Research Article **Open Access**

Restorative effect of bean ferritin iron on low hemoglobin level in premenopausal women with menstruation-induced anemia: A randomized, double-blind placebo-controlled intergroup trial

Ryuji Takeda[1*], Yuji Kuriyama[2], Yasushi Yoshida[2]

[1]Faculty of Health Sciences for Welfare, Department of Nutritional Sciences for Well-being, Kansai University of Welfare Sciences; [2]Department of General Medicine, Juntendo University School of Medicine.

***Corresponding author:** Ryuji Takeda, Faculty of Health Sciences for Welfare, Department of Nutritional Sciences for Well-being, Kansai University of Welfare Sciences.

Submission Date: December 7th, 2023; **Acceptance Date:** December 13th, 2023; **Publication Date:** March 13th, 2024

Please cite this article as: Takeda R., Kuriyama Y., Yoshida Y. Restorative effect of bean ferritin iron on low hemoglobin level in Premenopausal Women with Menstruation-Induced Anemia: A randomized, double-blind placebo-controlled intergroup trial. *Functional Foods in Health and Disease* 2024; 14(3):169-183. DOI: https://www.doi.org/10.31989/ffhd.v14i3.1331

ABSTRACT

Background: Recently, bean ferritin has been attracting considerable attention as a new source of iron, that is available to vegetarians. Although high rates of iron absorption and bioavailability from this protein have been reported, clinical data on its efficacy remain scarce.

Objective: In this study, we administered bean ferritin iron to premenopausal Japanese women for nine weeks, starting immediately after menstruation, to evaluate their recovery from low hemoglobin levels as one sign of anemia.

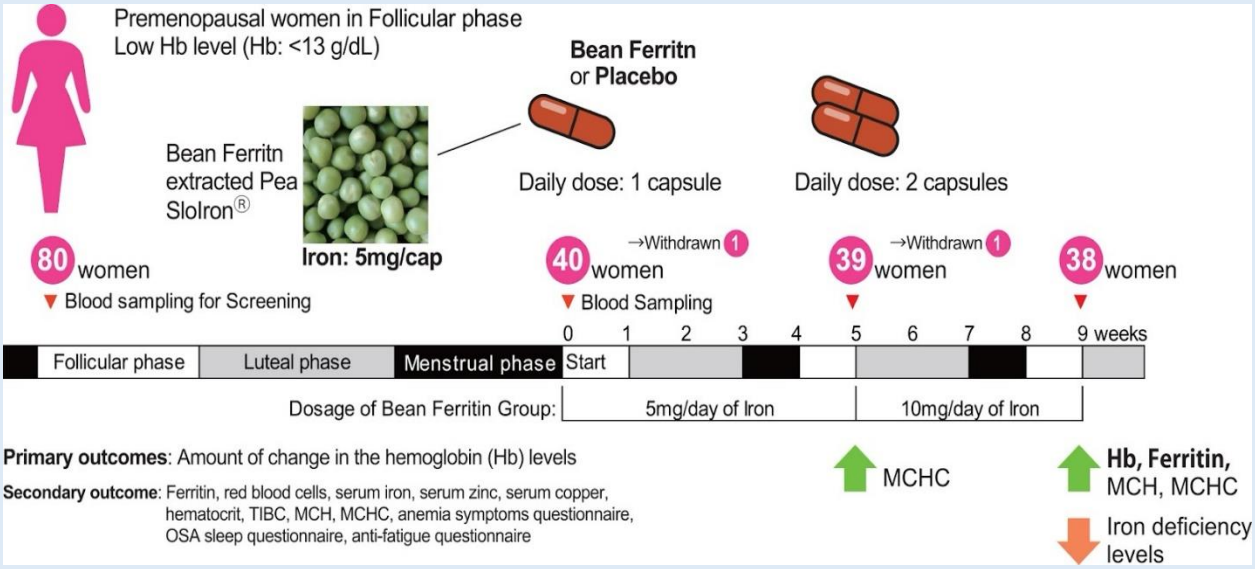
Methods: Participants in the test supplement group received an iron intake of 5 mg from one capsule containing bean extract (SloIron®) for five weeks, which was increased to 10 mg (i.e., two capsules) from the 6th to the 9th week. The study evaluated the change in hemoglobin levels as the primary endpoint, and hematocrit, red blood cell count, serum iron, MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), serum ferritin, TSAT (TIBC), serum zinc, serum copper, anemia symptoms questionnaire, OSA sleep inventory, and the anti-fatigue questionnaire as the secondary endpoints.

Results: Our results showed a significant difference (P=0.03) in the change in hemoglobin levels between the groups after nine weeks of intake, confirming the restorative effect of bean ferritin on low hemoglobin levels caused by menstruation. Moreover, a significant difference (P=0.01) was observed in the amount of change in MCHC between the two groups after five weeks of intake, and after nine weeks of intake, a significant difference in the change in both MCH (P=0.02) and MCHC

(P<0.01) was observed between the groups. A significant difference (P=0.03) was observed in the change in serum ferritin levels after nine weeks of intake.

Conclusion: The study confirmed that iron supplementation from bean ferritin is an effective treatment for low hemoglobin and low ferritin levels caused by menstruation.

Keywords: Ferritin, Bean ferritin, Menstruation, Serum iron, Hemoglobin



INTRODUCTION

Iron is an important component of a healthy diet, especially for menstruating women. In recent years, studies have been conducted using iron-containing foods to confirm iron supplementation, Yan et al. reported that daily consumption of the iron-fortified gummy over eight weeks could help build up blood iron levels to generate hemoglobin, relieve iron-deficiency anemia and related symptoms in young Chinese women. Intake of iron through gummy on a daily basis is an effective method to gradually increase the participants' iron [1].

The recommended daily intake for iron in menstruating adult women in Japan is 10.5 mg [2]. A survey conducted by the Japanese Ministry of Health, Labor, and Welfare in 2017 revealed that the daily iron intake of Japanese adult women was 7.2 mg, which is approximately 70% of the recommended value [3], indicating a high prevalence of iron deficiency among Japanese adult women. In 1992, Uchida et al. estimated the prevalence of various types of iron deficiency from the results of a survey of 3,015 Japanese women (iron-deficiency anemia: 8.5%; latent iron deficiency: 8.0%; storage iron deficiency: 33.4%; normal: 43.6%; and other: 6.5%). The study showed that half of the women who participated in the survey were iron-deficient or potentially iron-deficient [4]. In this study, the criteria for diagnosing anemia was determined from the Hb levels derived from the average values of healthy adults (i.e., transferrin saturation ≥16% and serum ferritin levels ≥12 ng/ml). One of the reasons for the high prevalence of iron deficiency among Japanese adult women may be that the recommended daily intake for iron in Japan is

considerably lower than the reference iron intake in the U.S. (18 mg/day) [5].

