



Reduced-dose alternate-day oral iron supplementation in women with iron deficiency: a randomized controlled trial

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Abstract

Purpose Oral iron supplementation is frequently limited by gastrointestinal side effects and reduced absorption associated with hepcidin-mediated regulation. This randomized, parallel-group, active-controlled study evaluated whether a reduced-dose alternate-day oral iron supplementation strategy could improve iron status in women with iron deficiency.

Methods Sixty-six iron-deficient women of reproductive age (18–50 years; serum ferritin < 30 ng/mL) were randomized to receive, for 42 days, either micronized, dispersible ferric pyrophosphate providing 21 mg of elemental iron every 48 h combined with a daily gut microbiota modulator, or sucrosomial ferric pyrophosphate providing 30 mg of elemental iron per day. The primary endpoint was serum ferritin, while secondary endpoints included serum iron, transferrin, transferrin saturation (TSAT), total iron-binding capacity (TIBC), hemoglobin, and red blood cell count.

Results Serum ferritin increased significantly over time in both groups ($p < 0.001$), with no significant differences between treatments. Comparable improvements were observed for serum iron, transferrin, TSAT, and TIBC, while hemoglobin remained stable throughout the study. Both interventions were generally well tolerated, with only mild transient gastrointestinal adverse events and no clinically relevant changes in inflammatory markers.

Conclusion A reduced-dose alternate-day oral iron supplementation strategy achieved improvements in iron status comparable to standard daily supplementation despite lower cumulative iron exposure. These findings support the potential clinical relevance of physiologically informed low-dose iron supplementation strategies in women with iron deficiency.

Keywords Iron deficiency · Oral iron supplementation · Ferritin · Iron bioavailability · Alternate-day dosing · Gut microbiota

Introduction

Iron deficiency is the leading cause of anemia worldwide. Anemia is diagnosed when hemoglobin levels are below 130 g/L in men, below 120 g/L in women, or below 110 g/L during pregnancy [1]. Iron deficiency anemia (IDA) is diagnosed when anemia is present and laboratory findings confirm iron deficiency. Anemia has an overall prevalence of 24.8% worldwide, while IDA affects approximately 2 billion people globally, with young children and women of reproductive age being at the highest risk [2]. Dietary iron is derived from two main sources: heme iron, primarily found in animal-based foods such as meat and fish, which is highly bioavailable, and non-heme iron, present in plant-based foods, whose absorption is lower and strongly influenced by dietary components and intestinal regulatory mechanisms [3]. Overall, only 10–15% of ingested iron is absorbed in the

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gut and, in healthy adults, this corresponds to approximately 1–2 mg/day [2, 4, 5]. Nutritional deficiencies, infectious diseases, chronic illnesses, and genetic predispositions may all contribute to the onset of iron deficiency [2, 6]. Iron supplementation administered orally or intravenously represents a common therapeutic strategy; however, several limitations exist, particularly with oral administration. First, oral iron intake is frequently linked to gastrointestinal side effects such as nausea, abdominal pain, constipation, diarrhea, dyspepsia, mainly due to iron-induced intestinal toxicity [7, 8]. Second, because humans lack a specific mechanism for iron excretion, systemic iron homeostasis is tightly regulated by hepcidin, a peptide hormone produced by the liver capable of binding to the iron exporter ferroportin in enterocytes, inducing its internalization and degradation. Consequently, hepatic hepcidin synthesis inhibits iron release from enterocytes into the circulation, thereby reducing intestinal iron absorption [9]. It has been demonstrated that iron supplementation at doses of 60 mg induces an increase in hepcidin levels for up to 24 h, leading to a reduction in fractional iron absorption by 35–45% in the following hours [10]. In addition, a randomized double-blind study comparing iron supplementation at a dose of 60 mg/day for 14 days with a different regimen of 60 mg administered on alternate days for 28 days showed a significantly higher iron absorption in the alternate-day group, suggesting that this alternate dosing strategy may be preferable to daily supplementation [11, 12]. The gut microbiota represents a key regulator of iron metabolism. Specific bacterial taxa, including short-chain fatty acid (SCFA)-producing bacteria, have been associated with improved iron bioavailability, possibly through modulation of intestinal pH and epithelial function [13, 14]. Conversely, dysbiosis characterized by an increased abundance of Enterobacteriaceae (including *Escherichia coli*), *Fusobacterium*, and *Clostridium* species has been associated with impaired iron metabolism and intestinal inflammation, conditions that may further compromise iron absorption [13]. Iron supplementation itself may exacerbate dysbiosis. Oral non-heme iron has been reported to reduce beneficial bacterial families such as Lactobacillaceae and Bifidobacteriaceae and to promote the expansion of siderophilic pathobionts, including members of the Enterobacteriaceae family (e.g., *Escherichia*, *Salmonella*, *Shigella*) and Clostridiaceae [15]. Luminal iron excess may favor microbial overgrowth and contribute to intestinal inflammation, ultimately affecting iron absorption [15–18]. Several studies have shown that the use of probiotics, prebiotics, and postbiotics may enhance iron absorption, contributing to both the treatment and prevention of anemia [19, 20], supporting the important role of the gut microbiota in iron metabolism. Collectively, these observations suggest that modulating the gut microbiota during iron supplementation may represent a

