〈Brief Note〉

Variations in iron status linked to menstrual cycles among Japanese female athletes

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Summary Iron deficiency anemia is a prevalent nutritional insufficiency. Premenopausal women lose iron via menstrual bleeding that could affect iron status, but the influence of the menstrual cycle on iron status is controversial. A model has recently been proposed to explain serum iron and transferrin saturation rebound at the end of menses that stabilizes during the luteal phase. Here, we aimed to determine the iron status of four healthy, Japanese, female collegiate cyclists during menstrual cycles during the off-season. We monitored iron status at menses, and the mid follicular and luteal phases over two normal menstrual cycles. The influence of the menstrual phase on iron status was assessed using generalized estimating equation models. None of the women had anemia at entry into the study, but all of them developed iron insufficiency at least once over the two menstrual cycles. The estimated marginal means of iron parameters showed menstrual-cycle associated fluctuations in red blood cell counts, and levels of hemoglobin, hematocrit, and serum ferritin that rebounded after menses and stabilized during the luteal phase. Iron parameters varied in female collegiate cyclists, putting them at risk for iron deficiency. Therefore, regular monitoring linked to the menstrual cycle and effective iron supplementation might be necessary for such athletes.

Key words: ferritin, transferrin saturation, hemoglobin, menses, anemia

1. Introduction

The prevalence of iron deficiency anemia is 22.7% among women of reproductive age in industrialized countries[1]. As iron deficiency impairs efficient oxygen delivery, it negatively affects exercise performance[2]. Therefore, the iron status of

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female athletes with or without anemia should be regularly monitored[3].

Premenopausal women lose 10–40 mg of iron via menstrual bleeding per cycle, which might affect iron status[4]; however, the influence of the menstrual cycle on iron status is controversial. The menstrual cycle essentially comprises a follicular phase including menses and a luteal phase[5]. A previous study found that mean values of hemoglobin (Hgb), transferrin saturation (TfSat), and serum ferritin (SF) are minimal during menses and maximal during the luteal and late luteal phases (10–16 and 17–30 days after menstruation, respectively)[6]. Another study that examined the iron status of iron-depleted but non-anemic women for 15 non-consecutive days during a five-week period found no systemic changes in Hgb, SF, and serum transferrin receptors[7]. Lainé et al. recently noted that hepcidin and iron-status parameters decreased during menses, then increased at mid-cycle before stabilizing during the luteal phase[8]. They provided a model that explained serum iron and TfSat rebound after the end of menses that stabilizes during the second half of the cycle[9].

Here, we investigated the iron status of healthy Japanese female collegiate cyclists during two menstrual cycles in the off-season, with or without iron supplementation.

2. Materials and Methods

2.1 Participants

Four healthy female collegiate cyclists with normal menstrual cycles participated in this study during the off-season between October and December 2017. Table 1 shows the age, height, weight and usual menstrual cycles of the participants. The purpose, methods, potential results, and review of the trial protocol, as well as the protection of personal information, potential benefits, and disadvantages of participating in the trial were explained to each athlete. All understood that participation was voluntary and that they could withdraw at any time, and they provided written, informed consent to participate.

The Ethics Committee of Juntendo University Graduate School of Sports and Health Sciences approved the protocol (Approval #28-113), which was implemented according to the Declaration of Helsinki.

2.2 Study design

The iron status of the participants was monitored at a clinic in the morning 2, 10 and 22 days (± 4 days) after menstrual bleeding started. From the onset of the second menstrual bleed until the last measurement, they received supplements containing 2.5 mg/day of iron, which satisfied the Japanese recommended daily allowance (RDA) of 10.5 mg/day of iron according to a preliminary assessment. The supplement (In Jelly – Multi Mineral; Morinaga & Co. Ltd., Tokyo, Japan) contained 2.5 mg of iron as ferric pyrophosphate, 2.3 mg of zinc as zinc gluconate, 0.2 mg of copper as copper gluconate, 233 mg of calcium as calcium lactate and 35 mg of magnesium as magnesium sulfate per pack (180g, 90 kcal). The supplements did not contain any substances prohibited by the World Anti-Doping Agency. Each participant ingested the supplement daily at any time. The measured iron parameters were red blood cell count (RBC), hematocrit (Hct), Hgb, serum iron, SF, and TfSat. Red blood cell count, Hgb and Hct were assessed using a Sysmex XE-2100 automated hematology analyzer (Sysmex

Table 1 Characteristics of the participants

Participant	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Regular Menstrual cycle (day)
A	20	160	55	21.5	21
B	20	164	65	24.2	33
C	19	155	52	21.6	28
D	19	157	61	24.7	34

Corporation, Hyogo, Japan). Serum ferritin, iron and total iron binding capacity (TIBC) were measured using latex agglutination turbidimetry, direct colorimetry and 2-nitroso-5-(*N*-propyl-*N*-sulfopropylamino) phenol (nitroso-PSAP), respectively, and a JCA-BM8060 automatic analyzer (JEOL Ltd., Tokyo, Japan). Transferrin saturation was calculated as serum iron × 100/TIBC). One participant (participant B) withdrew from the study after the fourth set of measurements.

