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Anemia in Female Collegiate Athletes: Association with Hematological Variables, Physical Activity and Nutrition

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Authors' contributions

This work was carried out in collaboration between all authors. Author YY was the chief investigator of the study and analyzed the data. Author MK made the protocol of the study and wrote the manuscript. Authors AN and NM was responsible for collecting participants at Nara Women's University and Tenri University, respectively. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The current study aims to clarify the prevalence of anemia in collegiate athletes in relation to various hematological variables, physical activity and nutrient intake.
Study Design: A cross sectional, retrospective study.
Place and Duration of Study: This study was done at two universities in Nara, Japan, between April and September, 2013.
Methodology: Fifty-eight female athletes and 65 female non-athletes were enrolled. Hematological variables, physical activities based on the definition of the International Physical Activity Questionnaire, and nutrients intake using one week's dietary records were measured.
Results: The prevalence of anemia (<12 g/dl of hemoglobin) and hypoferritinemia (<12 ng/ml of ferritin) was comparable between athlete and non-athlete groups. Reticulocytes percentages or haptoglobin levels were statistically higher or lower respectively, in the athlete group. There was no

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statistical difference in the intake of micronutrients related to erythropoiesis between the two groups. Multiple regression analysis revealed that hemoglobin levels were significantly associated with an irregular menstruation status in both groups, and were associated with the physical activity only in the athlete group.

Conclusion: Although the prevalence of anemia in female collegiate athletes is identical to that of non-athletes, they may be at risk for anemia when their strength of physical activity will increase. Since the signs of hemolysis are more common in the athlete group, a measure of minimizing hemolysis such as the introduction of a pad on the sole of the foot may be considered to prevent the occurrence of overt anemia.

Keywords: Female; adolescent; sports anemia; physical activity; nutrient intake.

ABBREVIATIONS

RBC; red blood cell, RDA; recommended dietary allowance, IPAQ; International Physical Activity Questionnaire, BMI; body mass index, Hb; hemoglobin, Ht; hematocrit, MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC; mean corpuscular hemoglobin in concentration, WBC; white blood cell, FFQ; Food Frequency Questionnaire.

1. INTRODUCTION

Anemia is a well recognized problem among athletes [1,2]. The mechanisms of anemia in athletes, or so-called "sports anemia", are thought to be quite diverse, including iron deficiency caused by either increased demand or loss of iron, intravascular hemolysis, expansion of blood volume, and poor intake of nutrients, particularly those related to red blood cell (RBC) production [3,4]. There have been several reports on the occurrence and characteristics of sports anemia in female athletes. However, these reports are mainly focused on professional and/or top-level athletes. For example, Landahl et al. studied 28 Swedish female soccer players, called up for the national team, and found that 57% had iron deficiency and 29% had iron-deficiency anemia [5]. Among 103 top-level basketball players belonging to national basketball teams, the prevalence of iron-deficiency anemia was higher in female players (14%) than in male players (3%) [6]. Hemolysis by footstrike also causes anemia in long-distance runners [7]. In contrast, the results of studies in non-professional athletes or non-athletes are controversial. In the study of Italian non-professional female athletes, the prevalence of both anemia and iron deficiency was comparable between athletes and sedentary controls [8]. Woolf et al. demonstrated lower serum ferritin concentration and mean cell hemoglobin in women with highly purposeful physical activity than in sedentary women [9].

Nutritional status has a pivotal effect on the occurrence of iron deficiency and anemia in athletes [10]. Due to an increase in the demand for various nutrients particularly in adolescent athletes, balanced intake of nutrients is essential for preventing sports anemia. For example, Noda et al. reported shortages of minerals (calcium and magnesium) and vitamins (A, B_1, B_2 and C) in comparison with the Japanese recommended dietary allowances (RDAs) in male collegiate soccer players [11]. In contrast, the research by Kim et al. in female Judo athletes demonstrated that calcium and iron intakes were below RDAs, but that vitamins (B_1 and B_2), protein, and energy intakes were sufficient. They failed to find any association with the occurrence of anemia [12]. However, these studies had limited numbers of subjects and/or lacked objective evaluation of physical activity. These inadequacies led us to examine the occurrence of anemia in female athletes belonging to university sports clubs. In the present study, we evaluated physical activity objectively using the International Physical Activity Questionnaire (IPAQ) [13] as well as the study of nutrient intakes.