To improve this iron deficiency, several Japanese women are commonly prescribed iron preparations and iron supplements. Iron preparations contain ferrous sulfate and ferric citrate, while iron supplements contain ferric citrate, iron pyrophosphate, and heme iron. In 1987, Sczekan and Joshi isolated ferritin, the most important iron-storing protein, from soybean seeds [6], and a research team at the University of California led by Professor Elizabeth C. Theil, a leading expert in the field of ferritin research, started basic research on bean ferritin [7-14]. Moreover, in recent years properties such as iron absorption kinetics and bioavailability, as well as the practical evaluation of the protein as a dietary iron source, has also been explored [15-31]. Because the iron absorption capacity and bioavailability of ferritin are similar to those of ferrous sulfate, a highly absorbable iron preparation used in pharmaceutical products, the protein began to be marketed as a new highly absorbable iron supplement in 2019. While most studies, to date, have been carried out on laboratory samples, human clinical trials using widely available iron sources have not yet been conducted. In 2017, Perfecto et al. examined the stability of ferritin during digestion and the changes in its absorption mechanisms using ferritin isolated from widely available plants, such as peas [32].

Currently, soybean- and pea-derived ferritin iron is circulating in the market. However, due to the structural properties wrapped in apoferritin, when compared to those of iron sulfate used in pharmaceuticals, the merits of ferritin iron are supported due to its few adverse effects, such as an extremely low iron taste and gastrointestinal disorders. Moreover, it has high absorbency despite being a non-heme iron, which is an important consideration for vegan and vegetarian patients.

According to the Dietary Reference Intakes for Japanese (2020) issued by the Ministry of Health, Labor, and Welfare, blood iron loss during menstruation is estimated to be 3.06 mg/day for women aged 10–17 years and 3.64 mg/day for those aged ≥18 years [2]. Moreover, Kumagai et al. reported a decrease of 30% or more in ferritin levels during menstruation [33]. The severity of iron deficiency in adult menstruating women may vary depending on the timing of their menstrual cycle. For this reason, it is worth noting that an accurate evaluation of adult menstruating women who receive oral iron supplements is only possible if blood sampling is carried out within a consistent amount of time from the end of the menstrual cycle. Therefore, the careful management of participants is required. This study aimed to verify the restorative effect of bean ferritin iron on iron deficiency status caused by menstruation in Japanese women. The administration of trial supplements and hematologic evaluation in healthy premenopausal women who experienced anemia symptoms due to low hemoglobin levels was performed at the same time during their menstrual cycle. This was done to control for menstrual cycle phases. Consumption of trial supplements was started within one week from the end of menstruation. Blood samples were collected five and nine weeks after the late follicular to the luteal phase of the menstrual cycle to assess the restorative effect of bean ferritin (SloIron®) on low hemoglobin levels caused by menstruation. The amount of iron in the test supplement was increased from 5 mg/day to 10 mg/day after five weeks of intake, and the dose dependency was also evaluated. Because of the intake by individuals with anemia symptoms, the iron deficiency status is expected to recover from the low hemoglobin level after nine weeks of intake. To evaluate the latent iron deficiency and the storage iron deficiency, this study also evaluated the recovery of the serum ferritin level, which is usually slow to recover in a short time period.

METHODS

Trial supplements: The study participants were divided into two groups. One group received a test supplement containing bean ferritin, which is the evaluation target of this study, and the other group received a placebo control

supplement. The bean ferritin in the test supplement used in the study is distributed under the name of SloIron (provided by SloIron Inc.), a pea extract containing approximately 5% iron. In contrast, the control supplement did not contain any iron. Both the test and control supplements were packed as porcine gelatin capsules with a content weight of 280 mg per capsule, and to maintain blindness, the test supplement capsules were colored white with titanium dioxide.

Corn starch was used as a basic ingredient for both trial supplements. The test supplement contained 5 mg of iron per capsule (100 mg of bean extract), while in the control supplement, the bean extract was replaced with an equal amount of corn starch.

Ethical considerations: This study was conducted in accordance with the Declaration of Helsinki for experiments involving human participants. This study was approved by the Institutional Review Board of Tsukuji Futaba Clinic, Hikobae-kai Medical Corporation (approval no.#20210601D) on July 17, 2021. Moreover, an outline of the study was registered in the public database UMIN Clinical Trials Registry (ID: UMIN000045253). The study was conducted in accordance with the CONSORT 2010 Statement (Consolidated Standards of Reporting Trials 2010 Statement) for reporting randomized controlled trials.

Subjects: The participants were recruited among paid volunteers (i.e., those who registered with feileB Co. Ltd.) based on the inclusion and exclusion criteria outlined below, and 38 out of 80 women who gave their consent to participate in the study were selected (Fig. 1). The target number of participants was determined based on our previous studies or similar reports. On July 22, 2021, the feileB Co. Ltd. website published an outline of the study and launched an email recruitment campaign. Overall, 80 participants were selected by the principal investigator (Hiroyuki Shimizu, Hasegawa Clinics, Seishukai Medical Corporation) based on the laboratory values of hemoglobin at screening and the inclusion and exclusion criteria described below. The participants were randomly assigned to two groups through stratified randomization using numbered solid bags. The assignment order was kept secret until the end of the study to ensure the blinding of participants, intervention providers, and outcome assessors. Synapse Planning Co. Ltd. was in charge of developing the assignment order and assigning participants to each group, while the principal investigator was in charge of enrolling eligible participants.

After the completion of the study, the allocation order and numbering of the participants were disclosed, and the groups of participants that received the bean extract and the control supplement were designated as the S group and P group, respectively.

Inclusion criteria:

1. Women who experienced symptoms of low hemoglobin level (anemia) on a daily basis
2. Premenopausal women
3. BMI of less than 30 kg/m2
4. Menstrual cycle timing falls within the following time frames: The timing of the menstrual cycle is for those who fall within the specified time period.
5. With a relatively stable menstrual cycle
6. Provided written consent to participate in the study.
7. Hb levels of less than 13 g/dL on a previous blood test.

Exclusion criteria:

1. Women who are allergic to beans (e.g., soybean, pea, and other common pulses)
2. Currently on medication for iron-deficiency anemia and receiving medication for any disease.
3. Consume food (e.g., soy milk) and health food products containing the ingredients of the test supplement used in the study.
4. Irregular menstrual cycles

5. Serious diseases affecting glucometabolic health, lipid metabolism, liver function, kidney function, heart, circulatory system, respiratory system, endocrine system, immune system, and nervous system, or mental disorders, and women with a history of such diseases.
6. Medication for a disease or with a history of a serious disease requiring medication.
7. Develop allergies related to the study.
8. Participating in other clinical research at the time of initiation of the study
9. Pregnant or plan to get pregnant or breastfeed during the study period.
10. Uterine myomas or endometriosis
11. Judged by the principal investigator to be unsuitable to participate in the study.

