = 0.013$). Iron levels were positively correlated with age in D2 ($rs = 0.415$, $p = 0.039$). EPO levels were positively correlated with running experience in D2 ($rs = 0.590$, $p = 0.002$). Haptoglobin and hs-CRP were positively correlated with weekly training distance in D1 (haptoglobin: $rs = 0.468$, $p = 0.018$; hs-CRP: $rs = 0.544$, $p = 0.005$).

Table 1
Participants' demographic characteristics ($n = 25$).

Parameter	Mean ± SD (range)
Age (y)	47.0 ± 9.2 (22–60)
Weight (kg)	64.9 ± 9.1 (49.9–94.6)
Height (m)	1.68 ± 0.08 (1.55–1.85)
BMI (kg/m^2)	23.1 ± 2.6 (18.3–29.2)
Years of running marathons	5.5 ± 3.4 (1–15)
Training distance (*n*)	
<40 km/wk	4
40–100 km/wk	14
>100 km/wk	7
Best marathon score (min)	211.2 ± 21.6 (167–239)
This ultramarathon score (min)	670.9 ± 86.9 (487–827)

BMI = body mass index; SD = standard deviation.

Table 2
Hematological parameters throughout the ultramarathon race (total participants, $n = 25$).

Parameter	Mean ± SD			Normal range
	Pre-race	Post-race		
		Immediate	24 h	
Weight (kg)	64.9 ± 9.1	63.7 ± 9.1*	64.6 ± 8.8	
CBC counts				
WBC (× 10^3/μL)	5.5 ± 1.1	14.8 ± 2.8*	7.6 ± 1.6*	4.5–11
RBC (× 10^6/μL)	4.6 ± 0.4	4.7 ± 0.4	4.4 ± 0.5*	4.6–6.2
Hb (g/dL)	14.3 ± 1.0	14.7 ± 0.8*	13.6 ± 0.8*	14–18
Hematocrit (%)	41.6 ± 2.7	42.5 ± 2.3	39.8 ± 3.3*	40–54
MCV (fL)	91.4 ± 6.2	91.8 ± 6.2*	91.2 ± 6.1	80–96
MCH (pg)	31.4 ± 2.4	31.7 ± 2.5*	31.3 ± 2.5	27.5–33.2
MCHC (g/dL)	34.3 ± 0.6	34.5 ± 0.7*	34.3 ± 1.0	33.4–35.5
RDW (%)	13.3 ± 1.0	13.3 ± 0.8	13.4 ± 0.9	11.5–14.5
Platelets (× 10^3/μL)	215.2 ± 53.9	253.5 ± 59.3*	211.8 ± 56.5	150–350
Oxidative stress molecules				
IL-6 (ng/mL)	0.83 ± 0.42	18.5 ± 3.39*	1.72 ± 0.89*	0–0.01
TNF-α (ng/mL)	0.91 ± 0.36	1.17 ± 0.40*	1.19 ± 0.28*	0–0.03
hs-CRP (mg/dL)	0.12 ± 0.24	0.82 ± 1.86*	3.75 ± 2.36*	0.05–25
EPO and hemolysis markers				
EPO (mU/mL)	10.3 ± 5.0	25.8 ± 11.9*	17.1 ± 8.4*	8.2–21.4
Plasma Hb (g/dL)	0.03 ± 0.02	0.04 ± 0.05	0.03 ± 0.02	0–3
Haptoglobin (mg/dL)	63.6 ± 30.6	29.6 ± 29.9*	63.6 ± 31.6	30–200
Iron panel				
Ferritin (ng/mL)	140.1 ± 116.4	182.9 ± 189.7*	188.1 ± 154.2*	38–280
Iron (μg/dL)	113.7 ± 42.1	59.6 ± 25.6*	154.4 ± 65.4*	35–200
TIBC (μg/dL)	199.3 ± 53.8	280.4 ± 49.5*	141.2 ± 78.6*	200–400

*$p < 0.05$ versus pre-race value.
CBC = complete blood cell; EPO = erythropoietin; Hb = hemoglobin; hs-CRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; RDW = red blood cell distribution; TIBC = total iron-binding capacity; TNF-α = tumor necrosis factor-alpha; WBC = white blood cell.

Hematological parameters of these individuals throughout this 100-km ultramarathon are shown in Table 2. In the body weight and CBC counts, decreases in body weight and increases in MCV, MCH, MCHC, and platelet values showed a statistically significant difference between immediate post-race compared to pre-race results. There were statistically higher values of WBC in both immediate and 24 hours post-race compared with pre-race specimens. Hb concentrations showed a statistically significant rise at the immediate post-race values, which dropped off rapidly at 24 hours post-race. Concentrations of RBC and hematocrit were only significantly lower at 24 hours post-race compared with pre-race. No significant change was observed in RDW either immediately or 24 hours post-race compared with pre-race values.