2.3 Statistical analysis

We assessed the influence of the menstrual phase and iron supplementation on iron status using generalized estimating equations (GEEs). Iron parameters were included as dependent variables and the following predictive variables were included: ID as a subject variable (factor), menstrual cycle (menses, mid follicular, and luteal phases; factor) and the total amount of supplemented iron (2.5 mg/day × number of days from the onset of the second menses; covariate) as a within-subject variable. Data were analyzed using SPSS ver. 19 (Japan IBM, Tokyo, Japan). Statistical significance was set at $P < 0.05$.

3. Results

3.1 Iron status during two menstrual cycles

Figure 1 shows individual variations in iron parameters. Each of RBC, Hgb, Hct and SF seemed to peak at ~ 10 days after the onset of menstrual bleeding, especially during the first menstrual cycle. In contrast, serum iron and TfSat peaked during the luteal phase.

Iron supplementation (2.5 mg/day × number of days) from onset of the second cycle affected the RBC, as well as Hgb and Hct levels during the last luteal phase of the second menstrual cycle.

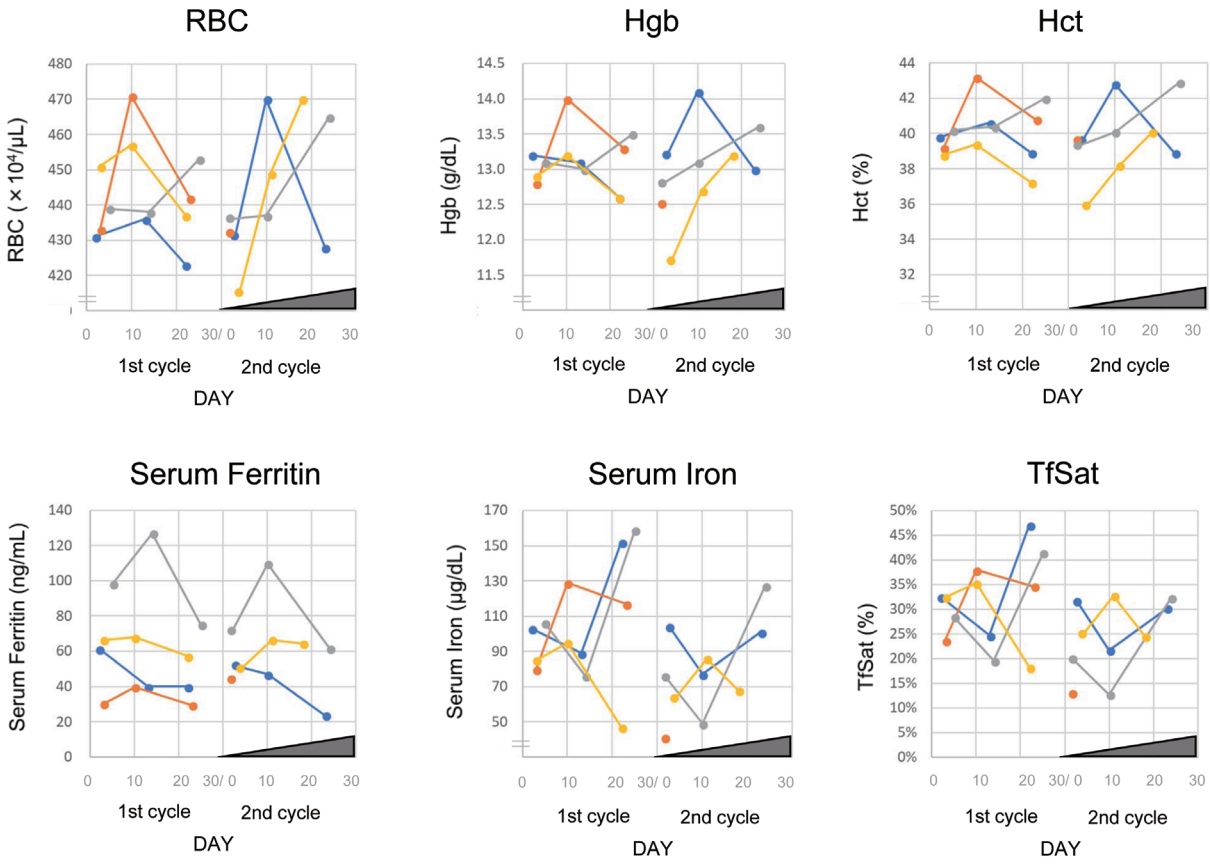

Fig. 1 Variations in iron parameters of individual participants during the study.
Colors represent participants: blue, A; orange, B; grey, C; yellow, D. Grey triangle, iron supplementation.
Abbreviations: Hct, hematocrit; Hgb, hemoglobin; RBC, red blood cell count; TfSat, transferrin saturation.

