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Hemolysis In Runners As Evidenced By Low Serum Haptoglobin: Implications For Preflight Monitoring Of Astronauts

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Hemolysis In Runners As Evidenced By Low Serum Haptoglobin: Implications For Preflight Monitoring Of Astronauts

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PRODUCT DISCLAIMER

ABSTRACT

Hematological parameters and serum haptoglobin were examined in 21 male employees of the Kennedy Space Center who were at three levels of physical activity: seven subjects regularly ran more than 40 Km (25 miles) per week (Group I), seven ran 13-24 Km (8-15 miles) per week (II), and seven were sedentary (III). Blood was drawn on a different day of the week for five weeks. Differences between day of the week, visit number, and activity level were examined. No differences were observed for day of week or visit number; thus, mean values for each variable were calculated for each subject. Variables did not differ among groups. However, trends with level of training were observed in some critical variables. Hemoglobin (Hb) and hematocrit (Hct) conformed to a staircase effect with Group I (14.5 $gm.dl^{-1}$ and 41.3%) lower than Group III (15.1 $gm.dl^{-1}$ and 42.9%). Reticulocyte count was higher and haptoglobin levels lower in Group I (1.35% and 75.7 $gm.dl^{-1}$) than Group III (.99% and 132.9 $gm.dl^{-1}$), with haptoglobin for the high mileage Group (I) in the clinically abnormal range. Since haptoglobin binds free Hb following RBC destruction, these results suggest that intravascular hemolysis occurs in trained male runners. These results may have special significance for astronauts training before long-duration spaceflights, since the further reduction in red blood cells which is reported to occur during spaceflight could become detrimental to their health and performance.

INTRODUCTION

Space travel has been reported to elicit anemia (15). Thus, the oxygen-carrying status of the blood of astronauts when they begin a space journey could be important to their well-being, especially during flights of long duration. Since most of the astronauts engage in some form of exercise, and some run long distances each week, the effects of long-distance running on the oxygen-carrying capacity of blood is a relevant issue for NASA.

Some researchers have reported low or low normal hematocrit (Hct) or hemoglobin (Hb) values in runners (4,7), a condition which has been termed "sports or runners' anemia." Their data may reflect a bona fide reduction in oxygen-carrying capacity or merely an increase in plasma volume with resultant reductions in hematocrit (Hct) and hemoglobin (Hb) concentration (17). Other researchers report no differences in hematological parameters between athletes and non-athletes (2). Even those investigators supporting a "true" sports anemia have failed to provide information about accompanying physiological or pathological changes which might explain the phenomenon.

The purpose of this study, therefore, was 1) to observe whether anemia is present in runners and, if so, whether it is related to level of exertion as determined by miles run, and 2) to determine whether any such anemia is related to red blood cell destruction. These data may be important in prescribing preflight exercise training programs for astronauts.

METHODS

SUBJECTS

Twenty-one male employees of the Kennedy Space Center between the ages of 24 and 57 years volunteered to participate in the study. This range includes the ages of the male astronauts. Subjects were divided into three groups based on their activity:

I. High mileage runners - average more than 40 Km (25 miles) weekly.
II. Middle mileage runners - average 13-24 Km (8-15 miles) weekly.
III. Controls - sedentary: do not run or participate in any other sports.

Subject characteristics for the three groups and comparisons among groups are provided in Table 1.

Runners were selected for the study based on their responses to a physical activity questionnaire filled out one month prior to the start of the study. All participants in Groups I and II were established runners who had maintained their weekly running mileage at the specified level for more than one month prior to the study and continued it throughout the study. Sedentary subjects were recruited from the Kennedy Space Center subject pool.

Prior to acceptance into the study, all subjects participated in a medical screening which included medical history, resting 12-lead electrocardiogram, blood analysis for chemistries and complete blood count, urinalysis, and a maximal treadmill stress test using the Bruce protocol (1). All subjects were free of disease and illness, none were taking medications other than vitamins, and none were cigarette smokers.