strategy to enhance iron bioavailability while limiting dysbiosis. Based on this rationale, we developed an iron-based supplement combining micronized, dispersible ferric pyrophosphate with BIOintestil[®], a food supplement containing Palmrose essential oil and ginger rhizome powder. BIOintestil[®] has already been tested in murine models of colitis and in patients with Irritable Bowel Syndrome (IBS), where it demonstrated the ability to modulate the gut microbiota by increasing the relative abundance of commensal bacteria, including SCFA producers, and reducing the abundance of pathobionts [21, 22]. The aim of this study was to assess whether a formulation combining a microbiota modulator with micronized, dispersible ferric pyrophosphate, administered at a lower dose on alternate days, could improve iron status, as assessed by serum ferritin and other prespecified iron-related and hematological parameters, compared with standard daily iron supplementation. To this end, we enrolled subjects with iron deficiency and randomized them into two groups: one received a formulation consisting of a microbiota modulator plus low-dose iron administered on alternate days (21 mg every 2 days), and the other received standard daily iron-based therapy (30 mg/day).

Materials and methods

Study design

This was a randomized, parallel-group, active-controlled study conducted over 42 days. The primary objective of this study was to evaluate the effect of an alternate-day supplementation regimen providing 21 mg of elemental iron every two days, provided as micronized, dispersible ferric pyrophosphate in combination with daily administration of a gut microbiota modulator (BIOintestil[®]), and to compare this regimen with 30 mg of elemental iron per day, provided as Sucrosomial[®] ferric pyrophosphate, which is considered one of the most effective oral iron preparations for the treatment of iron deficiency [23, 24]. Both supplements were commercially available and also contained vitamins and excipients. The potential contribution of individual vitamins or excipients was not analyzed separately, as the study was designed to compare the overall effects of two commercially available supplementation strategies.

The composition, declared amounts of the main functional ingredients and micronutrients, and administration regimen of both supplements are reported in Supplementary Table S1.

The primary endpoint of the study was the change in serum ferritin over time. Secondary endpoints included changes in the other measured biochemical iron-status parameters, namely serum iron concentration, total

iron-binding capacity (TIBC), serum transferrin, and transferrin saturation (TSAT%), as well as the hematological parameters hemoglobin concentration and red blood cell (RBC) count. Safety and tolerability were evaluated by monitoring adverse events, gastrointestinal symptoms, treatment discontinuations, serum C-reactive protein (CRP), and leukocyte count. Finally, we analyzed tolerability of the supplementation regimens by evaluating the incidence and nature of adverse events (AEs).

Study population

The study population consisted of healthy female subjects of Caucasian ethnicity, aged between 18 and 50 years, enrolled according to predefined inclusion and exclusion criteria. Inclusion criteria were: female sex, Caucasian ethnicity, childbearing age (18–50 years), ferritin levels below the normal range (<30 ng/mL) associated with symptoms of iron deficiency (e.g. fatigue and weakness), normal value of CRP and leukocyte count. Exclusion criteria were: acute, chronic, or progressive disease or any condition that, in the investigator's judgment, may influence the results of the study or may make iron intake risky for the participant; known food intolerances, food allergies, or hypersensitivity to any of the ingredients of the investigational supplements; current use of food supplements and/or products with the same or potentially overlapping activity as the study products, or absence of an adequate washout period of at least one month after their discontinuation; and participation in any other experimental clinical study involving the use of drugs or food supplements in the 3 months prior to enrolment. Menstrual status was recorded at enrolment as part of the clinical assessment. Baseline body weight and height were recorded at enrolment, and body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared (kg/m^2).

All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and was approved by the International Ethics and Integrity Committee, approval No. NT0001616/24, date of approval 17 September 2025.

Study treatments and randomization

Eligible participants were randomly assigned (1:1) to receive one of two oral iron supplements for 6 weeks. Product 1 (Eurekol Ferro®) consisted of micronized, dispersible ferric pyrophosphate providing 21 mg of elemental iron every other day in combination with a daily gut microbiota modulator. Product 2 (benchmark comparator) consisted of Sucrosomial® ferric pyrophosphate providing 30 mg of elemental iron per day. In the arm using micronized,

dispersible ferric pyrophosphate in combination with the gut microbiota modulator (Product 1), subjects followed a daily supplementation regimen consisting of two different capsules administered on alternating days. One capsule, taken on odd-numbered days, did not contain iron and included only the microbiota modulator, a gastro-resistant formulation based on Palmrose essential oil (*Cymbopogon martinii*) adsorbed on a ginger rhizome powder matrix. The other capsule, taken on even-numbered days, contained micronized, dispersible ferric pyrophosphate providing 21 mg of elemental iron, and also included the gut microbiota modulator. Thus, from the participant's perspective, the Product 1 regimen consisted of once-daily capsule intake, while iron exposure occurred every 48 h. This regimen ensured continuous daily administration of the gut microbiota modulator to support intestinal microbial homeostasis [22], while iron was administered every 48 h, in accordance with previous evidence suggesting that alternate-day dosing may limit the hepcidin-mediated reduction in iron absorption [10–12]. Capsules were taken orally once daily, at any time of the day, with a glass of water. In the benchmark arm (Product 2), subjects received one capsule of Sucrosomial® ferric pyrophosphate providing 30 mg of elemental iron daily [23]. Both supplements were produced in Italy, in accordance with the guidelines and regulations applicable in the field, and were available on the market. Further details on product composition and administration regimen are provided in Supplementary Table S1.

