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Anemia among freshmen of a faculty of physical education
― Risk factors among female students ―

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ABSTRACT

Purpose: The purpose of this study was to examine the frequency of anemia and to explore risk factors related to anemia among female students at admission. **Methods:** Participants were 2,361 freshmen in a faculty of physical education from 2008 to 2011. Risk factors for anemia were examined in 577 female students out of 2,361 students in total. Rates of anemia and iron storage depletion were examined in both male and female students, and risk factors for anemia were examined in a multiple logistic regression model. **Results:** The rate of anemia (less than 12 g/dl of hemoglobin) was 0.1% among males, 6.1% among females, and 1.7% among both sexes. The rate of anemia (less than 13 g/dl of hemoglobin) was 0.6% among males, 31.0% among females, and 8.0% among both sexes. The rate of ion storage depletion was 2.9% among males, 31.0% among females, and 25.3% among both sexes. A multiple logistic regression model indicated that sFe levels, TIBC, and CK levels were significant factors related to anemia among female students. **Conclusion:** The rate of anemia was 0.6% among male students (defined as hemoglobin <13 g/dl) and 6.1% among female students (defined as hemoglobin <12 g/dl) at admission. Results indicated that risk factors related to anemia in female students were an iron deficiency and elevated CK due to excessive exercise.

Key words; medical checkup, anemia, serum iron, ferritin, athlete

Background

Anemia is reduced hemoglobin and diminished sports performance due to decreased oxygen supply[1-3]. Anemia occurs as a result of decreased or increased destruction or loss of red blood cells ; the causes of iron deficiency are increased demand for iron, increased iron loss, and decreased iron intake or absorption[4]. Anemia is usually defined as hemoglobin less than 13 g/dl in males and as hemoglobin less than 12 g/dl in females by WHO[5].

Iron deficiency is common in anemia[6-8]. Iron is an essential element, and total iron in serum is

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referred to as serum iron (sFe). Iron is stored in organs of the reticuloendothelial system such as the liver and spleen and is measured in the form of ferritin. Hepcidin serves as a key regulator of the entry of iron into the circulation. Iron storage depletion is reported to potentially accelerate anemia although it does not directly influence sports performance[3].

Freshmen undergo a legally required medical checkup upon admission to a college in Japan. The freshmen of one faculty of physical education have undergone blood tests at the same time as a medical checkup to obtain more information on their own health since 2008.

Purpose

The purpose of this study was to examine the frequency of anemia and to examine risk factors related to anemia among female students at admission.

Methods

1. Participants

Participants were 2,361 of freshmen of a faculty of physical education from 2008 to 2011. Risk factors for anemia were examined in 577 female students out of 2,361 students in total.

All students belonged to one of four departments: department A, which fosters leaders in physical education and top athletes (984 students), department B, which fosters leaders and top athletes in Japanese martial arts (401 students), department C, which fosters teachers of physical education to children (331 students), and department D, which fosters emergency medical technicians (645 students). Students belonging to department D exercised in the same amount as ordinary students did.