The protocol was approved by the Kennedy Space Center Human Research Review Board. The project was carefully explained to each subject, and a consent form was signed before participation.

PROCEDURE

Testing was performed during the summer months (July - October) in Florida. Subjects came to the laboratory one morning a week for five consecutive weeks. Each visit was on a different day of the work week. Prior to their arrival at the laboratory, the subjects ate a low-fat breakfast of their choice (e.g., cereal and toast) with no coffee or tea. Blood was drawn after subjects sat at least five-minutes in the laboratory. The subjects kept a daily log of their running mileage for six weeks, starting one week prior to the five-week testing period.

TECHNIQUES

Twenty-five ml of blood were collected aseptically in an ethylenediaminetetraacetate (EDTA) tube and a plain clot tube. The EDTA tube was mixed and analyzed in duplicate for white blood cell count (WBC), red blood cell count (RBC), Hb, and mean corpuscular volume (MCV) using the Coulter Counter ZF5 System (5). Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated (19). A spun hematocrit was determined using an Adams microhematocrit centrifuge and reader (6). Percentage of reticulocytes was determined by counting (in duplicate) 1000 cells in smears stained with New Methylene Blue (6).

The plain tube was allowed to clot, then spun; and the serum was divided into two tubes and frozen at -16°C. Haptoglobin was analyzed by radial immunodiffusion (ICL Scientific Kit) on one of the frozen samples (11).

The second frozen sample was analyzed for total bilirubin (TBil) and iron using the Dupont ACA III (8).

Differences between days of week, week number, and group were tested by analysis of variance for each variable (9).

RESULTS

Since no differences were observed in day-of-week or week-to-week comparisons, mean values were calculated from the five weeks of data for each subject for each variable. These means are reported in Table 2. Normal hematology values for an adult male are listed in Table 3.

No variables differed significantly among the groups, due to large intragroup variations. However, certain trends are notable. Hct and Hb were lower in the high-mileage runners than in the middle-mileage runners and lower in the middle-mileage runners than in the control nonrunners. The reticulocyte count was higher in the high-mileage group than in the middle-mileage group or the control group, with these data also showing a staircase effect among the groups. Haptoglobin was lowest in the high-mileage group, with the mean for this group in the clinically abnormal range. Blood iron level was also lower in the high-mileage runners.

DISCUSSION

Sports anemia was defined by Pate as clinical anemia in an athlete or physically active person (16). Clinical anemia is the condition in which there is a reduced number of circulating erythrocytes and a smaller-than-normal erythrocyte mass or a reduced concentration of Hb in the peripheral blood (14).

In this study, we have seen lower (but not significantly lower) Hb and Hct levels in the high-mileage male runners as compared with the sedentary men. However, the hematology values for all three groups studied fall within the normal range (Table 3); thus, clinical anemia is not a finding in these runners. Data from other studies support this finding. In a study of members of the 1972 Australian Olympic team, Hb levels, erythrocyte counts, and Hct volumes were in the lower half of the normal range, but none of the athletes could be classified as anemic (21). Bunch warned physicians to be aware of this, so they would not misdiagnose disease and/or prescribe unnecessary invasive medical procedures (4). However, a study of the hematological values for a large population of runners would be required to determine if the normal values for runners as a class fall in the lower range of normal and whether it would be desirable to establish a new normal range for runners.

The lowered hematological values in runners have been attributed to several causes. Two of the most commonly proposed are increased plasma volume (2,17) and intravascular hemolysis (10,13). A combination of hemodilution and intravascular hemolysis may occur as a result of exercise.

The haptoglobin value in the runners in this study, which was below the normal range, supports the theory of intravascular hemolysis. Haptoglobin is a serum protein which combines with free Hb in the blood to form a complex molecule (3,16). In the presence of red blood cell destruction, circulating free haptoglobin levels are lowered as are Hb levels. Thus, serum haptoglobin level may indicate whether reduced Hb levels are due to red blood cell destruction. We observed an inverse relationship between haptoglobin levels and mileage. Although the group differences in haptoglobin levels were not significant in this study, a power analysis indicated that with an additional 25 subjects there would be a 92 percent chance of observing a significant difference in haptoglobin. In a previous study, we observed significantly lower haptoglobin levels in high-fit men and women (12 males and 12 females) as compared with more sedentary men and women (20).