Study procedures

The study consisted of a screening and baseline visit at T0, and two follow-up visits after 14 ± 2 days of treatment (T14), and after 42 ± 2 days of treatment (T42). At the baseline visit (T0), eligibility was verified according to the predefined inclusion and exclusion criteria, demographic data were collected, and written informed consent was obtained. Subjects were then randomized to receive one of the two food supplements and were provided with the assigned study product. Participants were instructed not to change their usual daily routine or lifestyle throughout the intervention period, and no specific dietary modification was prescribed. Fasting blood samples for baseline laboratory assessments were collected the following morning (T0+1), prior to starting iron supplementation. At the follow-up visit (T14), eligibility criteria were re-evaluated, treatment compliance was assessed by counting returned capsules, and adverse events and treatment discontinuations were recorded. Subjects received additional study products to complete the treatment period. Fasting blood samples were collected the following morning (T14+1), prior to product intake. At the final visit (T42), treatment compliance was again assessed

by counting returned capsules, and any adverse events or treatment discontinuations were recorded. Study procedures are summarized in Fig. 1.

Blood collection, sample preparation, and laboratory analyses

Fasting venous blood samples were collected in the morning at baseline (T0+1), after 14 ± 2 days of treatment (T14+1), and after 42 ± 2 days of treatment (T42+1). At T0+1 and T14+1, blood samples were collected before product intake. Blood samples for biochemical assessment of iron status and CRP were collected in serum tubes and processed according to standard clinical laboratory procedures. After clotting and centrifugation, serum was used for the measurement of ferritin, serum iron, transferrin, total iron-binding capacity (TIBC), transferrin saturation (TSAT), and C-reactive protein (CRP). Whole blood collected in EDTA tubes was used for hematological analyses, including hemoglobin concentration, red blood cell count, and leukocyte count. Laboratory analyses were performed according to routine clinical laboratory procedures.

Compliance assessment

The principal investigator and co-investigators kept a record of the products delivered to the subjects at the beginning of the trial, and unused capsules were withdrawn and counted at the end of the trial. Subjects were defined as compliant if they used 80–120% of the treatment regimen over the 42-day period.

Safety assessment

Clinical safety and tolerability were assessed throughout the study by monitoring adverse events, treatment discontinuations, and gastrointestinal symptoms, together with serum

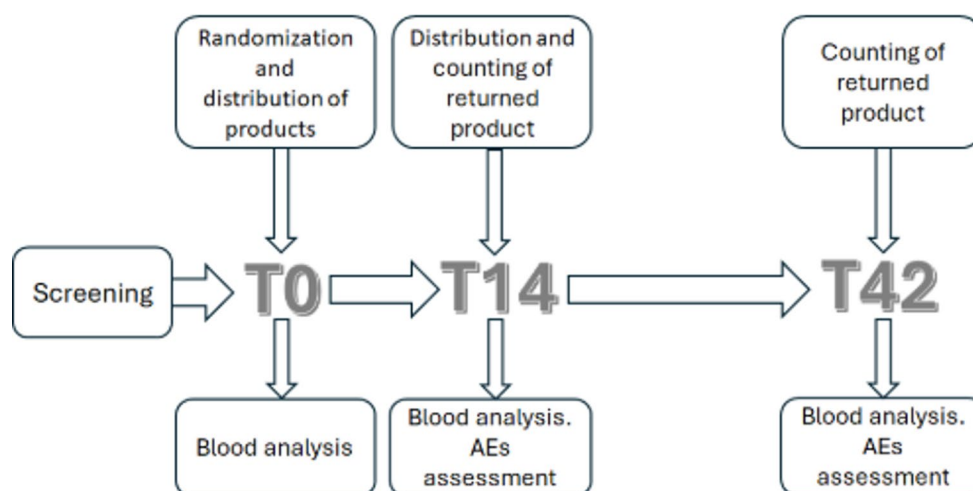
CRP and leukocyte count at T14 and T42. These laboratory parameters were used to support the clinical safety assessment by monitoring clinically relevant inflammatory changes during the intervention. AEs were recorded by the principal investigator and co-investigators. Subjects were asked to report any adverse events or gastrointestinal symptoms at each study visit or at any time during the study period. In case of reported events, the investigator evaluated their severity and their potential relationship to the study product based on clinical judgment, temporal association, and available medical information. Adverse events were classified according to their likelihood of causal relationship with the study product (very likely, likely, not clearly attributable, unlikely, or excluded). For each reported AE, information regarding onset, duration, intensity, outcome, and any action taken were recorded, and all AEs were followed until resolution or stabilization.