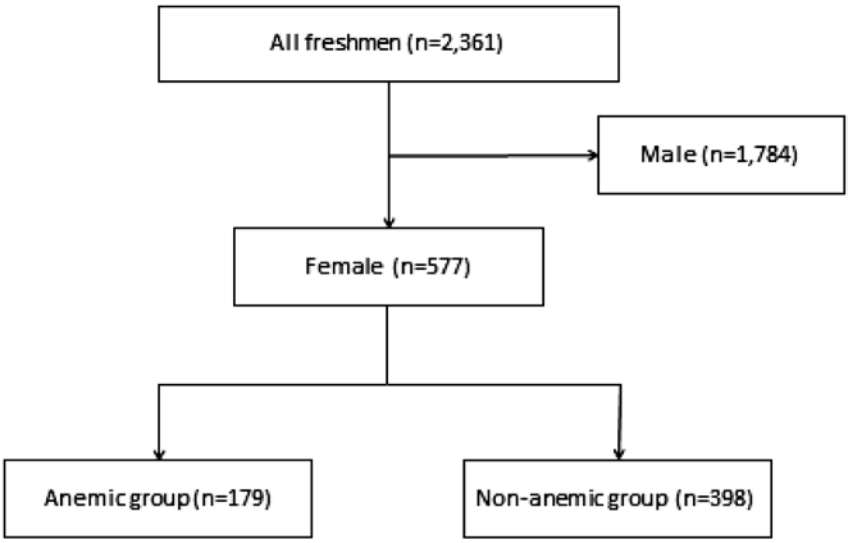


Figure 1. Flow chart of material selection

2．Definition

Anemia was defined as less than 13 g/dl of hemoglobin in female students when analyzed using a multiple regression model.

3．Medical checkup and blood tests

All 2,361 students underwent a health check and blood tests during the first week of admission in April. Height, body weight, and body mass index (BMI) were measured, and students were asked which department they belonged to. Blood tests involved a complete blood count (CBC) and serum biochemistry. CBC involved a red blood cell count, hemoglobin levels, hematocrit levels, a white blood cell count, and a platelet count. Serum biochemistry involved total protein levels, total bilirubin levels, AST levels, ALT levels, lactate dehydrogenase (LDH) levels, creatinine phosphokinase (CK) levels, triglyceride (TG) levels, HDL-cholesterol levels, blood urea nitrogen (BUN) levels, uric acid levels, sFe, ferritin levels, and total iron binding capacity (TIBC). Female participants were divided into two groups, an anemic group (hemoglobin <13 g/dl) and a non-anemic group (hemoglobin >=13 g/dl). Iron storage depletion in terms of sFe and ferritin was plotted for the anemic and non-anemic groups.

4．Statistical analysis

The characteristics of the two groups were examined and compared using a chi-square test and a student t-test.

A multiple logistic model was used to explore risk factors related to the anemia among female participants. The response variable was anemia, and explanatory variables were the 26 items in Table 3. The open source statistical software R (version 3.2.2) was used to perform analyses.

5．Ethical approval

This study was approved by the Research Ethics Committee of the Faculty of Physical Education (No. 24-07).

Results

1．Rates of anemia and iron storage depletion

Table 1 shows the characteristics of participants in the anemic group and in the non-anemic group. The rate of anemia (less than 12 g/dl of hemoglobin) was 0.1% among males, 6.1% among females, and 1.7% among both sexes. The rate of anemia (less than 13 g/dl of hemoglobin) was 0.6% among males, 31.0% among females, and 8.0% among both sexes. The rate of ion storage depletion was 2.9% among males, 31.0% among females, and 25.3% among both sexes.

2．Risk factors for anemia among female students

Characteristics of the anemic and non-anemic groups are shown in Table 2. The level of sFe and ferritin were plotted for anemic and non-anemic female students in Figure 2.

Multiple logistic regression indicated that sFe, TIBC, and CK were significantly related to anemia (Table 3). Risk factors for anemia did not differ significantly among students in the four departments (Table 3).