3.2 Generalized estimating equation model

The follicular phase was divided into menses (days 2–6) and mid follicular (around 10 days from the onset of bleeding) phases to assess variations associated with menstrual cycles. Iron parameters were compared among menses, mid follicular and luteal phases using the GEE model controlled for the total amount of supplemented iron during the second phase as a covariate.

Figure 2 shows values for the iron-related parameters. Values for RBC, Hgb, and Hct were lowest during menses and highest during the mid follicular phase, whereas SF values were the lowest during the luteal phase. No particular trends were identified for serum iron and TfSat.

The supplemented iron (2.5 mg/day × number of days) positively contributed to RBC and Hct levels, but negatively to SF, serum iron and TfSat, whereas the supplemented iron did not significantly contribute to Hgb levels (Table 2).

4. Discussion

Iron deficiency anemia is a prevalent nutritional state among young women. The World Health Organization (WHO) defines anemia as Hgb < 12 g/dL or Hct < 36%, and iron deficiency as SF < 15 ng/mL in non-pregnant women (aged > 15 years)[1]. Peeling et al.[10] proposed three stages of iron deficiency among female athletes defined according to Hgb, SF and TfSat values as:

Stage 1: Iron depletion (Hgb > 11.5 g/dL, SF < 35 ng/dL, TfSat > 16%);

Stage 2: Iron deficient erythropoiesis (Hgb > 11.5 g/dL, SF < 20 ng/dL, TfSat < 16%);