Sample size: Before conducting this study, the changes in Hb were preliminarily examined. Consequently, an increase of 0.7g/dL was observed, and SloIron was ingested in nine weeks. Based on this, we determined that the ingestion of our test food would result in 0.5g/dL compared with the placebo, with a standard deviation of 0.5g/dL. The sample size was calculated assuming 80% power and was evaluated in 17 participants per group. Furthermore, considering the possibility of dropouts, the number of participants per group was planned to be 21.

Study design and methods: The study, which used two kinds of supplements, was designed as a randomized, double-blind placebo-controlled intergroup trial conducted from August 2 to November 10, 2021, at Hasegawa Clinics, Seishukai Medical Corporation. The screening process began on July 22, and the enrolled participants started receiving the trial supplements on August 2 or 15. The study schedule and menstrual cycle of participants are shown in Figure 1.

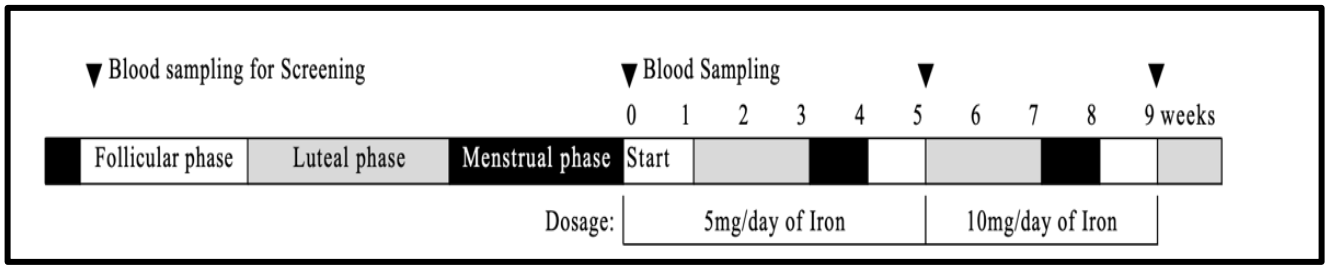


Figure 1. Study schedule and menstrual cycle of participants.

Both trial supplements were distributed at the time of the arrival in the hospital to maintain blindness and were administered as follows: one capsule per day (5 mg of iron per day), preferably taken at breakfast with water or lukewarm water on an empty stomach. The participants received the trial supplements for nine weeks; from the end of the fifth week, the dose was doubled to two capsules per day (10 mg of iron/day). The reason for increasing the dosage was to verify the difference between the 5 mg per day dosage and the 10 mg per day dosage. The 5 mg per day dosage was intended to supplement the approximately 4.1 mg per day dosage that Japanese adult women lack, while the 10 mg/day dosage was intended to supplement the recommended intake for adult women in Japan. Bean ferritin iron is structurally unaffected by catechins because it does not produce tannin iron, and because it is absorbed by endocytosis, there is no need for reduction by vitamin C or other reducing agents. Therefore, dietary amounts were not considered, as there is no need to be overly concerned about the intake of catechins or vitamin C. During the period of intake of trial supplements, the participants were asked to record daily their menstrual cycle phases, living conditions, dietary composition, and whether they took medications or the trial supplements on an Internet blog. Subsequently, the participants were asked to visit the hospital before, at five, and nine weeks after intake, or upon discontinuation, and as may be necessary, and were then asked to complete a web-based questionnaire on the anemia symptoms before, at five, and nine weeks after the intake of trial supplements.

Next, blood samples were collected to evaluate the efficacy and gather observational items (e.g., adverse events, body weight, BMI, blood pressure, pulse, and biochemical and hematological tests) before, at five, and nine weeks after intake. In addition, biochemical and hematological tests were also performed.

Primary outcome: In this study, the amount of change in the Hb levels was used as a primary outcome for screening and efficacy evaluation.

Secondary outcome

Indicators relating to anemia: In this study, the red blood cell count, serum iron, MCH, MCHC, serum ferritin, and TSAT (TIBC), which are common indicators of iron deficiency, serum zinc and copper were also measured as secondary outcomes to evaluate the zinc and copper levels in the blood as the absorption of these two elements may be antagonized by iron intake. Additionally, questionnaire surveys on anemia symptoms, OSA sleep, and anti-fatigue were conducted to evaluate changes in quality of life.

Besides statistical analysis, the mean, standard deviation, standard error, median, and minimum and maximum values were calculated for all endpoints.

QOL questionnaire: The participants completed QOL questionnaires using anemia symptoms, OSA sleep, and anti-fatigue were conducted to evaluate changes in quality of life.

Safety analysis: The following safety endpoints were established. Body weight, body mass index (BMI), and blood biochemistry tests were measured during physical examinations. Fluctuations in body weight, blood biochemistry tests, and BMI were observed during the intake period to check for any abnormal changes; the participants recorded a web diary during the weeks of the intake.

Statistical analysis: Statistical analysis for efficacy evaluation was carried out by Kansai University of Welfare Sciences. The primary endpoint of the study, the change in Hb, was evaluated using the student's t-test adjusted by a closed Fisher combination to account for the multiplicity of tests. Therefore, the order of priority for the tests was evaluated in order of changes in nine weeks and changes in five weeks. As a reference, the student's t-test was also used for the values before ingestion (zero weeks). The secondary outcomes were also tested using the same closed-form Fisher combination. The tests were applied assuming the normality of each dataset (while multiplicity was not considered) with a significance level of 5% (two-tailed test). SAS 9.4 (SAS Inc.) was used for the statistical analysis.

RESULTS

A flow diagram of the progress from screening to analysis is shown in Figure 2. Two participants who violated the exclusion criteria after inclusion were excluded from the analysis. The participants' background characteristics are shown in Table 1, and no significant differences between the two groups in terms of age, BMI, hemoglobin, or ferritin were noted. In Table 2A, the participants were classified based on the six degrees of severity of iron deficiency proposed by Uchida et al. No bias was observed in any of the two groups, and the data composition in the participants' low hemoglobin levels was uniform. Since the study also considers the timing of the menstrual cycle phases, Table 2B shows the number of days from the end of menstruation. In this case, no substantial bias was observed in both groups.

However, despite controlling for menstrual cycle phases (i.e., from the late follicular through the luteal phase), after nine weeks, the time that elapsed from the end of menstruation to blood sampling in the S group became shorter than in the P group, making it difficult to assess recovery from a low hemoglobin level. No participants with menorrhagia were observed.