Sample size calculation and statistical analysis

Sample size estimation was based on data derived from a previous reference study [25] using a similar iron supplementation strategy and was anchored to the expected mean change in serum ferritin and the corresponding variability between groups. Assuming a statistical power of 95% and a two-sided significance level of 0.05, the minimum required sample size was estimated at 10 subjects per group (20 in total). Considering the shorter duration of the present intervention (6 weeks compared with 6 months in the reference study) and the inclusion of additional components intended to support gut function and modulate iron absorption, the sample size was conservatively increased threefold to 60 participants (30 per group). To further account for potential dropouts, an additional 10% was planned, resulting in total target enrolment of 66 subjects.

Inferential statistical analyses were performed on the efficacy endpoints, including ferritin, serum iron, TIBC,

Fig. 1 Study design flowchart



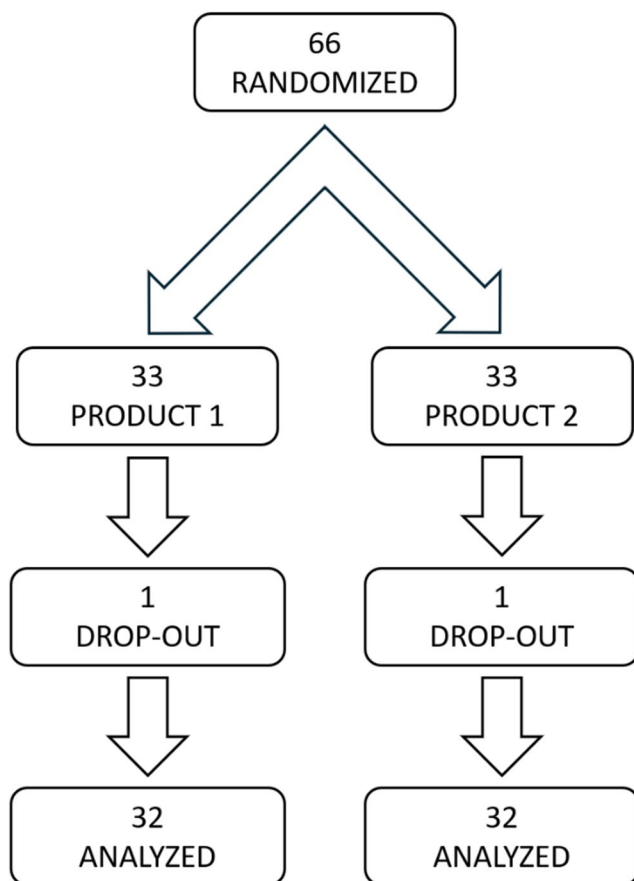


Fig. 2 Study enrollment and participant disposition flowchart

TSAT%, transferrin, hemoglobin (Hb), RBC. After verifying the assumptions for applicability, a parametric approach was applied. A two-way mixed-design ANOVA was used to analyze the quantitative variables. When a significant effect of the time variable occurred, post hoc comparisons were performed using Bonferroni-corrected pairwise t-tests. The threshold of significance, α level, was established at 0.05. Statistical analysis was performed using RStudio (Posit Software, Boston, MA, USA).

Results

Enrolled cohorts

A total of 66 participants were enrolled and randomized in a 1:1 ratio to Product 1 or Product 2, with 33 participants allocated to each treatment group (Fig. 2). Two participants discontinued the study prematurely due to mild gastrointestinal adverse events, one in each treatment group. Therefore, 64 participants completed the study according to the protocol and were included in the per-protocol analysis, with 32 participants in each treatment group. At baseline, the mean

Table 1 Baseline demographic, anthropometric, and clinical characteristics of the study population (T0)

	Product 1 (n=32)	Product 2 (n=32)
Age (years)	33.8±1.4	33.7±1.5
Female sex, n (%)	32 (100)	32 (100)
Caucasian ethnicity, n (%)	32 (100)	32 (100)
Body weight (kg)	61.2±0.9	60.3±0.9
Height (cm)	163.6±0.8	162.8±1.0
BMI (kg/m ²)	22.9±0.3	22.8±0.3
Ferritin (ng/mL)	19.8±1.3	20.7±1.2
Serum iron (µg/dL)	67.9±4.9	65.5±4.9
Transferrin (µg/dL)	316.3±5.4	320.3±7.0
Total iron-binding capacity (µg/dL)	373.7±7.7	381.3±9.4
Transferrin saturation (%)	16.7±1.4	16.4±1.3
Hemoglobin (g/dL)	12.9±0.1	12.6±0.2
Red blood cells (10 ⁶ /µL)	4.5±0.1	4.5±0.1
C-reactive protein (mg/L)	0.5±0.1	0.8±0.2
Leukocyte count (10 ³ /µL)	6.8±0.3	7.1±0.3