Stage 3: Iron deficiency anemia (Hgb < 11.5 g/dL,

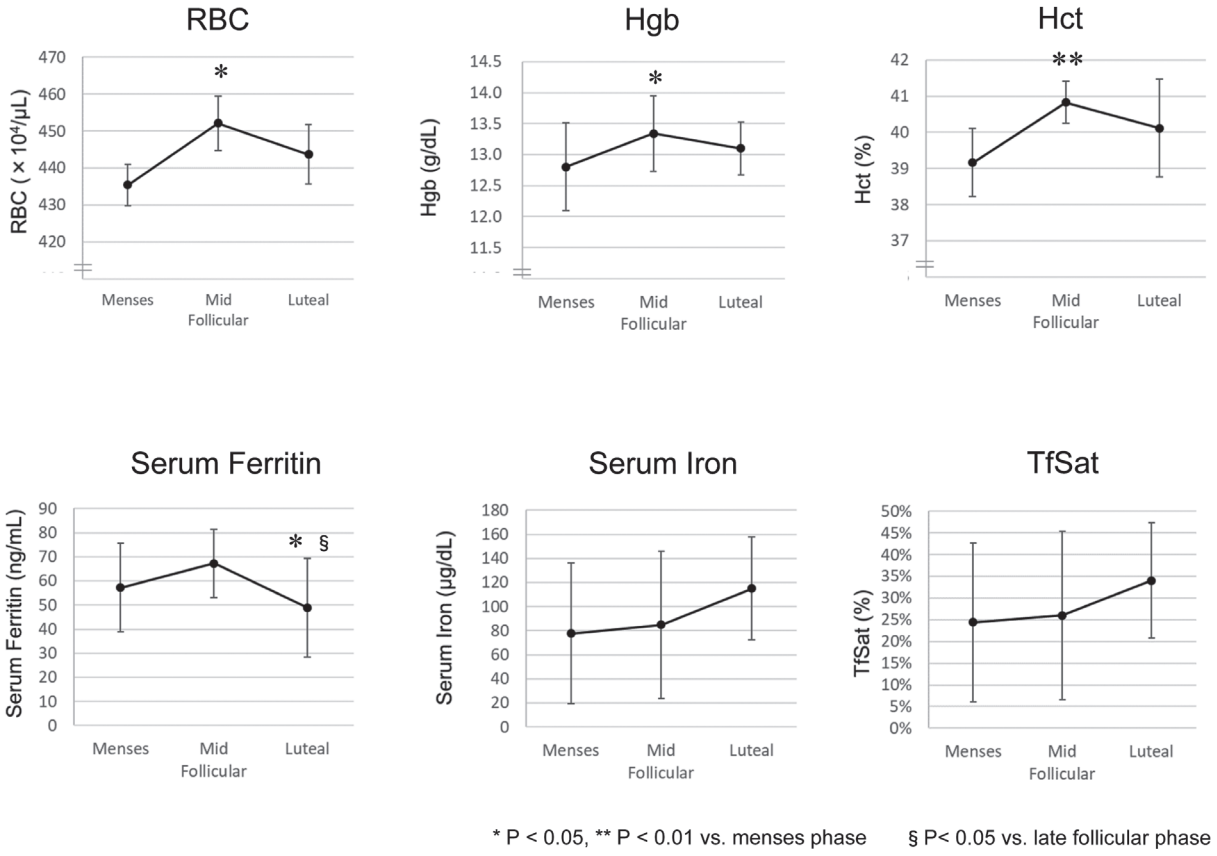

Fig. 2 Variations in iron parameters (estimated) during one menstrual cycle.
Marginal means and standard errors were estimated from general estimating equation models controlled for iron supplementation.
Abbreviations: Hct, hematocrit; Hgb, hemoglobin; RBC, red blood cell count; TfSat, transferrin saturation.

Table 2 Parameter estimates of supplemented iron (2.5 mg × days from the onset of second menses) in Generalized Estimating Equations to explain iron parameters.

Dependent variable	beta	standard error	95 % Wald Confidence Interval		P	
			Lower	Upper		
RBC	0.2214	0.0226	0.1771	0.2658	0.0008	**
Hgb	0.0033	0.0024	-0.0015	0.0081	0.1822	
Hct	0.0129	0.0039	0.0053	0.0204	0.0008	**
SF	-0.2062	0.0731	-0.3496	-0.0629	0.0048	*
Serum iron	-0.4813	0.2265	-0.9253	-0.0373	0.0336	*
TfSat	-0.0015	0.0007	-0.0029	-0.0001	0.0388	*

* P < 0.05, ** P < 0.001

SF < 12 ng/dL, TfSat < 16%).

None of the participants in the present study were classified as having anemia according to the WHO criteria, whereas the SF of participant B was < 35 ng/mL, indicating Stage 1 iron depletion. The Hgb level of participant D fell below the 12.0 g/dL cutoff for anemia at the menses phase of the second menstrual cycle, and the iron status was categorized as Stage 1 or worse according to the Peeling criteria at least once in all participants. Therefore, collegiate female cyclists were at risk of iron deficiency even in the off-season, indicating that iron status should be regularly monitored in such athletes.

One report has described that levels of Hgb, TfSat, and SF reach a nadir during menses and a zenith during the luteal or late luteal phases[6], whereas another did not associate iron parameters with menstrual cycles[7]. Others have proposed that serum iron and TfSat increase after menses and then stabilize during the luteal phase[8,9]. The present findings showed that RBC, Hgb, Hct and SF peaked after menses, then decreased during the luteal phase, whereas no particular trends were evident for serum iron and TfSat. Although this discrepancy could be due to the limited amount of data generated in the present study, we could nevertheless confirm that iron parameters fluctuate during the menstrual cycle. Therefore, the menstrual cycle should be considered when assessing the iron status of premenopausal women.

Iron deficiency is treated by supplementation with 100–300 mg/day of iron[11,12] However, these dosages are higher than those ingested during normal dietary intake and the risk of gastrointestinal side effects such as loose stools/diarrhea, hard stools/constipation and abdominal pain is increased[13]. Therefore, the athletes received low-dose supplementation (2.5 mg/day) during the second menstrual cycle, which complied with the RDA (10.5 mg/day). The findings of the GEE model indicated that supplementation could improve RBC and Hct levels. The coefficient beta of Hgb was positive (0.0033) but the 95% confidence interval (-0.0015–0.081) crossed zero, indicating that the contribution was insignificant. This could be due to the limited amount of data; further study is required to clarify the effects of supplementation. Despite the insignificance of Hgb and the negative influence of SF, serum iron and TfSat, our results indicated that iron supplementation improved RBC and Hct, and thus should be considered to improve iron status.

The present study proceeded after the competition season, so the participants did not engage in regular training. Therefore, the results should be applicable to female non-athletes as well as athletes.

The small sample size is the major limitation of the present study. However, we linked fluctuating iron status to the menstrual cycle. Further investigation is warranted to clarify variations in iron parameters linked with the menstrual cycle. The absence of a dietary assessment also blurred the effect of iron supplementation. This should also be examined in a future study. Knowing serum hepcidin concentrations and amounts of iron loss during

menstrual bleeding will help to clarify the underlying mechanism(s) of variations in iron parameters linked with the menstrual cycle.

In conclusion, despite the limited sample size, we determined that female collegiate cyclists are at risk for iron deficiency and that iron parameters linked with menstrual cycles vary. Therefore, healthy young female athletes should be regularly monitored during menstrual cycles and receive iron supplements when necessary.

Conflicts of interest

The authors declare no conflict of interests.

Acknowledgement

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