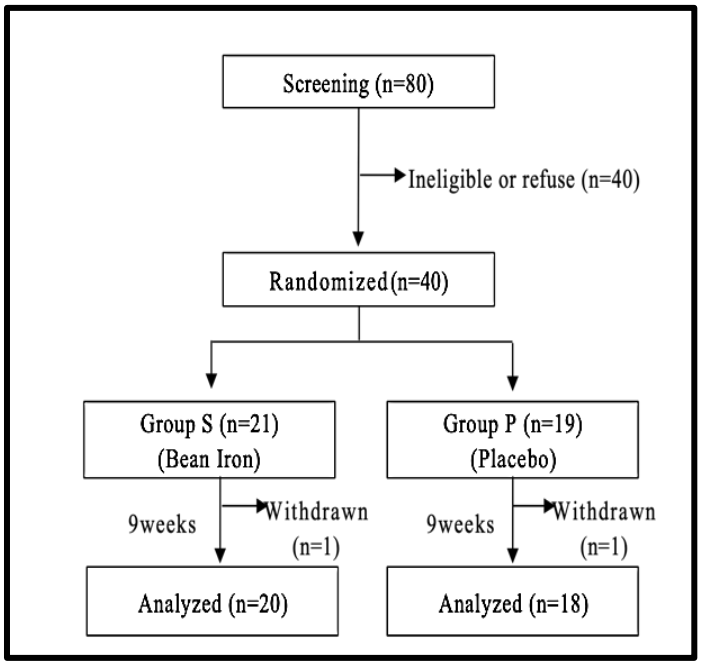


Figure 2. Flow diagram of the progress from screening to analysis

Table 1. Baseline characteristics of subjects and timings of blood sampling in the SlloIron and Placebo groups.

Variable	SloIron(n=20)	Placebo(n=18)	p-value*
Age (years)	32.4 ± 9.4	34.9 ± 9.3	0.3995
BMI (kg/m^2)	19.4 ± 2.5	20.6 ± 2.7	0.1596
Hb (g/dL)	11.2 ± 1.9	11.4 ± 1.7	0.7623
RBC (/μL)	417.8 ± 27.4	412.7 ± 37.8	0.6324
Ht (%)	37.4 ± 4.7	37.3 ± 4.4	0.9917
Ferritin (ng/ml)	15.0 ± 19.9	18.6 ± 31.8	0.6705
TSAT (%)	15.2 ± 0.1	13.4 ± 7.8	0.5482

Mean±SD. * Student's t test

Table 2A. Classification of iron deficiency (Uchida, 1992)

	SloIron(n=20)	Placebo(n=18)
Iron deficiency anemia	9	8
Latent iron deficiency	3	2
Pre-latent iron deficiency	4	4
Low TSAT	1	2
Non iron deficiency	3	0
Others	0	2

* The classification based on Uchida (1992) [3)]

Table 2B. Number of days from end of menstruation to blood sampling

Sampling date		SloIron(n=20)	Placebo(n=18)
0weeks	0 days	0	0
	1-7 days	0	0
	8-14 days	0	0
	14- days	20	18
5weeks	0 days	2	0
	1-7 days	2	3
	8-14 days	9	7
	14-21 days	7	8
9weeks	0 days	0	2
	1-7 days	5	9
	8-14 days	8	4
	14-21 days	7	3

Primary outcome: Table 3 shows the values of the primary outcome before (0 week), at five, and nine weeks after intake, as well as the amount of change from before at five and nine weeks after intake. The amount of change in the primary outcome of Hb levels showed significant intergroup differences were observed after nine weeks of intake.

Table 3 Primary outcome of subjects in the SloIron and Placebo groups.

		SloIron(n=20) MEAN±SD	Placebo(n=18) MEAN±SD	*P*-Value* Between group
Primary outcome				
Hb	0w	11.2 ± 1.8	11.4 ± 1.6	0.78
(g/dL)	5w	11.3 ± 1.8	11.5 ± 1.6	-
	9w	11.8 ± 1.9	11.4 ± 1.6	-
	5w-0w	0.1 ± 0.6	0.1 ± 0.5	0.75
	9w-0w	0.6 ± 0.8	0.1 ± 0.7	0.03

*Student's t test adjusted by closed Fisher combination

Secondary outcome: Table 4 shows the values of indicators relating to anemia, secondary outcome, before (0 week), at five, and nine weeks after intake, as well as the amount of change from before, at five, and nine weeks after intake.

The amount of change in MCH (P=0.02) and MCHC (P<0.01) levels between groups observed significant differences after nine weeks of intake. The other items showed that no significant intergroup differences were observed. Figure 3 shows changes in the serum ferritin levels for each participant before and after intake of trial supplements, and Figure 4 shows the amount of change after five and nine weeks of intake. Many participants in the S group showed an increase in ferritin levels, indicating a significant difference between the two groups after nine weeks of intake.

Table 4 Secondary outcome of subjects in the SloIron and Placebo groups.