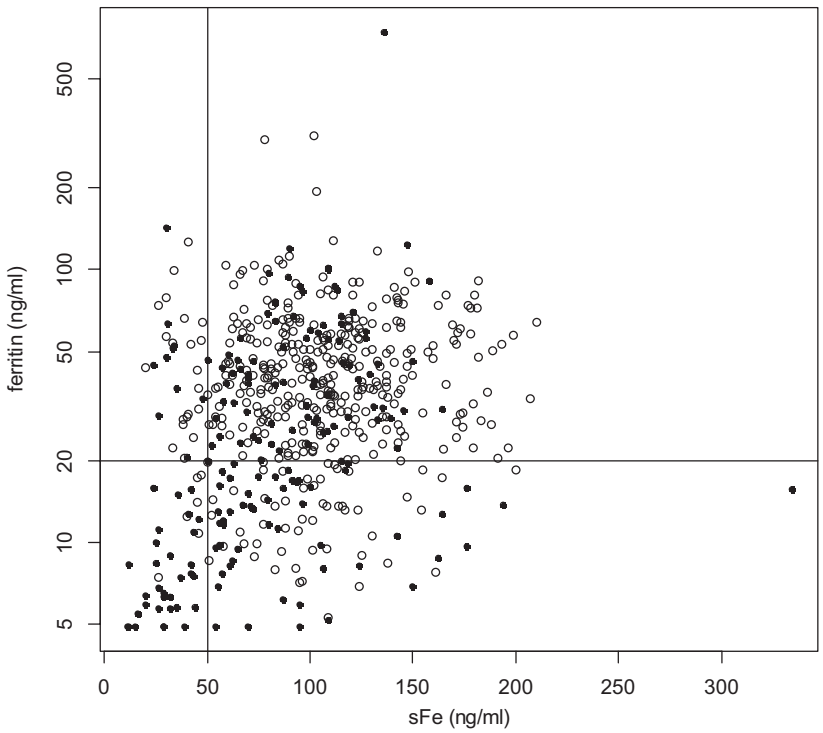
Discussion

The rate of anemia was 0.6% among male students and 6.6% among female students when anemia was defined as hemoglobin less than 13 g/dl in male athletes and as hemoglobin less than 12 g/dl in

Table 1. Frequency of anemia and iron storage depletion by sex and hemoglobin

Sex	total n	Hemoglobin <12 g/dl n(%)	hemoglobin <13 g/dl n (%)	iron storage depletion n (%)
Male	1,784	2(0.1)	11 (0.6)	56 (2.9)
Female	577	38(6.6)	179(31.0)	146(25.3)
Total	2,361	40(1.7)	190 (8.0)	202 (9.6)

Figure 2. Plot of sFe and ferritin



The vertical line indicated the lower limit of sFe (=50 ng/ml) and the horizontal line indicated the lower limit of ferritin (=20 ng/ml). Closed circles mean the anemic group and open circles mean non-anemic group.

Table 2. Characteristics of both groups

variable		Non-anemic group		Anemic group		p-value
		n=398		n=179		
	unit	Mean	SD	Mean	SD	
department A		107 (26.9%)		57 (31.8%)		0.39040
B		38 (9.5%)		21 (11.7%)		
C		114 (28.6%)		42 (23.5%)		
D		139 (34.9%)		59 (33.0%)		
age	years old	18.1	0.5	18.1	0.8	0.24035
height	cm	160.0	5.4	160.5	5.1	0.15116
weight	kg	56.7	9.4	57.0	7.8	0.29409
BMI	kg/m2	22.1	3.0	22.0	2.5	0.31293
total bilirubin	mg/dl	0.7	0.3	0.6	0.3	0.0314
GOT	IU/L	22.3	10.2	22.3	8.5	0.31738
GPT	IU/L	16.1	10.8	15.4	7.8	0.21435
LDH	IU/L	214.2	50.1	217.4	53.7	0.21911
r-GPT	IU/L	16.3	7.4	15.6	5.2	0.13026
CK	IU/L	197.7	331.7	261.2	346.0	0.06051
amylase	IU/L	82.3	28.9	85.4	27.4	0.12939
blood sugar	mg/dl	89.5	8.3	89.1	6.2	0.24202
HDL cholesterol	mg/dl	70.5	14.5	69.7	14.3	0.23116
triglyceride	mg/dl	63.4	39.2	60.6	35.8	0.1913
uric acid	mg/dl	4.5	1.1	4.6	1.2	0.15663
BUN	mg/dl	13.8	3.2	14.0	3.8	0.23083
white blood cells	/mm^3	6706	1485	6214	1336	0.02117
platelet count	10^4/mm^3	26.4	4.9	26.9	5.1	0.16448
sFe	μg/dl	104.5	37.5	80.3	43.4	0.00695
TIBC	μg/dl	356.3	41.9	372.1	58.0	0.02251
RBC	10^4/mm^3	465.1	23.9	434.7	27.4	0.0018
hemoglobin	g/dl	13.8	0.6	12.2	0.8	0.00048
hematocrit	%	42.7	1.8	38.8	2.0	0.00061