2. MATERIALS AND METHODS

2.1 Study Population

One hundred forty-one female students at two universities in Nara Prefecture were initially enrolled. Considering the loss of red blood cells and iron after menstruation, 16 students were excluded since the date of the blood examination was within 10 days after the end of menstruation. Two students were also excluded since the volume of drawn blood was not sufficient for the examination. None of the students had any acute or chronic disease at the time of examination. Among 123 students finally enrolled in the present study, fifty-eight students were members of athletic clubs at the university. These sports clubs included basketball (25), lacrosse (10), Aikido (8), track and field (7), Naginata (5), and soccer (3). The number of participants belonging to each sport club is shown in parentheses. This study was done between April and September, 2013.

2.2 Questionnaire

Before blood examination, a questionnaire was distributed to all subjects. Data gathered by the questionnaire included age, height, weight, menstruation period, history of chronic diseases, and present health status including taking medicine. Based on the height and weight data obtained by the questionnaire, body mass index (BMI) was calculated by the formula of weight (kg) divided by the square of height (m).

2.3 Blood Examination

Venous blood samples were drawn under the fasting condition in the morning. Examination items included RBC counts, hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin in concentration (MCHC), reticulocyte count, white blood cell (WBC) count, platelet count, iron, zinc, ferritin, haptoglobin and erythropoietin. The assays were done by Mitsubishi Chemical Medience Corporation (Tokyo, Japan). The reference values of these variables were established by the company using more than several hundred healthy adult volunteers.

2.4 Measurement of Physical Activity

To evaluate physical activity, we used the IPAQ, short version, whose reliability and validity has been established by previous research. The physical activity of each subject was calculated by the short version of IPAQ providing information on the time spent walking, in vigorous- and moderate-intensity activity and in sedentary activity during the usual one week within the previous two months. The scores of 3.3 METs for walking, 4 METs for the moderate-intensity activity, and 8 METs for the vigorous-intensity activity were given. The total physical activity in a given subject was estimated as duration × frequency/week × METs intensity scores [13].

2.5 Measurement of Nutrients Intake

We asked the participants to keep dietary records for any one week within the previous two months. The estimations of nutrient intakes were done using Excel Eiyoukun Ver. 5.0 (Kenpakusha FFQ g Ver 3.0, Tokyo, Japan). This software was developed based on Standard Tables of Food Composition in Japan, Fifth Revised and Enlarged Edition (Ministry of Education, Culture, Sports, Science and Technology) and Dietary Reference Intakes for Japanese, 2010 (Health, Labor and Welfare Ministry) [14]. The Food Frequency Questionnaire Based on Food Groups (FFQ) evaluating daily diet contents with questions about 29 food groups and 10 cooking methods was administered. The validity of the FFQ has been established in comparison with the diet records of consecutive 7 days [15].

2.6 Analysis

The differences in the continuous variables between athlete and non-athlete groups were done by the Mann-Whitney U test. The differences in the categorical variables between the two groups were analyzed by the Chi-square test. Multiple regression analysis was used to assess the relation between Hb levels and various variables including age, BMI, menstruation status, physical activity and nutrition intakes. *P* values less than 0.05 were considered significant. All statistical analyses were carried out on a personal computer using Excel Statistics version 2010 software (Tokyo, Japan).

3. RESULTS

3.1 Basic Characteristics of the Study Population

As indicated in Table 1. age, menstruation status, and high-sensitivity CRP levels were comparable among athlete and non-athlete groups. Based on the data of Japanese women, the menstrual cycle between 28 and 35 days is defined as “regular”, and that of less than 28 days or more than 35 days is defined as “irregular” [16]. On the other hand, the athlete group showed significantly greater height, weight, and BMI than the non-athlete group.

3.2 Comparison of Hematological and Other Biochemical Variables between Athlete and Non-Athlete Groups

Table 2.shows that the prevalence of anemia (hemoglobin levels less than 12 g/dl) and hypo-ferritinemia (ferritin levels less than 12 ng/ml) [17] was slightly higher in the athlete group, but the differences were not statistically significant. Hb, MCV, MCHC, serum iron, serum zinc, erythropoietin and ferritin levels were comparable among the two groups. In contrast, the athlete group showed significantly higher reticulocyte percentages and lower haptoglobin levels than those in the non-athlete group. RBCs were also marginally lower in the athlete group.