		SloIron(n=20)	Placebo(n=18)	*P*-Value*
		MEAN±SD	MEAN±SD	Between group
RBC	0w	420.3 ± 29.1	414.4 ± 37.5	0.58
(/μL)	5w	422.3 ± 26.3	420.9 ± 32.3	-
	9w	433.1 ± 30.3	418.6 ± 31.6	-
	5w-0w	2.0 ± 22.6	8.2 ± 20.6	-
	9w-0w	15.3 ± 23.2	5.9 ± 26.0	0.25
Ht	0w	37.4 ± 4.5	37.4 ± 4.3	0.98
(%)	5w	36.8 ± 4.3	37.7 ± 4.2	0.52
	9w	38.2 ± 4.6	37.5 ± 4.3	-
	5w-0w	-0.6 ± 1.9	0.3 ± 2.2	-
	9w-0w	0.9 ± 2.2	0.1 ± 2.3	-
MCV	0w	89.0 ± 9.7	90.2 ± 5.9	0.64
(fL)	5w	87.0 ± 8.9	89.4 ± 6.2	-
	9w	88.3 ± 9.5	89.4 ± 6.8	-
	5w-0w	-1.9 ± 1.8	-1.0 ± 2.4	-
	9w-0w	-1.1 ± 1.9	-0.9 ± 2.2	0.82
MCH	0w	26.6 ± 4.0	27.4 ± 2.7	0.51
(pg)	5w	26.7 ± 4.0	27.3 ± 2.9	-
	9w	27.2 ± 4.1	27.3 ± 2.9	-
	5w-0w	0.1 ± 0.5	-0.2 ± 0.5	0.08
	9w-0w	0.5 ± 1.0	-0.2 ± 0.8	0.02
MCHC	0w	29.8 ± 1.7	30.3 ± 1.4	0.35
(%)	5w	30.5 ± 1.8	30.5 ± 1.3	-
	9w	30.7 ± 1.9	30.4 ± 1.1	-
	5w-0w	0.7 ± 0.6	0.1 ± 0.8	0.01
	9w-0w	0.9 ± 0.7	0.1 ± 0.7	<0.01
Ferr	0w	14.4 ± 19.6	19.0 ± 31.0	0.57
(ng/ml)	5w	15.9 ± 21.4	21.9 ± 47.6	-
	9w	18.8 ± 17.9	18.5 ± 33.0	-
	5w-0w	1.5 ± 12.1	3.3 ± 18.7	-
	9w-0w	3.8 ± 13.9	-0.1 ± 6.2	0.28
TSAT	0w	15.1 ± 10.0	13.5 ± 7.6	0.57
(%)	5w	16.8 ± 11.1	15.5 ± 9.9	-
	9w	17.1 ± 10.2	15.4 ± 10.6	-
	5w-0w	1.7 ± 7.0	2.1 ± 5.9	-
	9w-0w	1.8 ± 8.0	2.0 ± 8.3	0.96
Serum	0w	53.4 ± 30.1	50.1 ± 26.1	0.72
Iron	5w	60.9 ± 33.5	61.4 ± 36.5	-
(μg/dL)	9w	64.9 ± 39.9	62.8 ± 43.0	-
	5w-0w	7.5 ± 26.6	11.2 ± 23.1	-
	9w-0w	11.8 ± 32.9	12.6 ± 36.1	0.94
Serum	0w	102.0 ± 9.9	104.7 ± 23.9	0.64
Copper	5w	105.1 ± 17.7	111.9 ± 26.9	-
(μg/dL)	9w	107.5 ± 15.2	114.2 ± 23.4	-
	5w-0w	3.0 ± 14.3	6.4 ± 10.7	-
	9w-0w	5.3 ± 14.8	8.6 ± 9.4	0.42
Serum	0w	93.9 ± 14.5	95.4 ± 11.9	0.72
Zinc	5w	90.7 ± 13.8	85.4 ± 12.1	-
(μg/dL)	9w	95.0 ± 12.5	93.5 ± 10.4	-
	5w-0w	-3.2 ± 12.7	-10.1 ± 10.6	-
	9w-0w	2.0 ± 13.2	-2.0 ± 10.6	0.94

*Student's t test adjusted by closed Fisher combination

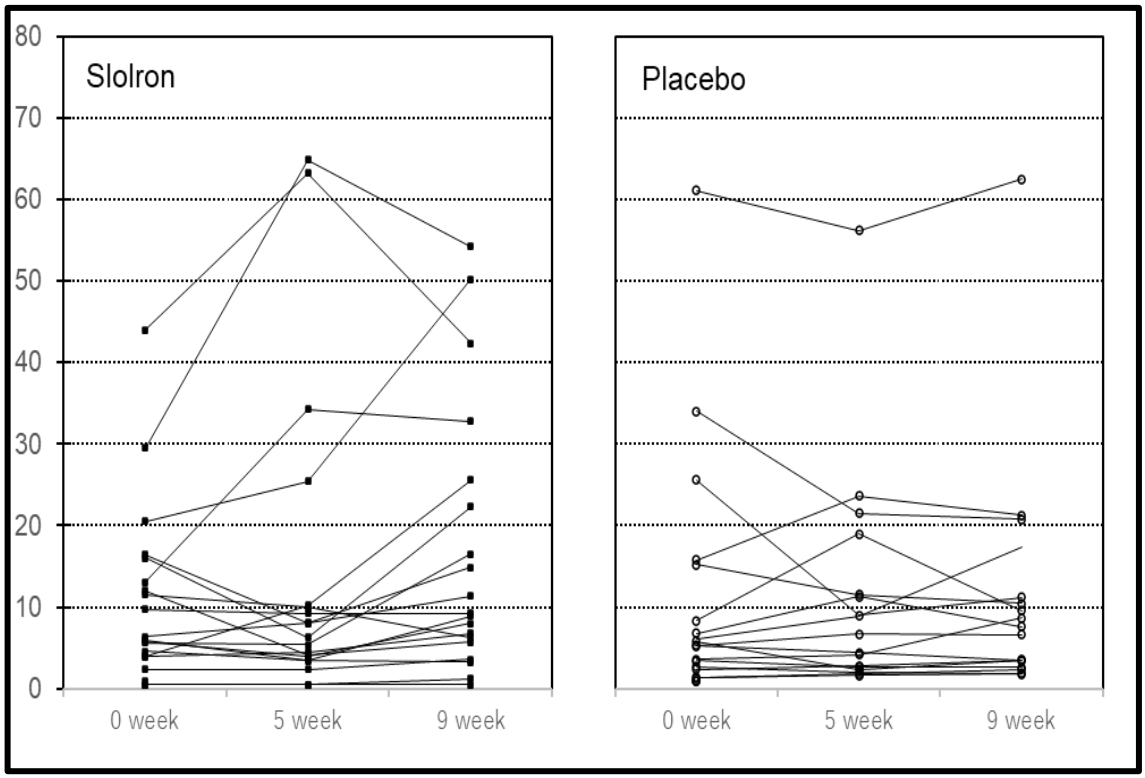


Figure 3. Changes in ferritin in each participant

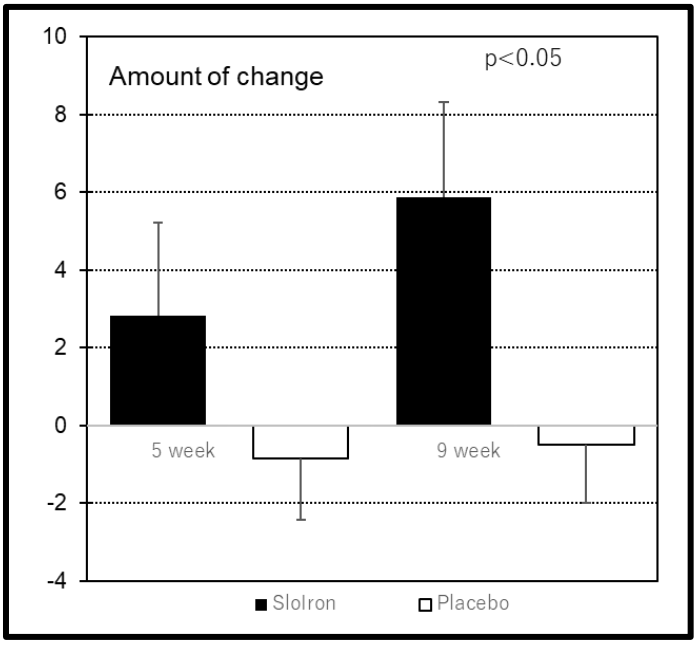


Figure 4. The amount of change in ferritin levels of SloIron and Placebo groups after five and nine weeks of intake

Various questionnaires: Significant intragroup differences in the amount of change in several items of the anemia symptoms questionnaire (e.g., subjective feeling of iron deficiency symptoms, sense of fatigue, sense of vertigo or dizziness, sleepiness, facial complexion, discomfort during exercise, unpleasant feelings of restlessness in the lower limbs, and taste perception) were observed. Notably, significant intragroup differences in such items, such as the subjective feeling of iron deficiency symptoms, sense of

fatigue, sense of vertigo or dizziness, and facial complexion, were observed in both the S and P groups. Significant intergroup differences in taste perception were observed only after three and seven weeks of intake. No significant intergroup differences were observed in the OSA sleep inventory or anti-fatigue questionnaire (data not shown).