female athletes. However, 31.0% of female students had hemoglobin lower than 13.0 g/dl, and 25.3% of female students had iron storage depletion as indicated by ferritin measurements. The normal range of hemoglobin is calculated based on a healthy population. Iron storage depletion was 31.0% among female students, placing them at potential risk of developing anemia due to excessive exercise. The progression from iron storage depletion to iron deficiency can be divided into three stages: a negative iron balance, iron deficient erythropoiesis, and iron deficiency anemia[4]. The ferritin level was over 200 ng/ml in four students (Figure 2), indicating excessive intake of iron supplements.

Since female students in three departments were expected engage in more exercise, the anemic group was defined as having hemoglobin <13 g/dl, which is the same level as in male students. This definition was presumably in line with the physical activity of female students.

Table 3. Result of multiple logistic regression

Variable	unit	Estimate	Std. Error	z value	Pr(>|z|)	
age	year	0.0463836	0.1473707	0.315	0.75296	
height	cm	0.0268371	0.0192106	1.397	0.16242	
BMI		-0.0098273	0.0380145	-0.259	0.79601	
department	A	0.4905958	0.2790361	1.758	0.07872	.
	B	0.4756460	0.4110691	1.157	0.24723	
	C	0.1035513	0.2651766	0.390	0.69617	
LDH	IU/L	0.0001868	0.0021847	0.086	0.93185	
r-GPT	IU/L	-0.0244912	0.0207827	-1.178	0.23862	
sFe	mg/L	-0.0162382	0.0029864	-5.437	0.00194	**
CK	IU/L	0.7470623	0.2591189	2.883	0.00394	**
ferritin	ng/ml	-0.0027168	0.0038103	-0.713	0.47584	
TIBC	ng/ml	0.0068836	0.0024447	2.816	0.00487	**
BUN	mg/dl	0.0104843	0.0314470	0.333	0.73884	
blood sugar	mg/dl	-0.0187118	0.0138929	-1.347	0.17802	
total bilirubin	mg/dl	-0.2464248	0.3917022	-0.629	0.52928	
amylase	IU/L	0.0024044	0.0034907	0.689	0.49096	
HDL cholesterol	mg/dl	-0.0115439	0.0077005	-1.499	0.13384	
triglyceride	mg/dl	-0.0044738	0.0029549	-1.514	0.13002	
uric acid	mg/dl	0.1496179	0.1032772	1.449	0.14742	
platelet count	10^4/L	0.0045391	0.0207806	0.218	0.82709	

** p<0.01

In female students, sFe, TIBC, and CK were significant factors related to anemia. A decrease in sFe and an increase in TIBC coincided with iron deficiency anemia in this study. The level of sFe represents the amount of circulating iron bound to transferrin, and the TIBC is an indirect measure of circulating transferrin[4]. Iron deficiency needs to be prevented and treated in female students. The Ministry of Health, Labor, and Welfare recommends a daily iron intake of 10.5 mg for menstruating females and 6.0 mg for non-menstruating females ages 18-29 years and for pregnant and lactating females[9], but that recommendation does not mention female athletes. Dietary iron exists in heme and non-heme forms. Heme iron is present with hemoglobin or myoglobin molecules from animal sources, and non-heme iron is bound to other food components, usually in ferric form[3].

Elevated CK was also a factor for anemia. Creatine phosphate is formed from ATP and creatine when muscles relax and demands for ATP decrease. Creatine phosphate constitutes a major energy reserve in muscle. Creatine kinase catalyzes the phosphorylation of creatine and is a muscle-specific enzyme that is clinically useful in detecting acute or chronic diseases of the muscles and brain[10]. There are three different isoenzymes of CK: CK-BB, CK-MB, and CK-MM. The isozymes of CK were not measured in the current study. That said, none of the students had active cerebral or cardiac disease during the medical checkup, and elevated CK was presumably due to excessive exercise.