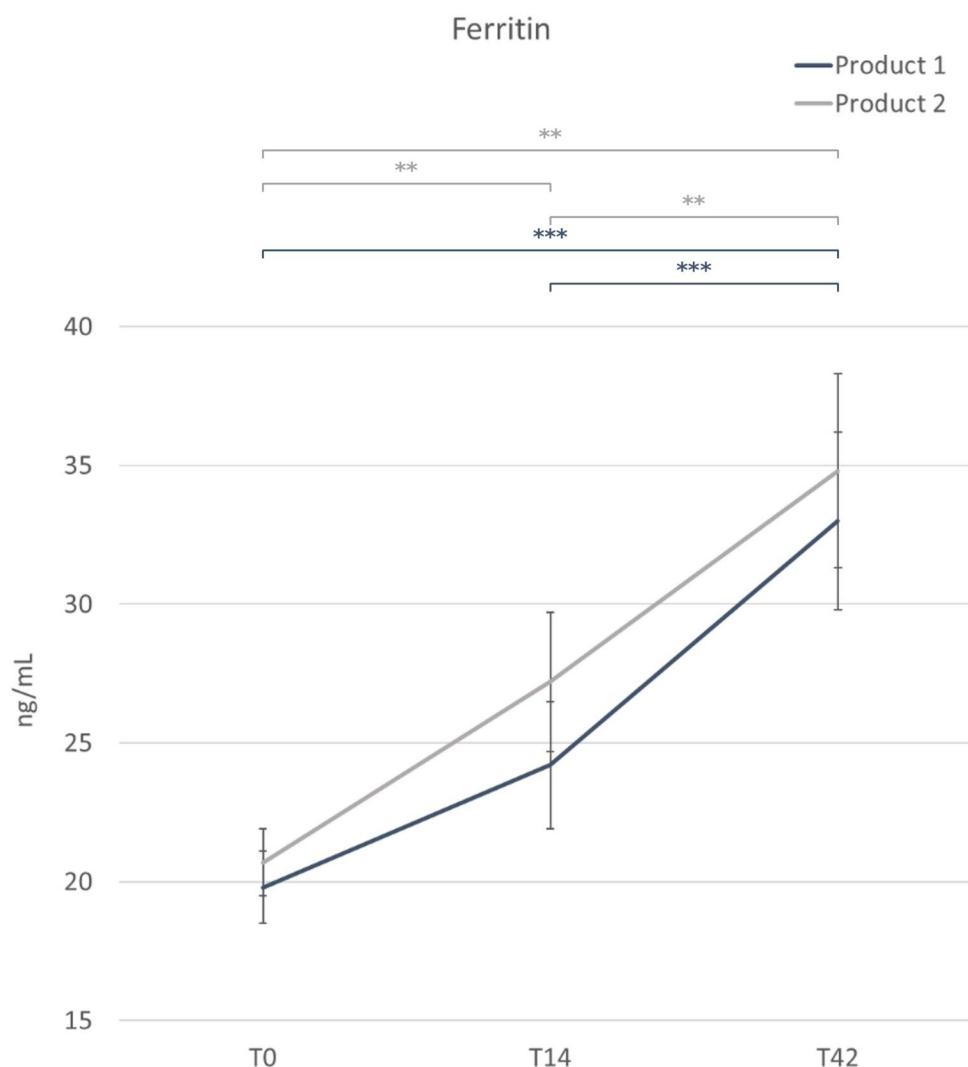
age of the study population was 33.8±1 years (range 19–49 years). BMI values were within the normal-weight range in all participants included in the final analysis (Product 1: 19.3–24.9 kg/m²; Product 2: 19.0–24.8 kg/m²). Menstrual status was comparable between the two treatment groups. Regular menstrual cycles were reported by 28/32 subjects in the Product 1 group and 26/32 subjects in the Product 2 group, whereas irregular cycles were reported by 4/32 and 6/32 subjects, respectively. No women with self-reported heavy menstrual bleeding were present in either treatment group. Baseline demographic, anthropometric, menstrual, and clinical characteristics are summarized in Table 1.

Continuous variables are expressed as mean±SEM; categorical variables are reported as n (%). Treatment compliance was high in both arms (mean 99% in both groups), with all subjects included in the per-protocol analysis meeting the predefined compliance criteria.

Effects of the interventions on serum ferritin

In both treatment groups, mean serum ferritin levels increased progressively over time (Fig. 3). No significant differences between treatments were observed ($p=0.537$), whereas a significant effect of time was detected ($p<0.001$), with no significant treatment × time interaction ($p=0.696$), indicating a comparable temporal pattern between the two interventions. Post hoc analyses showed a significant increase in serum ferritin between baseline (T0) and the end of the study (T42) in both groups. In the Product 1 group, the increase was significant between T0 and T42 ($p<0.001$) and between T14 and T42 ($p<0.001$), while the T0–T14 comparison approached statistical significance ($p=0.051$). In the Product 2 group, significant increases were observed

Fig. 3 Mean serum ferritin levels (ng/mL) in Product 1 and Product 2 groups, across all time points ($\pm$ SEM). Horizontal brackets indicate significant post hoc comparisons between time points within each treatment group (blue: Product 1; grey: Product 2). ** $p < 0.01$; *** $p < 0.001$



between all-time points (T0–T14, T0–T42, and T14–T42; all $p \leq 0.002$).