3.3 Comparison of Physical Activity and Nutrient Intake between Athlete and Non-Athlete Groups

The physical activity in the athlete group was significantly higher than that in the non-athlete group as judged by the METs and the total physical activity grading. The intake of energy and micronutrients (iron, zinc, copper, vitamin B_6, vitamin B_{12}, vitamin C and folic acid) that are thought to be associated with the occurrence of anemia did not show any significant differences among the two groups (Table 3).

Table 1. Basic characteristics of subjects

Variables	Athletes	non-Athletes	*p* values
Number of subjects	58 (47.2)*	65 (52.8)	
Age (year)	20 [18-22]**	21 [18-26]	0.35†
Height (cm)	159 [147-174]	156 [145-173]	0.0097†
Weight (kg)	51 [39-74]	48 [33-65]	< 0.01‡
Body mass index (kg/m2)	20.9 [17.8-25.6]	20.1 [14.3-24.8]	< 0.001‡
Menstruation status			
regular§	18 (31.0)	16 (24.6)	0.43†
irregular	40 (69.0)	49 (75.2)	
High sensitivity CRP (mg/dl)			
< 0.01	29 (50.0)	32 (49.3)	0.63†
0.01-0.05	24 (41.4)	30 (46.1)	
> 0.05	5 (8.6)	3 (4.6)	

** Data in parentheses indicate percentages.;** Medians and ranges in square brackets are shown.;† Chi-square test ‡ Mann-Whitney U test; § Regular menstrual cycle indicates between 25 and 38 days as indicated in Ref. 16.*

Table 2. Comparison of hematological and other biochemical variables between athletes and non-athletes

Variables	Athletes (n=58)	non-Athletes (n=65)	*p* values	Reference value(range)
Hb(g/dl)	13.0[8.1-14.5]	13.2[8.4-14.9]**	0.28‡	11.5-15.0
RBC(x10000/μl)	419[374-525]	439[380-541]	0.067‡	380-500
MCV(fl)	92[77-100]	93[63-104]	0.29‡	85-102
MCHC (%)	32.1[28.1-33.6]	32.2[27.1-33.7]	0.67‡	30.2-35.1
Reticulocyte (‰)	11[6-27]	9[4-25]	0.047‡	4-19
WBC(μl)	5950[3000-9100]	5900[1300-10700]	0.84‡	3300-9000
Platelet (x10000/μl)	24.9[9.6-33.2]	24.4[16.1-35.9]	0.85‡	14.0-34.1
Ferritin(ng/ml)	31.4[2.7-143]	28.5[1.7-239]	0.57‡	14.0-64.2
Erythropoietin (mIU/ml)	18.2[8.7-72.6]	18.6[9.8-281]	0.15‡	9.1-32.8
Haptoglobin (mg/dl)	55[10-145]	70[10-221]	0.041‡	25-218
Iron (μg/dl)	90[30-234]	88[18-202]	0.93‡	40-180
Zinc (μg /dl)	87[53-110]	87[63-103]	0.95‡	64-110
Anemia positivity§	9/58 (15.5)	6/65 (9.2)*	0.43†	
Hypo-ferritinemia positivity§	14/58 (24.1)	13/65 (20.0)	0.58†	

Hb=hemoglobin, RBC=red blood cell, MCV=mean corpuscular volume, MCHC=mean corpuscular hemoglobin concentration, WBC=white blood cell; Data in parenthesis indicate percentages.;** Medians and ranges in square brackets are shown.;† Chi-square test ‡ Mann-Whitney U test; § Anemia and hypo-ferritinemia are defined as < 12 g/dl Hb and < 12 ng/ml ferritin, respectively.*

3.4 Multiple Regression Analysis of Hb Levels in Association with Anthropometric Factors, Physical Activity and Nutrient Intake in the Athlete and Non-Athlete Groups

An irregular menstrual status was negatively associated with Hb levels in both groups. METs showed a statistically significant negative association with Hb levels only in the athlete group. As for nutrient intakes, zinc intake was significantly associated only in the non-athlete group (Table 4).

4. DISCUSSION

Anemia is one of the deteriorative problems in athletes, especially in females [1,2]. This type of anemia has been well documented in professional or top-level athletes [5,6,18-20]. However, reports on sports anemia in non-professional or ordinary athletes are still limited. Anemia impairs oxygen transport, thereby inducing fatigue and loss of concentration. Long-term anemia also has an effect on cardiac function, cognitive performance and growth in children [17]. Therefore, early detection of sports anemia and intervention, if necessary, are potentially important.