Haptoglobin, by binding free Hb in the circulation, preserves the iron for erythropoiesis (4,16). The lack of significance in the difference in serum iron among the three groups in this study indicates the degree of hemolysis is small. The haptoglobin was not completely depleted in any subject and appears able to conserve all of the iron from RBC breakdown. Rapid or increased reutilization of the iron in bone marrow erythropoiesis may result in increased reticulocyte release into the circulation, which could explain the increased reticulocyte count found in the high mileage runners as compared with the controls.

Total bilirubin was not different in the three groups. Since the serum bilirubin level is dependent both on the rate bilirubin is formed and on the efficiency with which it is excreted by the liver, a low-grade hemolysis may not be accompanied by an increased serum bilirubin concentration (22).

Plasma volume was not measured in this study; however, a four-percent difference in Hb between the high mileage and control groups was observed, while a 42-percent difference in haptoglobin was observed. The large difference in haptoglobin cannot be explained solely on the effects of dilution due to the increased plasma volume, since that would require practically a doubling of plasma volume. Furthermore, if no hemolysis were present, an increase in haptoglobin level might be seen, since acute phase reactions (stress, trauma, and other inflammatory conditions characterized by tissue injury and repairs) cause an increase in plasma haptoglobin. Such a post-exercise acute phase reaction was observed by Liesen, Dufaux, and Hollman (12), with elevation in serum haptoglobin levels as long as 48 hours after the end of strenuous runs of two to three hours.

Another possible explanation for reduced haptoglobin in Group I might be a disorder associated with intravascular hemolysis. Such conditions as pernicious anemia in relapse, paroxysmal nocturnal hemoglobinuria, paroxysmal cold hemoglobinuria, malaria, polycythemia vera, chronic liver disease, portal cirrhosis or mononucleosis might be considered but are unlikely for this population, since all subjects were initially screened with no disease processes observed (18).

In conclusion, for the range of training mileage studied (8-60 miles per week), no clinical symptoms of anemia were present in the runners, and all values analyzed, except haptoglobin, were within the clinically normal range. Haptoglobin trends of the runners were below the normal range, indicating a slight intravascular hemolysis may exist. These data suggest that clinical anemia is not a common characteristic of male runners.

An astronaut preparing for spaceflight is in a special situation, however. He is about to embark on a mission which may further decrease Hct and Hb. Therefore, even modest changes in Hct and Hb preflight may have increased importance. We, therefore, recommend periodic monitoring of blood characteristics of astronauts to assure that their training program is not inducing clinical anemia preflight.

In summary, 1) in male distance runners (13-97 Km [8-60 miles] per week) the hematological parameters (Hb, Hct, MCV, MCHC, MCH, retic, bilirubin) all fall within the normal range; 2) several values for the hematological parameters of the runners trend towards differing from those of the sedentary control group, which may be suggestive of a different normal range; 3) there is suggestive evidence of a greater intravascular hemolysis related directly to the amount of training; and 4) astronauts should be monitored periodically to assure they are not compromising their hematological status preflight.

Additional research with extremely high mileage runners, as seen in world class distance runners averaging more than 70 miles per week, is needed to completely rule out the possibility of an anemic condition in athletes.

REFERENCES

1. American College of Sports Medicine. Guidelines for Graded Exercise Testing and Exercise Prescription, Ed. II, 1980.

2. Brotherhood, J., B. Brozovic, and L.G.C. Pugh. Hematological status of middle-and-long distance runners. Clinical Science and Molecular Medicine. 48:139-145, 1975.

3. Brus, I., and S.M. Lewis. The haptoglobin content of serum in haemolytic anaemia. Brit. J. Haemat. 5:348-355, 1959.

4. Bunch, T. Blood Test Abnormalities in Runners. May Clinic Proc; February 1980, Vol. 55.

5. Coulter Counter Model ZF, 4201032D November 1979, Coulter Electronics, Hialeah, FL, 1972. Coulter Hemoglobinometer, Instructional Manual 4202049, Coulter Electronics, Healeah, FL, 1978.