Effects of the interventions on iron-status parameters

Overall, iron-status parameters showed significant time-dependent changes in both treatments, with comparable temporal patterns between groups. Mean transferrin levels decreased over time in both groups (Fig. 4A), with a significant main effect of time ($p < 0.001$) but no significant difference between treatments ($p = 0.760$) and no significant differences between groups over time ($p = 0.051$). Post hoc analyses revealed a significant reduction in transferrin between baseline (T0) and T42 in the Product 1 group ($p = 0.002$), while no significant differences were observed between T0 and T14 ($p = 0.216$) or between T14 and T42 ($p = 0.345$). In the Product 2 group, significant reductions were detected between T0 and T14 ($p < 0.001$) and between

T0 and T42 ($p < 0.001$), whereas the comparison between T14 and T42 was not statistically significant ($p = 0.172$). Transferrin saturation (TSAT%) increased significantly over time in both treatment groups (Fig. 4B). Two-way mixed-design ANOVA revealed a significant effect of time ($p < 0.001$), with no significant difference between treatments ($p = 0.927$) and no significant differences between groups over time ($p = 0.829$). Post hoc analyses demonstrated a significant increase in TSAT% between T0 and T42 in the Product 1 group ($p = 0.008$), with no significant differences between T0 and T14 ($p = 1.000$) or between T14 and T42 ($p = 0.110$). Similarly, in the Product 2 group, TSAT% increased significantly between T0 and T42 ($p = 0.006$), whereas comparisons between T0 and T14 ($p = 0.326$) and between T14 and T42 ($p = 0.371$) were not statistically significant.

Mean serum iron levels increased progressively over time in both groups (Fig. 4C). Two-way mixed-design ANOVA showed a significant effect over time ($p < 0.001$), with no