EPO, IL-6, TNF-α, and hs-CRP, as shown in Table 2, showed significant increases both immediately after and 24 hours post-race compared with pre-race values. EPO and IL-6 increased immediately post-race and dropped off at 24 hours post-race.

With regards to the hemolysis markers shown in Table 2, plasma Hb did not change immediately and 24 hours after running. Haptoglobin decreased at the immediate post-race values, which returned to baseline values at 24 hours post-race.

In terms of iron tests in Table 2, ferritin levels showed significant increases in both immediate and 24 hours post-race compared with pre-race values. Comparing post-race to the pre-race values, iron levels dropped immediately in post-race testing but rose rapidly within 24 hours. By contrast, TIBC increased immediately post-race and decreased at 24 hours post-race.

The pre-race, immediately following, and 24 hours post-race qualitative tests for urine occult blood are presented in Table 3. Compared with the pre-race values, in general, there was a statistically significant difference ($p = 0.002$) in immediate values and no significant difference ($p = 0.112$) in 24 hours post-race values. Further analyzing the immediately following values, a statistically significant difference ($p < 0.05$) was observed in both "−" and "+++" subitems compared with pre-race results. The association between plasma haptoglobin and urine occult blood in immediately following species are shown in Fig. 1. The significance was 0.851.

Comparison of the qualitative results for fecal occult blood between pre-race and post-race are shown in Table 4. In terms of overall result, there was a statistically significant difference ($p = 0.014$) between the pre- and post-values. As for the subitems, no statistically significant differences ($p = 0.774$) in negative results were noted, and significant differences ($p = 0.02$) in strong positive results were observed.

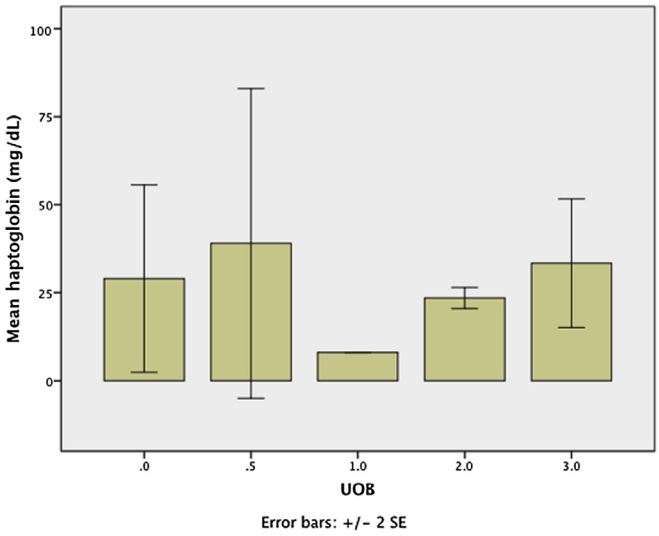

Fig. 1. A bar chart with standard error (SE, multipler = 2) for comparing the plasma haptoglobin across various categories of urine occult blood (UOB) immediately post-race.

4. Discussion

The association of our participants' demographics and their hematological/cytokine changes show that runners with additional training would more frequently have raised EPO levels, which could be explained as an increased ability of the body to provide Hb production. It is also interesting to note that the level of change in TIBC is negatively correlated with running age and experience, which could be explained as a generalized enhancement of circulatory hemodynamic endurance, similar to the phenomenon in which baseline heart rate is lower in athletes versus non-athletes.

Similar to previous reports, immediate post-race hemoconcentration with decreased body weight and increased mean red cell count and Hb level were noted in the athletes immediately after running.[13,14] The three indicators of anemia, RBC count, Hb, and hematocrit, significantly decreased below normal 24 hours after the 100-km race. By contrast, the long-lasting increase in EPO (shown by an immediate post-race

Table 3
Urine occult blood (UOB) ($n = 25$).

UOB	Pre-race (n)	Post-race (n)		p pre-race vs. post-race	
		Immediate	24 h	Immediate	24 h
−	25	8*	19	0.002	0.112
+/−	0	2	0		
+	0	2	1		
++	0	2	3		
+++	0	11**	2		

*$p = 008$ immediate post- versus pre-race value.
**$p < 0.001$ immediate post- versus pre-race value.

Table 4
Qualitative results of test for fecal occult blood (FOB) ($n = 25$).

FOB	Pre-race (n)	Post-race (n)	p
Negative*	20	7	0.014
Weak positive	2	1	
Moderate positive	1	8	
Strong positive**	2	9	

*$p = 0.774$ post- versus pre-race value.
**$p = 0.020$ post- versus pre-race value.

increase lasting 24 hours after the run) and the immediate rise in MCH (indicative of presenting younger cells) after the run were indicative of erythropoietic response. It can therefore be thought that anemia stimulated bone marrow activation.[15,16] Post-race values of RBC count, Hb, hematocrit, and EPO were significantly modified by the strenuous event, and compatible with the observation that anemia occurred in our study population.