6. Davidson, I., and J.B. Henry, MD, Editors. Todd-Sanford Clinical Diagnosis by Laboratory Methods, 15:1387-1388. W.B. Saunders Co., Philadelphia.

7. Dressendorfer, R., Ph.D., C. Wade, Ph.D., and E. Amsterdam, M.D. Development of pseudoanemia in marathon runners during a 20-day road race. JAMA, 246:11, September 11, 1981.

8. DuPont Company. ACA III Test Methodology IRN and TBIL, Automatic Clinical Analysis Division, Wilmington, DE. IRN - PN 703713B, TBIL - PN 703720B.

9. Glass, G.V. and J.C. Stanley. Statistical Methods in Education and Psychology. Prentice-Hall, Inc. Englewood Cliffs, NJ, 1970, pp. 383-388.

10. Hanson, P.G., B. Buhr, R. Sarnwick and N.A. Shahidi. Exercise Hemolysis in Trained and Untrained Runners. Biodynamics Laboratory and Departments of Medicine and Pediatrics. University of Wisconsin reported at Wake Forest University.

11. ICL Scientific, RID System Fahey and Mancini Technics, ICL, Fountain Valley, CA.

12. Liesen, H., B. Dufaux, and W. Hollman. Modifications of serum glycoproteins the days following a prolonged physical exercise and the influence of physical training. Eur. J. Appl. Physiol. 37:243-254, 1977.

13. Lindermann, R., R. Ekanger, P.K. Opstad. M. Nummestad, R. Ljosland. Hematological changes in normal men during prolonged severe exercise. Amer. Corr. Ther. J. 107-111, 1978.

14. Maile, J.B. Laboratory Medicine Hematology, C.V. Mosby Company, St. Louis. 5:448, 667, 1977.

15. Nicogossian, A.E. and J.F. Parker, Jr. Space Physiology and Medicine. National Aeronautics and Space Administration, 1982.

16. Pate, R. Sports anemia: A review of the current research literature. The Physician and Sports Medicine. 11:2:115-126, February 1983.

17. Refsum, H.E., G. Jordfald, and S.B. Stromme. Hematological changes following prolonged heavy exercise. Medicine Sport, Advances in Exercise Physiology. 9:91-99.

18. Ritzmann, S. and J. Daniels. Serum Protein Abnormalities Diagnostic and Clinical Aspects. Little, Brown and Company, Boston, 1975, pg. 219-222.

19. Simmons, A. Technical Hematology. 3:82, 1980, J.B. Lippincott Company, Philadelphia, PA.

20. Spitler, D.L., W.C. Alexander, G.W. Hoffler, D.F. Doerr, and P. Buchanan. Haptoglobin and serum enzymatic response to maximal exercise in relation to physical fitness. Med. Sci. Sports and Exercise. 16:4:366-320, 1984.

21. Stewart, G.A., J. Steel, A. Toyne, and, M. Stewart. Observations of the hematology and the iron and protein intake of Australian Olympic Athletes. Med. J. of Australia. 2:1339-1343, 1972.

22. Wintrobe, M.M. Clinical Hematology. 7:726, 1974, Lea & Febiger, Philadelphia.

TABLE 1

PHYSICAL CHARACTERISTICS OF THREE ACTIVITY GROUPS
WITH MEAN VALUES (X) AND STANDARD DEVIATIONS (S.D.)