Mechanical hemolytic anemia is due to mechanically induced damage to red blood cells, results in hemoglobinuria and is observed in prolonged marching, marathon running (footstrike hemolysis),

barefoot ritual dancing and even in intense playing bongo[3]. LDH is abundant in red blood cells, and is often used as a marker of hemolysis. The multiple logistic model showed no significance related with the anemia in LDH and the divisions in this study although there were students of long-distance runners in the department A and those of kendo players who played with bare feet on the wooden floor in the department B.

The current study had several limitations. First, this study was a cross-sectional study, so it cannot determine the cause of anemia. Second, this study did not examine the type, intensity, or interval of sports the participants engaged in, and such aspects might influence the level of CK. Third, this study did not ask students about their use of iron supplements[3].

Conclusion

The rate of anemia was 0.6% among male students (defined as hemoglobin <13g/dl) and 6.1% among female students (defined as hemoglobin <12 g/dl) at admission to a faculty of physical education. Risk factors related to anemia were iron deficiency and increased creatine kinase due to excessive exercise in female students.

Competing interests

All authors of this manuscript declare that they have no conflict of interest.

Authors' contributions

I. Kuboyama helped with the study concept and design. A. Maki helped to collect data. N. Nagai helped to perform statistical analysis and interpret data. N. Nagai and I. Kuboyama helped to draft the manuscript. All authors read and approved the final manuscript.

References

1) Kathryn E Mylhre, Bryant J Webber, Thomas L Cropper, Juste N tchandja, Dale M Ahrendt, Christopher A Dillon, Roy W Hass, Samantha L Guy, Mary T Pawlak, Suzan P Federinko. Prevalence and Impact of Anemia on Basic Trainees in the US Air Force. Sports Medicine 2：23, 2016.
2) James O Plumb, James M. Otto, Michael PW, Grocott. 'Blood doping' from Armstrong to prehabilitation：manipulation of blood to improve performance in athletes and physiological reserve in patients. ExremePhysiol Med 5：5, 2015.
3) Ieva Alaunyte, Valentina Stojceska, Andrew Plunkett. Iron and the female athlete：a review of dietary treatment methods for improving iron status and exercise performance. jissn 12：38, 2015.
4) Kasper, Fauci, Hauser, Longo, Jameson, Loscalzo. Harrison's principles of internal medicine 19th edition. 625-658, McGraw Hill education, New York, 2015.
5) Organization WH. Worldwide prevalence of anaemia 1993-2005. WHO global database on anaemia 2008.
6) Georgie Bruinvels, Richard Burden, Nicola Brown, Toy Richards, Charles Pedlar. The prevalence and impact of heavy menstrual bleeding (Mmenorrhagia) in elite and non-elite athlets. PLOS one DOI：10.137, 2016.
7) Joseph J. Knapik, Ryan A. Steelman, Sally S. Hoedebecke, Krista G. Austin, Emily K. Farina, and Harris R. Lieberman. Prevalence of Dietary Supplement Use by Athletes：Systematic Review and Meta-Analysis. Sports Med. 46：103–123, 2016.
8) Charles R Pedlar, Carlo Brugnara, Georgie Bruinvels, Richard Burden. Iron balance and iron supplementation for the female athlete：A practical approach. European Journal of Sport science, 2017. doi.

org /10.1080/17461391.2017.1416178.
9） Ministry of Health, Labour and Welfare. Overview of Dietary Reference Intakes for Japanese (2015). P35, 2015. http://www.mhlw.go.jp/file/06-Seisakujouhou-10900000-Kenkoukyoku/Overview.pdf
10） Robert K Murray, David A Bender, Kathleen M Botham, PeterJ Kennelly, Victor W Rodwell, P Anthony Weil. Harper's Illustrated Biochemistry 29th edition, McGraw Hillm Medical, NewYork, 2012.