Safety and side effects: No adverse events were reported during the entire study, which was successfully completed. In addition, no common side effects associated with iron supplementation (e.g., heartburn, nausea, abdominal pain, constipation, etc.) were reported. No significant differences were observed in such observational items as body composition and biochemical and hematological tests, and changes for all the events were within the items' acceptable value.

DISCUSSION

The study showed significant intergroup differences in the primary endpoint of Hb levels, as well as in MCH and MCHC, which are used to measure the red blood cell quality, and serum ferritin, an indicator of the body's iron stores. Table 4 shows the change in iron anemia symptoms to a low hemoglobin level before and after the consumption of trial supplements in participants who were classified based on the six degrees of severity of iron deficiency proposed by Uchida et al. The results show that approximately half of the participants in the S group recovered from low hemoglobin levels. Moreover, while some participants in the P group recovered, although in a smaller number than in the S group, the number of participants who deteriorated was significantly higher in the P group than in the S group. These results confirm that iron intake from bean ferritin can effectively lead to recovery from a low hemoglobin level or a low ferritin level caused by menstruation.

The serum ferritin levels were slightly higher in the S group, which continued to receive bean iron at a dose of 5 mg/day for five weeks but were not significantly different from those in the P group. Conversely, after nine weeks of intake, there was a significant difference between the amount of change observed in the S group, which continued to receive bean iron at a dose of 10 mg/day for an additional four weeks (for a total of nine weeks), and that observed in the P group. At the same time, the serum ferritin levels of participants in the S group increased approximately 1.6-fold on average compared to the beginning of the study, also suggesting an increase in the body's iron stores.

Some studies have reported that serum ferritin levels decrease by 30% or more during menstruation, and in particular, the follicular phase during and immediately after the end of menstruation is thought to be characterized by a decrease in ferritin levels [34]. Asakura et al., in 2009, reported that the main cause of iron deficiency in Japanese women is not insufficient intake but iron loss through menstruation [35]. Although iron stores are expected to be restored after menstruation through dietary intake or other sources, it is worth noting that most absorbed iron is transferred to the blood and used for hemoglobin formation. Consequently, body iron stores are believed to remain unchanged or even slightly reduced, as shown by the changes observed in the P group in our study. Moreover, in patients with iron-deficiency anemia treated with oral iron supplements, reticulocyte crisis (i.e., the rapid increase of the number of reticulocytes) occurs within 7–10 days of the start of treatment after an increase in serum iron levels and is followed by an increase in Hb levels. It can take from three to four months (12 to 16 weeks) to fully replete iron stores (i.e., serum ferritin) [36]. To restore the serum ferritin levels after menstruation and recover from iron-deficiency anemia or latent iron deficiency, iron should be supplied regularly from sources other than food. For iron supplements derived from beans, such as the one used in this study, a daily intake of 10 mg or more is recommended to ensure a more effective recovery. Moreover, the slight increase in the amount of change

observed at a daily intake of approximately 5 mg, albeit not significant, suggests that continuous iron intake over a prolonged period (e.g., 12 weeks or longer) may lead to gradual recovery from a low hemoglobin level.

In patients with low hemoglobin levels, such as women during menstruation, elevated expression of iron homeostasis proteins, such as divalent metal transporter 1 (DMT1), Dcytb, ferroportin, and hephaestin is observed, and iron absorption from the digestive tract tends to increase. According to the National Health and Nutrition Survey conducted by the Ministry of Health, Labour, and Welfare, approximately 70% of the iron consumed by Japanese from food sources is non-heme iron [3]. It also should be noted that higher consumption of iron resulting from the concomitant intake of supplements and food sources is known to inhibit the intestinal uptake of zinc and copper due to the competitive antagonism between these elements on the DMT1. To evaluate the inhibitory effect of iron from bean ferritin on copper and zinc uptake during iron absorption, in addition to the serum iron, we also evaluated serum zinc and copper. The results of our study showed no clear inhibitory trends, with no significant intergroup differences, although significant intragroup differences were observed in the P group. Since both groups consumed iron from food sources daily and the iron intake from test supplements in the S group was lower (5–10 mg) than that from commercially available supplements (prescription drugs supply an iron intake of 100–200 mg/day), the differences were not considered significant. To evaluate the inhibitory effect of iron from bean ferritin, an intake of 40 mg or more (i.e., the acceptable daily intake reported for Japanese women) would be necessary.

In the anemia symptoms questionnaire, several items showed significant intragroup differences in both groups. Since these changes include the restorative effect of iron supplementation from dietary sources, the intragroup differences observed in both groups were significant. Moreover, while all P-values in the S group were smaller, indicating slightly stronger recovery trends, the difference between the groups was not large enough to be considered significant. Because responses to questionnaires are highly subjective and may easily reflect some placebo effect, statistically significant differences were difficult to derive due to the substantial number of participants with no iron-deficiency anemia included in the analysis.

According to the Nutritional and Dietary Guidelines for athletes issued by the Sports Medicine and Science Committee of the Japan Sports Association, the iron intake in athletes should be 1.5 times the recommended amount for general adult women (15 mg/day), considering iron loss due to sweating and intestinal bleeding caused by exercise. As mentioned above, the recommended iron intake for adult women in the U.S. is 18 mg/day. It might be preferable for women who are menstruating or immediately after menstruation and iron-deficient women who have had or experienced symptoms of iron deficiency to consume iron at a dose of 15–18 mg/day, which is the recommended intake for athletes and adult women in the U.S. Since the average consumption of iron in adult Japanese women is 7.2 mg/day, supplementation with 8–10 mg/day of iron from sources other than food (e.g., supplements) would be advisable. These recommended values for iron-deficient women are also supported by the results of this study.