In our study, we noted that known hematological biomarkers such as WBC, hs-CRP, IL-6, and TNF-α all increased at the immediate post-race time point after the 100-km ultramarathon. This is compatible with an immediate inflammatory and oxidative response.[17,18] In a study by Banzet et al,[19] IL-6 is involved in an exercise-induced increase of hepcidin gene expression, which had been implicated in training-induced iron deficiency. Furthermore, our data also show a significant increase in serum ferritin immediately after the race and 24 hours post-race. Although the mean values of ferritin remained within the normal reference limits, this statistically significant increase is consistent with an acute phase response to inflammation. Although further direct evidence is needed, our results are compatible with the hypothesis that oxidative stress response plays a role in sports anemia.[6,10,12]

Exercise-induced hemolysis is a widely observed phenomenon during long-distance running. An athlete's typical blood profile includes a decrease of haptoglobin and an increase of plasma Hb levels.[1,8] Although the concentration of plasma Hb in our study remained <0.05 g/dL, there was a significant and acute post-race decrease of haptoglobin, which confirmed the presence of hemolysis. Regarding the iron parameters, hemolysis induced by running a marathon is likely caused by promoting iron release from Hb.[20] Our findings, however, showed a decrease of iron and an increase of TIBC levels immediately post-race, while the 24 hour timepoint measurement in our study showed a rebound increase of iron and decrease of TIBC. The fact that iron levels decreased immediately post-race and further rebounded is very interesting. Although no apparent explanation for this result can be proposed at this point, the fact is that 24 hours post-race, our data show an increase in iron and decrease in TIBC. This trend and the amount of increase are in line with a study by Robach et al,[1] where those results were generated over a longer race duration (36 ± 5 hours). The timepoint of our measurement (24 hours post-race) is close to 36 hours. Although this is not directly comparable, our results imply that physiological adaptation to strenuous exercise for approximately 36 hours may result in an increase in iron and a decrease in TIBC levels. This might be due to the longer duration and heavier load of an ultramarathon than of regular marathon races. Also, it is likely that the timepoint of measurement of iron elevation and TIBC depression could not reflect the most obvious change. Additionally, the interpretation of the iron loss might also be due to gastrointestinal bleeding, hematuria, and inflammation response.[9]

It should be noted that in the study by Robach et al,[1] they quantified actual RBC volume and RBC mass and found that these parameters were largely unchanged. The authors proposed that plasma expansion resulting in hemodilution could explain the drop in Hb concentration levels. However, the authors also observed a hemolysis effect that was supported by several parameters, and therefore concluded that hemolysis was present, although the actual RBC amount was unchanged. The authors then further speculated that although hemolysis was present, it was probably unrelated to the circulating RBC pool and may have limited physiological significance. In our study, due to the study design, we did not directly measure RBC volume or mass. Our data clearly shows that hemolysis is present, consistent with previous studies. However, a determination as to whether or not hemodilution and/or hemolysis cause a drop in Hb concentration levels cannot be concluded by our data.

In addition to these serological changes, hematuria is further evidence of hemolysis during intense sporting activities. All 25 pre-race tests for urinary occult blood in our athletes had negative results; there were 17 athletes who had varied hematuria immediately after the race ($p = 0.002$). However, increased blood loss in the urine may not only be the consequence of hemolysis, but also the effect of mechanical trauma in the glomerulus or microscopic bleeding owing to the movement of the bladder during running.[9,21] However, no link between the plasma haptoglobin and urine occult blood found in our data represented this proposition.

During acute, intense physical activity, visceral blood flow will be reduced because of enhanced muscular perfusion, which may lead to an increased susceptibility to gastric lesions, impaired gastrointestinal absorption, and potential bleeding.[10,22] The significant presence ($p = 0.014$) of occult blood in the stool of our athletes after the race supported this thesis. Gastrointestinal bleeding will further aggravate anemia in these athletes.

We acknowledge that there are some limitations of our study. The relatively small sample size and the observational design limit the overall strength of the study's conclusions. By contrast, ultramarathon is an exclusive sport with far fewer participants compared to regular marathon races, and we believe our study, albeit a small sample size is of noteworthy importance. Second, only male runners were enrolled in our study, which may account for sex differences in regards to hemogram data. However, our data might not be representative of potential hematological parameter changes in female runners. Third, we did not follow-up the individuals with serological tests subsequent to the 24-hour measurement, and therefore were not able to assess the long-term effects of the 100-km ultramarathon on these runners. A final limitation is that occult blood in urinary and fecal samples was only detected by the qualitative measurements, not by the quantitative methods, which would likely offer more precise results.