VARIABLE	UNITS	GROUP I HIGH MILEAGE		GROUP II MIDDLE MILEAGE		GROUP III CONTROL		SIGNIFICANCE		
		X	S.D.	X	S.D.	X	S.D.	I vs II	I vs III	II vs III
AGE	year	37.4	9.1	45.1	9.8	41.0	10.7	S	NS	NS
HEIGHT	cm	177.7	6.4	179.3	5.6	176.6	5.3	NS	NS	NS
WEIGHT	kg	69.5	10.9	76.2	18.5	77.8	18.6	NS	S	NS
MILEAGE	$Km.day^{-1}$	8.1	3.2	2.6	0.6	0	0	S	S	S
VO_2	$ml.kg^{-1}.min^{-1}$	53.9	4.9	45.6	5.2	31.8	4.9	S	S	S

TABLE 2

HEMATOLOGICAL VARIABLES OF THREE ACTIVITY GROUPS
WITH MEAN VALUES (X) AND STANDARD DEVIATIONS (S.D.)

		GROUP I		GROUP II		GROUP III	
VARIABLE	UNITS	HIGH MILEAGE		MIDDLE MILEAGE		CONTROL	
		$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
WBC	$x10^3$cells.mm^{-3}	5.1	1.2	5.5	1.1	5.8	1.3
RBC	$x10^6$cells.mm^{-3}	4.67	0.25	4.80	0.43	4.86	0.33
HB	gm.(100 ml)$^{-1}$	14.52	0.81	14.96	1.19	15.19	1.08
HCT	%	41.3	2.5	42.4	3.1	42.9	2.2
MCV	u^3	90.4	3.1	90.0	2.4	90.1	3.0
MCH	pg	31.1	1.2	31.2	1.0	31.2	1.0
MCHC	g.dl^{-1}	35.	1.0	35.2	1.1	35.4	1.3
RETIC	%	1.35	0.5	1.23	0.6	0.99	0.38
HAP	mg.dl^{-1}	75.7	56.7	110.0	60.6	132.9	59.8
IRON	ug.dl^{-1}	75.4	30.8	79.6	29.4	84.1	37.4
BILI	mg.dl^{-1}	.69	.16	.75	.23	.59	.18

TABLE 3

NORMAL HEMATOLOGICAL VALUES FOR ADULT MEN

RED BLOOD COUNT (6)	=	$4.6 - 6.2 \times 10^6/mm^3$
HEMOGLOBIN (6)	=	13.5 - 18 gm/dl
HEMATOCRIT (6)	=	40 - 54%
MEAN CORPUSCULAR VOLUME (6)	=	$83 - 97\ u^3$
MEAN CORPUSCULAR HEMOGLOBIN (19)	=	27 - 33 pg
MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (19)	=	32 - 37 gm/dl
RETICULOCYTE (6)	=	0.5 - 1.5%
HAPTOGLOBIN (11)	=	100 - 300 mg/dl
TOTAL BILIRUBIN (8)	=	0 - 1.5 mg/dl
IRON (8)	=	42 - 135 ug/dl

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15. Abstract

Hematological parameters and serum haptoglobin were examined in 21 male employees of the Kennedy Space Center who were at three levels of physical activity: seven subjects regularly ran more than 40 Km (25 miles) per week (Group I), seven ran 13-24 Km (8-15 miles) per week (II), and seven were sedentary (III). Blood was drawn on a different day of the week for five weeks. Differences between day of the week, visit number, and activity level were examined. No differences were observed for day of week or visit number; thus, mean values for each variable were calculated for each subject. Variables did not differ among groups. However, trends with level of training were observed in some critical variables. Hemoglobin (Hb) and hematocrit (Hct) conformed to a staircase effect with Group I (14.5 $gm.dl^{-1}$ and 41.3%) lower than Group III (15.1 $gm.dl^{-1}$ and 42.9%). Reticulocyte count was higher and haptoglobin levels lower in Group I (1.35% and 75.7 $gm.dl^{-1}$) than Group III (.99% and 132.9 $gm.dl^{-1}$), with haptoglobin for the high mileage Group (I) in the clinically abnormal range. Since haptoglobin binds free Hb following RBC destruction, these results suggest that intravascular hemolysis occurs in trained male runners. These results may have special significance for astronauts training before long-duration spaceflights, since the further reduction in red blood cells which is reported to occur during spaceflight could be become detrimental to their health and performance.

16. Key Words

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