In this study, participants were recruited using hemoglobin as a primary endpoint, but after the end of the study, we found that some participants exhibited higher serum ferritin levels than the reference upper limit (80 ng/ml) and did not follow its normal distribution were erroneously included in the analysis. In future studies, reference values for serum ferritin levels should be specified in the selection or exclusion criteria to allow a more accurate evaluation of the restorative effects of iron intake in iron-deficient patients.

While the study evaluated the restorative effect of iron intake on low hemoglobin levels and low ferritin

levels in Japanese women who experienced anemia symptoms of low hemoglobin levels after menstruation, in the future, the restorative effect of iron from bean ferritin should be compared with that of iron derived from other elements, and its effect on sports performance in athletes should be evaluated. Furthermore, from a theoretical standpoint, it would be important to devise some method to identify iron sources that are less likely to generate free radicals and do not put too much strain on the body.

Research on biofortified food crops has also attracted attention in recent years. As a representative example, studies from Rwanda [36,37] reported improvement of iron deficiency status through the consumption of iron-fortified beans. On the other hand, in these studies, iron-fortified beans were cooked by heating and consumed without any consideration of ferritin denaturation conditions reported by Perfecto et al. [32]. On these studies, it is assumed that the iron from the beans was not consumed as ferritin, which does not indicate the effectiveness of the bean-derived ferritin. Therefore, unlike the Rwandan study, this study is considered to demonstrate the efficacy of bean-derived ferritin. It was also suggested that ferritin iron extracted from iron-fortified beans could be also distributed in the future as a material with high absorption and safety.

The study did not evaluate the oxidative damages by radicals brought about by ferritin iron, because the dosage of iron it low. Iron sulphate has been reported to reduce gastrointestinal discomfort and other symptoms as oxidative damages when taken with fruit enzymes [38]. The bean-derived ferritin iron used in this study is considered less likely to cause gastrointestinal discomfort due to radicals because it is wrapped into protein, and no adverse events have occurred in our study. The occurrence of radicals in the body during ferritin iron ingestion is not well known and further research is needed.

CONCLUSION

The significant difference in the amount of change in Hb levels observed in the study after nine weeks of iron intake from bean ferritin confirmed that recovery from low hemoglobin levels or low ferritin levels caused by menstruation in Japanese women was possible. Also, an intake of 5 mg/day of bean ferritin for five weeks has been shown to improve red blood cell quality (MCHC). Significant differences were also shown in the change in ferritin levels after nine weeks of intake, suggesting that iron consumed at a dose of 10 mg/day is more likely to improve the low hemoglobin level and increase the body's iron stores than at a dose of 5 mg/day. Therefore, pea-derived bean ferritin intake is effective against anemia.

Acknowledgments and funding: This study was funded by the Anti-Aging Pro Corporation, Tokyo, Japan.

Competing interests: The test foods in this study were produced by Anti-Aging Pro Corporation., Tokyo, Japan. Other authors declare that there are no potential conflicts of interest.

Author contributions: Conceptualization, Y.K. and Y.Y.; methodology, R.T. and Y.K.; software, R.T.; validation, R.T.; formal analysis, R.T.; investigation, Y.K.; data curation, R.T.; writing—original draft preparation, R.T and Y.K.; writing—review and editing, Y.Y.; supervision, Y.Y.; project administration, R.T. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Yan Xiao, Bingjie Yang, Jiayi Ni, Ran Hu: Efficacy of an iron-fortified gummy on iron-deficiency anemia in young women. Functional Foods in Health and Disease 2024, 14(2):97-113. DOI: https://www.doi.org/10.31989/ffhd.v14i1.1239
2. Japanese Ministry of Health, Labour and Welfare. Dietary Reference Intakes for Japanese (2020)
3. Japanese Ministry of Health, Labour and Welfare. National Health and Nutrition Survey (2017).

4. Uchida T, Kawachi Y, Sakamoto Y, Igaki T, Ogasawara N, Kariyone S, Matsuda S, et al: Prevalence and pathogenesis of iron deficiency in Japanese women (1981-1991) (in Japanese). Rinsho Ketsueki 1992, 33(11):1661-1665.
5. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc: a Report of the Panel on Micronutrients. Washington, DC: National Academy Press; 2001. DOI: https://doi.org/10.17226/10026
6. Sczekan SR, Joshi JG: Isolation and characterization of ferritin from soyabeans (Glycine max). J Biol Chem 1987, 262(28):13780-13788.
7. Ragland M, Briat JF, Gagnon J, Laulhere JP, Massenet O, Theil EC: Evidence for conservation of ferritin sequences among plants and animals and for a transit peptide in soybean. J Biol Chem 1990, 25;265(30):18339-18344.
8. Ragland M, Theil EC: Ferritin (mRNA, protein) and iron concentrations during soybean nodule development. Plant Mol Biol. 1993, 21(3):555-560. DOI: https://doi.org/10.1007/bf00028813
9. Beard JL, Burton JW, Theil EC: Purified ferritin and soybean meal can be sources of iron for treating iron deficiency in rats. J Nutr. 1996, 126(1):154-160. DOI: https://doi.org/10.1093/jn/126.1.154
10. Burton JW, Harlow C, Theil EC: Evidence for reutilization of nodule iron in soybean seed development. J Plant Nutr. 1998, 21:913–927. DOI: https://doi.org/10.1080/01904169809365453
11. Theil EC: Ferritin: at the crossroads of iron and oxygen metabolism. J Nutr. 2003, 133(5 Suppl 1):1549S-1553S. DOI: https://doi.org/10.1093/jn/133.5.1549S
12. Theil EC: Iron, ferritin and nutrition. Annu Rev Nutr. 2004, 24:327-343. DOI: https://doi.org/10.1146/annurev.nutr.24.012003.132212
13. Liu X, Theil EC: Ferritin as an iron concentrator and chelator target. Ann N Y Acad Sci. 2005, 1054:136-140. DOI: https://doi.org/10.1196/annals.1345.016
14. Theil EC: Iron homeostasis and nutritional iron deficiency. J Nutr. 2011, 141:S724–S728 DOI: https://doi.org/10.3945/jn.110.127639
15. Murray-Kolb LE, Welch R, Theil EC, Beard JL: Women with low iron stores absorb iron from soybeans. Am J Clin Nutr 2003, 77(1):180-184. DOI: https://doi.org/10.1093/ajcn/77.1.180
16. Davila-Hicks P, Theil EC, Lönnerdal B: Iron in ferritin or in salts (ferrous sulfate) is equally bioavailable in nonanemic women. Am J Clin Nutr 2004, 80(4):936-940. DOI: https://doi.org/10.1093/ajcn/80.4.936
17. Lönnerdal B, Bryant A, Liu X, Theil EC: Iron absorption from soybean ferritin in nonanemic women. Am J Clin Nutr 2006, 83(1):103-107. DOI: https://doi.org/10.1093/ajcn/83.1.103
18. San Martin CD, Garri C, Pizarro F, Walter T, Theil EC, Núñez MT: Ca Absorption of Iron from Ferritin Is Independent of Heme Iron and Ferrous Salts in Women and Rat Intestinal Segment. J Nutr 2008, 138(4):659–666.
19. Theil EC, Chen H, Miranda C, Janser H, Elsenhans B, Núñez MT, Pizarro F, et al: Absorption of iron from ferritin is independent of heme iron and ferrous salts in women and rat intestinal segments. J Nutr 2012, 142(3):478-483. DOI: https://doi.org/10.3945/jn.111.145854
20. Khurana M, Fung EB, Vichinsky EP, Theil EC: Dietary nonheme iron is equally bioavailable from ferritin or ferrous sulfate in thalassemia intermedia. Pediatr Hematol Oncol. 2017, 34(8):455-467. DOI: https://doi.org/10.1080/08880018.2017.1395935
21. Zha G: Phytoferritin and its implications for human health and nutrition. Biochim Biophys Acta. 2010, 1800(8):815-823. DOI: https://doi.org/10.1016/j.bbagen.2010.01.009
22. Masuda T: Soybean Ferritin Forms an Iron-Containing Oligomer in Tofu Even after Heat Treatment. J. Agric. Food Chem. 2015, 63(40):8890–8895. DOI: https://doi.org/10.1021/acs.jafc.5b03080
23. Lv C, Zhao G, Lönnerdal B: Bioavailability of iron from plant and animal ferritins. J Nutr Biochem. 2015, 26(5):532-540. DOI: https://doi.org/10.1016/j.jnutbio.2014.12.006
24. Masuda T, Goto F, Yoshihara T, Mikami B: Crystal structure of plant ferritin reveals a novel metal binding site that functions as a transit site for metal transfer in ferritin. J Biol Chem. 2010, 5;285(6):4049-4059. DOI: https://doi.org/10.1074/jbc.m109.059790
25. Kalgaonkar S, Lonnerdal B: Effects of dietary factors on iron uptake from ferritin by Caco-2 cells. J Nutr Biochem. 2008, 19:33–DOI: https://doi.org/10.1016/j.jnutbio.2007.02.001
26. Kalgaonkar S, Lonnerdal B: Receptor-mediated uptake of ferritin-bound iron by human intestinal Caco-2 cells. J Nutr Biochem. 2009, 20:304–311. DOI: https://doi.org/10.1016/j.jnutbio.2008.04.003
27. San Martin CD, Garri C, Pizarro F, Walter T, Theil EC, Núñez MT: Caco-2 intestinal epithelial cells absorb soybean ferritin by mu2 (AP2)-dependent endocytosis. J Nutr. 2008, 138:659–656. DOI: https://doi.org/10.1093/jn/138.4.659