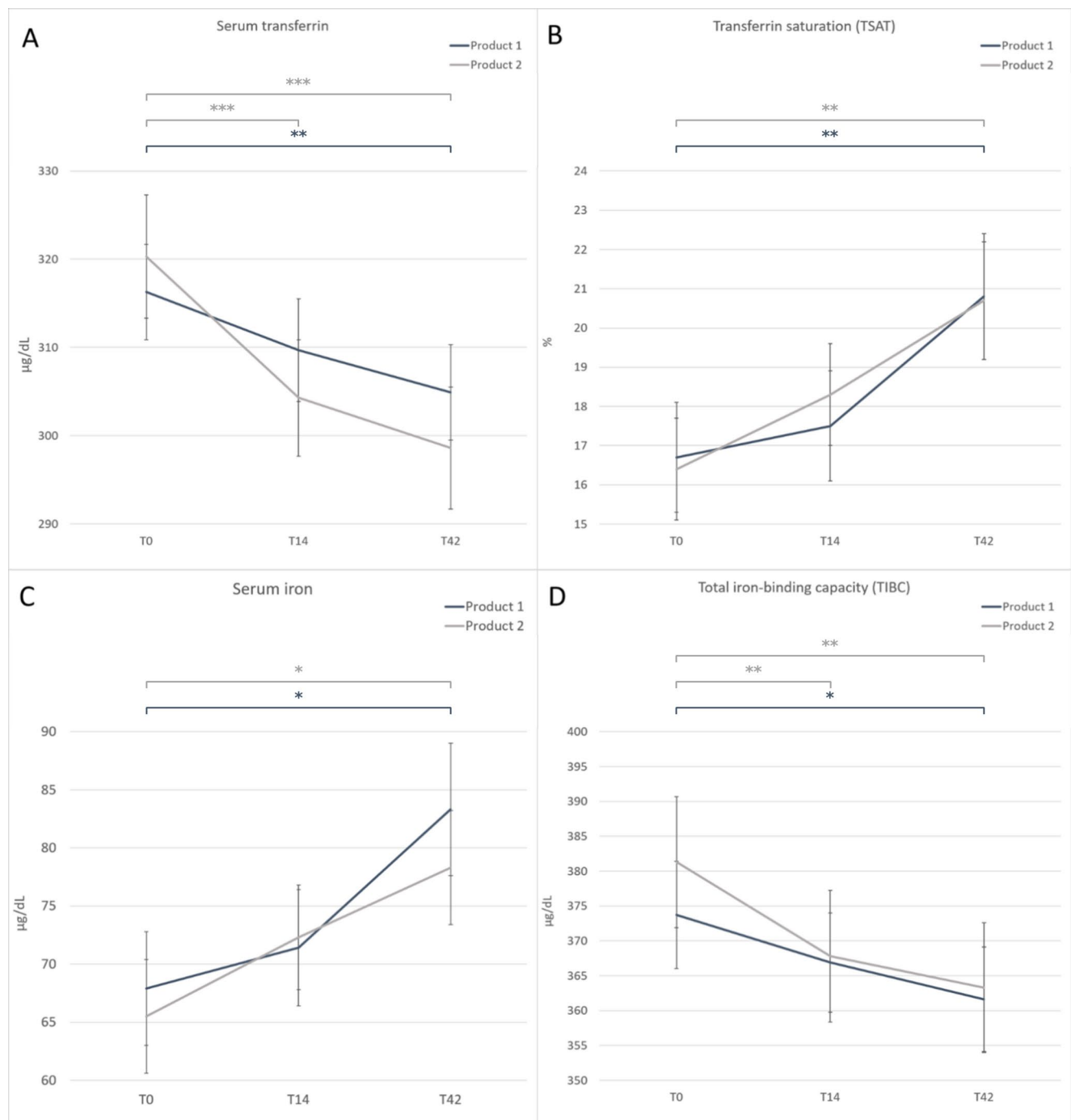


Fig. 4 Iron-status parameters in Product 1 and Product 2 groups across all time points. Mean serum transferrin concentration (Panel A), transferrin saturation (TSAT; Panel B), serum iron concentration (Panel C), and total iron-binding capacity (TIBC; Panel D) are shown at T0, T14,

and T42. Data are presented as mean $\pm$ SEM. Horizontal brackets indicate significant post hoc comparisons between time points within each treatment group (blue: Product 1; grey: Product 2). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

significant differences between treatments ($p=0.714$) and no significant differences between groups over time ($p=0.667$). Post hoc comparisons indicated a significant increase in serum iron between T0 and T42 in the Product 1 group ($p=0.010$), while differences between T0 and T14 ($p=1.000$) and between T14 and T42 ($p=0.082$) were not

significant. Similarly, in the Product 2 group, serum iron increased significantly between T0 and T42 ($p=0.040$), with no significant difference between T0 and T14 ($p=0.263$) or between T14 and T42 ($p=0.781$). TIBC decreased during the study period in both treatment groups (Fig. 4D). A significant main effect of time was observed ($p<0.001$), with

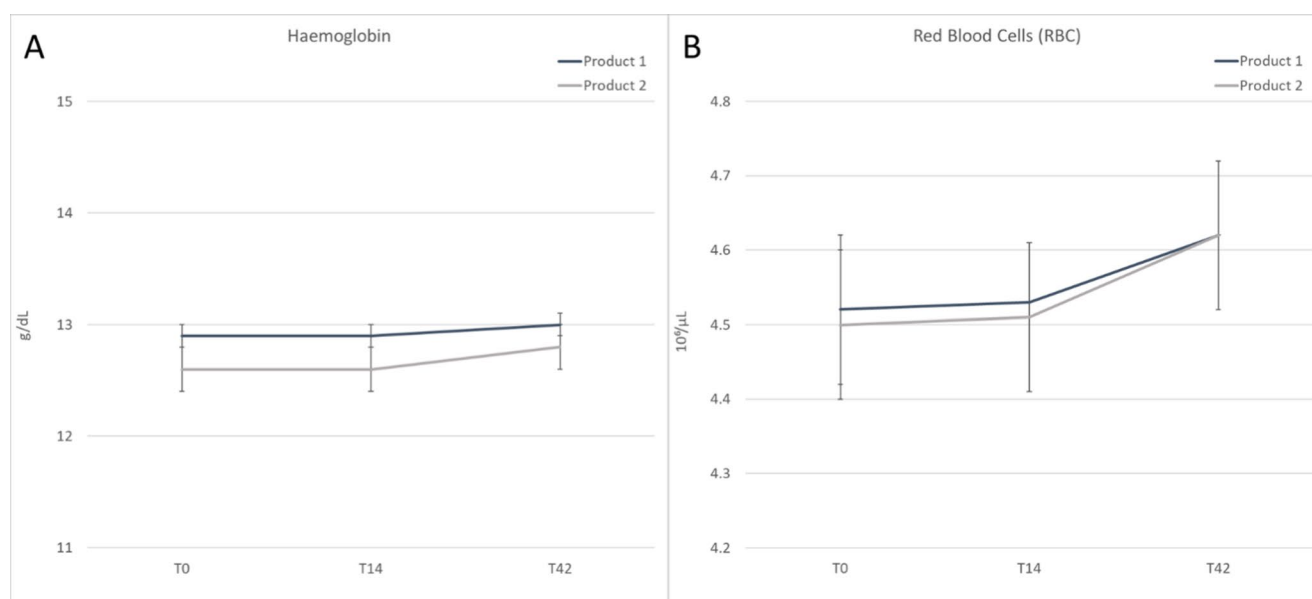


Fig. 5 Mean hemoglobin concentration (Panel **A**) and mean red blood cell count (Panel **B**) in Product 1 and Product 2 groups across all time points. Data are presented as mean $\pm$ SEM

no significant difference between treatments ($p=0.766$) and no significant differences between groups over time ($p=0.477$). Post hoc analyses showed a significant reduction in TIBC between T0 and T42 in the Product 1 group ($p=0.046$), whereas no significant differences were found between T0 and T14 ($p=0.331$) or between T14 and T42 ($p=0.472$). In the Product 2 group, significant reductions were observed between T0 and T14 ($p=0.006$) and between T0 and T42 ($p=0.001$), while the comparison between T14 and T42 was not statistically significant ($p=0.682$).