In conclusion, our data indicated that running a 100-km ultramarathon induced substantial sports anemia, which is contributed to by oxidative stress response, hemolysis, hematuria, and gastrointestinal bleeding.

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References

1. Robach P, Boisson RC, Vincent L, Lundby C, Moutereau S, Gergelé L, et al. Hemolysis induced by an extreme mountain ultra-marathon is not associated with a decrease in total red blood cell volume. *Scand J Med Sci Sports* 2014;**24**:18−27.
2. Dang CV. Runner's anemia. *JAMA* 2001;**286**:714−6.
3. Hu M, Lin W. Effects of exercise training on red blood cell production: implications for anemia. *Acta Haematol* 2012;**127**:156−64.
4. Shaskey DJ, Green GA. Sports haematology. *Sports Med* 2000;**29**:27−38.
5. Telford RD, Sly GJ, Hahn AG, Cunningham RB, Bryant C, Smith JA. Footstrike is the major cause of hemolysis during running. *J Appl Physiol* 2003;**94**:38−42.
6. Fallon KE, Sivyer G, Sivyer K, Dare A. Changes in haematological parameters and iron metabolism associated with a 1600 kilometre ultramarathon. *Br J Sports Med* 1999;**33**:27−31.
7. Smith JE, Garbutt G, Lopes P, Pedoe DT. Effects of prolonged strenuous exercise (marathon running) on biochemical and haematological markers used in the investigation of patients in the emergency department. *Br J Sports Med* 2004;**38**:292−4.
8. Lippi G, Schena F, Salvagno GL, Aloe R, Banfi G, Guidi GC. Foot-strike haemolysis after a 60-km ultramarathon. *Blood Transfus* 2012;**10**:377−83.
9. Ottomano C, Franchini M. Sports anaemia: facts or fiction? *Blood Transfus* 2012;**10**:252−4.
10. Reinke S, Taylor WR, Duda GN, von Haehling S, Reinke P, Volk HD, et al. Absolute and functional iron deficiency in professional athletes during training and recovery. *Int J Cardiol* 2012;**156**:186−91.
11. Duca L, Da Ponte A, Cozzi M, Carbone A, Pomati M, Nava I, et al. Changes in erythropoiesis, iron metabolism and oxidative stress after half-marathon. *Intern Emerg Med* 2006;**1**:30−4.
12. Latunde-Dada GO. Iron metabolism in athletes−achieving a gold standard. *Eur J Haematol* 2013;**90**:10−5.
13. Kratz A, Lewandrowski KB, Siegel AJ, Chun KY, Flood JG, Van Cott EM, et al. Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. *Am J Clin Pathol* 2002;**118**:856−63.
14. Dickson DN, Wilkinson RL, Noakes TD. Effects of ultra-marathon training and racing on hematologic parameters and serum ferritin levels in well-trained athletes. *Int J Sports Med* 1982;**3**:111−7.
15. Roecker L, Kowoll R, Fraszl W, Battal K, Brechtel L, Brachmann S, et al. Observation of serum erythropoietin concentrations in female athletes for up to eight days after a marathon run. *Clin Lab* 2006;**52**:511−3.
16. Schwandt HJ, Heyduck B, Gunga HC, Röcker L. Influence of prolonged physical exercise on the erythropoietin concentration in blood. *Eur J Appl Physiol Occup Physiol* 1991;**63**:463−6.
17. Bernecker C, Scherr J, Schinner S, Braun S, Scherbaum WA, Halle M. Evidence for an exercise induced increase of TNF-alpha and IL-6 in marathon runners. *Scand J Med Sci Sports* 2013;**23**:207−14.
18. Waskiewicz Z, Klapcinska B, Sadowska-Krepa E, Czuba M, Kempa K, Kimsa E, et al. Acute metabolic responses to a 24-h ultra-marathon race in male amateur runners. *Eur J Appl Physiol* 2012;**112**:1679−88.
19. Banzet S, Sanchez H, Chapot R, Bigard X, Vaulont S, Koulmann N. Interleukin-6 contributes to hepcidin mRNA increase in response to exercise. *Cytokine* 2012;**58**:158−61.
20. Buchman AL, Keen C, Commisso J, Killip D, Ou CN, Rognerud CL, et al. The effect of a marathon run on plasma and urine mineral and metal concentrations. *J Am Coll Nutr* 1998;**17**:124−7.
21. McInnis MD, Newhouse IJ, von Duvillard SP, Thayer R. The effect of exercise intensity on hematuria in healthy male runners. *Eur J Appl Physiol Occup Physiol* 1998;**79**:99−105.
22. Sanchez LD, Tracy JA, Berkoff D, Pedrosa J. Ischemic colitis in marathon runners: a case-based review. *J Emerg Med* 2006;**30**:321−6.