28. Li L, Fang CJ, Ryan JC, Niemi EC, Lebrón JA, Björkman PJ, Arase H, et al: Binding and uptake of H-ferritin are mediated by human transferrin receptor-1. Proc Natl Acad Sci U S A. 2010, 107(8):3505-310.
DOI: https://doi.org/10.1073/pnas.0913192107
29. Sakamoto S, Kawabata H, Masuda T, Uchiyama T, Mizumoto C, Ohmori K, Koeffler PH, et al: H-Ferritin Is Preferentially Incorporated by Human Erythroid Cells through Transferrin Receptor 1 in a Thresh-old-Dependent Manner. PLoS One. 2015, 10(10):e0139915.
DOI: https://doi.org/10.1371/journal.pone.0139915
30. Latunde-Dada GO, Pereira DIA, Tempest B, Ilyas H, Flynn AC, Aslam MF, Simpson RJ, et al: A nano-particulate ferritin-core mimetic is well taken up by HuTu 80 duodenal cells and its absorption in mice is regulated by body iron. J Nutr. 2014, 144(12):1896-1902.
DOI: https://doi.org/10.3945/jn.114.201715
31. Powell JJ, Bruggraber SFA, Faria N, Poots LK, Hondow N, Pennycook TJ, Latunde-Dada GO, et al: A nano-disperse ferritin-core mimetic that efficiently corrects anemia without luminal iron redox activity. Nanomedicine. 2014, 10(7):1529-1538.
DOI: https://doi.org/10.1016/j.nano.2013.12.011
32. Perfecto A, Rodriguez-Ramiro I, Rodriguez-Celma J, Sharp P, Balk J, Fairweather-Tait S: Pea Ferritin Stability under Gastric pH Conditions Determines the Mechanism of Iron Uptake in Caco-2 Cells. J Nutr. 2018, 148(8):1229-1235.
DOI: https://doi.org/10.1093/jn/nxy096
33. Kumagai E, Nakazono T, Nakayama ROM, Kumagai T: The Study on the Frequency and Pathogenesis of Anemia in College Students (in Japanese). Bulletin of Kumamoto University College of Medical Science 1993, 3:55-60.
34. Asakura K, Sasaki S, Murakami K, Takahashi Y, Uenishi K, Yamakawa M, Nishiwaki Y, et al: Iron intake does not significantly correlate with iron deficiency among young Japanese women: a cross-sectional study. Multicenter Study Public Health Nutr 2009, 12(9):1373-1383.
DOI: https://doi.org/10.1017/s1368980008004072
35. Japanese Society of Laboratory Medicine. JSLM Guidelines 2003.
36. Jere D Haas, Sarah V Luna, Mercy G Lung'aho, Michael J Wenger, Laura E Murray-Kolb, Stephen Beebe, Jean-Bosco Gahutu, Ines M Egli. Consuming Iron Biofortified Beans Increases Iron Status in Rwandan Women after 128 Days in a Randomized Controlled Feeding Trial. J Nutr. 2016;146(8):1586-92.
DOI: https://doi.org/10.3945/jn.115.224741
37. Sarah V Luna, Laura M Pompano, Mercy Lung'aho, Jean Bosco Gahutu and Jere D Haas. Increased Iron Status during a Feeding Trial of Iron-Biofortified Beans Increases Physical Work Efficiency in Rwandan Women. J Nutr. 2020; 150(5): 1093–1099.
DOI: https://doi.org/10.1093/jn/nxaa016
38. G Bertuccelli, F Marotta, N Zerbinati, A Cabeca, F He, S Jain, A Lorenzetti, H Yadav, M Milazzo, F Calabrese, C Tomella, R Catanzaro. Iron supplementation in young iron-deficient females causes gastrointestinal redox imbalance: protective effect of a fermented nutraceutical. J Biol Regul Homeost Agents. 2014;28(1):53-63.