The results from previous studies on the prevalence of anemia in female athletes, mostly young women or adolescents, are quite diverse. The probable reasons are differences in age, race, size of the study cohort, strength of physical activity, and the definition of anemia. As for the definition of anemia and iron depletion status, several studies have adopted the WHO standard, i.e. Hb<12 g/dl or ferritin <12 ng/ml [6,8,20]. However, other studies have adopted higher cut-off values, for examples Hb<14 g/dl and ferritin <22 ng/ml [19] or ferritin <30 ng/ml [18]. Consequently, these studies reported higher prevalence of sports anemia or iron depletion than those using the WHO criteria. Other investigators compared absolute values of Hb or ferritin among athletes and sedentary controls [9,21]. Therefore, in the present study, we used two measures (the WHO standard and direct comparison of absolute values) for evaluating the occurrence of anemia or iron-depletion status.

Regardless of the definition of anemia, previous studies on professional or top-level female athletes have shown prevalence of approximately 15-38% [5,6,19,20]. The prevalence of iron depletion have been in the range of 18-57% [5,6,19,22]. Such prevalence in female athletes is significantly higher than the values, assessed in male athletes [18].However, a recent investigation of females at a Swedish senior high school indicated that the prevalence of anemia was not significantly different among top-level athletes (5/57;8.6%) and non-athletes (3/92;3.3%) [23]. Although studies of non-professional female athletes are limited, Santolo et al. demonstrated that the prevalence of anemia or hypoferritinemia tended to be higher in athletes than in a sedentary group, but the difference was not significantly different [8]. However, they did not calculate the exact physical activity in the study cohort.

Table 3. Comparison of physical activity and nutrient intake between athletes and non-athletes

Variables	Athletes (n=58)	non-Athletes (n=65)	*p* values
	Physical activity		
METs	5870 [452-12700]**	693 [66-5190]	< 0.001‡
Grade of total physical activity			< 0.001†
Low	7 (12.1)*	33 (50.8)	
Moderate	8 (13.8)	27 (41.5)	
High	43 (74.1)	5 (7.7)	
	Nutrient intake(/day)		
Energy (kcal/kg)	32.3 [16.8-51.6]	32.6 [18.3-51.7]	0.86‡
Iron (mg)	5.9 [2.8-11.4]	5.5 [3.0-10.1]	0.39‡
Zinc (mg)	6.6 [3.3-13.1]	6.4 [3.4-10.1]	0.54‡
Copper (mg)	0.81 [0.38-1.68]	0.80 [0.39-1.32]	0.84‡
Vitamin B6 (mg)	0.76 [0.36-1.68]	0.77 [0.36-1.50]	0.47‡
Vitamin B12 (mg)	3.7 [0.83-11.7]	3.6 [0.65-12.6]	0.73‡
Vitamin C (mg)	51 [17-155]	68 [19-240]	0.091‡
Folic acid (μg)	180 [63-462]	213 [76-523]	0.095‡

Data in parentheses indicate percentages.;Medians and ranges in square brackets are shown.;† Chi-square test ‡ Mann-Whitney U test*

Table 4. Comparison of multiple regression analysis in athletes and non-athletes groups

Variables	Athletes (n=58)	*p* values	non-Athletes (n=65)	*p* values
Age (years)	0.077 [-0.22~0.38]*	0.61	-0.058 [-0.023~0.11]	0.49
Body mass index(kg/m2)	0.12 [-0.095~0.33]	0.27	0.081 [-0.13~0.29]	0.44
Menstruation status**	-0.73 [-1.38~-0.09]	0.024	-0.86 [-1.52~-0.19]	0.013
METs	-0.071 [-0.044~-0.098]	0.048	0.027 [-0.72~0.77]	0.94
Energy (kcal/kg/day)	0.047 [-0.66~0.75]	0.89	-0.026 [-0.84~0.79]	0.95
Iron (mg/day)	-0.11 [-0.54~0.34]	0.65	-0.15 [-0.59~0.29]	0.51
Zinc (mg/day)	-0.037 [-0.095~0.021]	0.44	0.042 [0.019~0.063]	0.027
Vitamin B6 (mg/day)	0.14 [-0.42~0.68]	0.67	0.08 [-0.22~0.38]	0.36
Vitamin B12 (mg/day)	-0.18 [-0.39~0.029]	0.091	0.014 [-0.22~0.25]	0.91
Vitamin C (mg/day)	0.002 [-0.21~0.02]	0.98	-0.011 [-0.033~0.013]	0.39
Folic acid (□g/day)	-0.057 [-0.017~0.052]	0.29	-0.054 [-0.016~0.055]	0.32

*Multiple regression analysis was performed by hemoglobin as an objective variable and several variables listed in the table as explanatory variables;*Correlation coefficients and their 95% CI in brackets are shown; **Subjects with a regular menstrual cycle were allocated "0" and those with an irregular cycle were allocated "1".*

Nutritional assessment is essential for considering the etiology of sports anemia. Weight et al. [10] presented that female athletes, but not male athletes, failed to meet the RDA with regard to iron (< 15 mg/day) and folate (<100 mg/day). Therefore, these authors speculated an iron-poor diet was a factor in iron deficiency, especially in female athletes. In the assessment of food habits in 33 high-level adolescent soccer players, in 48% the iron deficiency without anemia was found in spite of sufficient provision of dietary iron [24]. A recent Japanese study of 31 male collegiate soccer players demonstrated that the mean intake of iron was higher than the Japanese RDAs at the same age, but two athletes experienced iron depletion without anemia. They also showed that intakes of calcium, magnesium and vitamins A, B_1, B_2, C were lower than the Japanese RDAs [11].

In the current study, the prevalence of anemia and hypoferritinemia was comparable between the athlete and non-athlete group, suggesting that sports activity at the level of university sports clubs may not cause overt anemia. In contrast, absolute Hb levels were negatively correlated with the intensity of physical activity in the athlete group. Furthermore, significant hemolysis as defined by higher reticulocyte counts and lower haptoglobin [25], were found in the athlete group. Four athletes belonging to lacrosse (2), Basketball (1) and track and field (1) Clubs showed strong signs of hemolysis, i.e. haptoglobin levels less than 10 mg/dl. We speculated the severe foot-strike during exercise as the reason for this phenomenon [7]. In nutritional assessment, the intakes of energy and micronutrients that are thought to be related to erythropoiesis were comparable among the two groups. Among various nutrients, zinc intake was only found to be positively correlated with Hb levels in the non-athlete group. Although a role of zinc intake in the occurrence of anemia is well known, the exact interpretation of this result remains undetermined.

There are several limitations in the current study. First, the enrolled subjects belonged to quite diverse sports clubs. Schumacher et al. demonstrated that endurance-training sports may cause reduction of Hb and RBC more frequently than strength- or mixed-training sports [26]. However, no significant differences in hematological variables such as Hb, RBC, ferritin and transferrin among different sports groups were found in 84 Serbian female top-level athletes [20]. Second, we asked the subjects to record their physical activity during a typical week as done in the original version [27]. We are not sure whether the investigation of any one week is sufficient for evaluating the physical activity in a given subject. Finally, in order to check the iron status more accurately, other hematological variables such as transferrin and soluble transferrin receptor may need to be examined [28]. On the other hand, our present study has the following strengths: (i) Enrollment of more than 100 study subjects, (ii) Objective assessment of physical activity, and (iii) Simultaneous implementation of nutritional assessment. However, we failed to detect any evidence of an increase of anemia or iron depletion due to sports activity at the university level.

5. CONCLUSION

The main findings of the current study in collegiate female athletes were three-folds; (i) The prevalence of anemia or hypo-ferritinemia based on the definition of WHO was comparable between the athlete and non-athlete group, (ii) Absolute Hb levels were found to decrease with an increase of physical activity only in the athlete group and (iii) Significant hemolysis was observed in the athlete group. These results altogether indicate the possibility that collegiate female athletes may be at risk for anemia, even if they have not been diagnosed with such a disorder at present. Therefore, we recommend the introduction of a pad on the sole of the foot to minimize the occurrence of hemolysis due to foot-strike. In addition, more intakes of iron, folate and vitamin B_6 should be recommended to all female students for prevention of nutrient-deficient anemia, since their intakes were found to be below RDAs.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. We obtained written informed consent from all subjects beforehand. This study was approved by the ethical committee for epidemiological study at Nara Women's University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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