Hemoglobinemia and RBC count

Mean hemoglobin levels remained stable over time in both treatment groups, with no substantial differences after the interventions (Fig. 5A). Two-way mixed-design ANOVA revealed no significant differences between groups ($p=0.114$), no significant effect of time ($p=0.064$), and no significant differences between groups over time ($p=0.648$), indicating that hemoglobin levels did not change significantly over the course of the study in either group. A slight increase in mean RBC count was observed over time in both treatment groups (Fig. 5B); however, this change was modest and post hoc Bonferroni-corrected pairwise comparisons did not reveal statistically significant differences between individual time points in either group. Two-way mixed-design ANOVA showed no significant differences between treatments ($p=0.900$) but a significant main effect of time ($p=0.005$), with no significant differences between groups over time ($p=0.937$), indicating a comparable temporal pattern between the two interventions. In the Product

1 group, no significant differences were observed between T0 and T14 ($p=1.000$), T0 and T42 ($p=0.257$), or T14 and T42 ($p=0.221$). Similarly, in the benchmark group (Product 2), no significant differences were detected between T0 and T14 ($p=1.000$), T0 and T42 ($p=0.111$), or T14 and T42 ($p=0.076$).

Safety and adverse events (AEs)

CRP and blood leukocyte count remained within the normal range (CRP <10 mg/L; leukocyte count $4.5\text{--}11 \times 10^3/\mu\text{L}$) in all subjects at all observation times. Mean serum CRP values remained low throughout the study in both groups (Product 1: 0.46 ± 0.09 , 0.56 ± 0.14 , and 0.44 ± 0.10 mg/L at T0, T14, and T42; Product 2: 0.76 ± 0.24 , 0.53 ± 0.16 , and 0.38 ± 0.08 mg/L, respectively). Mean leukocyte counts also remained within the normal range (Product 1: 6.78 ± 0.29 , 6.61 ± 0.30 , and $6.66 \pm 0.28 \times 10^3/\mu\text{L}$ at T0, T14, and T42; Product 2: 7.11 ± 0.29 , 6.93 ± 0.28 , and $6.72 \pm 0.22 \times 10^3/\mu\text{L}$, respectively). Only mild gastrointestinal adverse events were reported in both treatment groups. In the Product 1 group, six subjects reported transient symptoms such as nausea, stomach irritation, and/or bloating, which mainly occurred during the first days of treatment and resolved spontaneously or after taking the product with meals. One subject in the Product 1 group discontinued the study due to gastrointestinal intolerance. In the Product 2 group, five subjects reported similar mild gastrointestinal symptoms, with one subject discontinuing the study due to gastrointestinal intolerance. All reported adverse events were classified as mild in severity and were considered related to the study

products based on their temporal association and known gastrointestinal tolerability profile of oral iron supplements. No serious adverse events were reported. Overall, the supplements showed a comparable safety profile and were well tolerated.

Discussion

Although iron deficiency is rarely a life-threatening condition, its management represents a significant clinical challenge [26]. This paradox arises from the unique biology of iron homeostasis: iron is an essential micronutrient, yet its absorption is tightly restricted by regulatory mechanisms, and its excess, particularly at the intestinal level, is inherently toxic. Unlike most other dietary minerals, iron is a redox-active transition metal capable of catalyzing the generation of reactive oxygen species and lacks a dedicated excretory pathway. As a result, intestinal iron absorption is stringently controlled by the hepcidin–ferroportin axis, and unabsorbed iron remains in the gut lumen and may exert deleterious effects on the gut epithelium and on the intestinal microbiota. These features make oral iron supplementation intrinsically difficult to manage, as increasing the administered dosage does not necessarily translate into greater absorption but may instead amplify gastrointestinal intolerance and dysbiosis. Intravenous iron supplementation is generally more effective than oral iron, particularly in conditions associated with impaired absorption or inflammation, but it is not devoid of adverse reactions. Moreover, it requires administration in a clinical setting and is associated with substantially higher costs [27]. Recently, novel oral formulations including lipophilic iron donor ferric maltol, sucrosomial iron (ferric pyrophosphate encapsulated in lipophilic micelles), and liposomal iron (iron incorporated into phospholipid vesicles) have been developed to enhance iron absorption and improve its gastrointestinal tolerability by limiting luminal exposure to free iron [28]. In our study a reduced dose of elemental iron, combined with a microbiota-modulating component previously shown to affect intestinal microbiota composition, achieved improvements in ferritin and iron-status parameters comparable to those observed with sucrosomial iron, thus representing a valid alternative strategy. This reduction in iron dosage was also achieved by using alternate-day administration, a strategy designed on the basis of previous evidence on the hepcidin response to oral iron dosing [10–12]. Using this strategy, improvements in iron-status parameters were broadly comparable at the end of the study in the two arms, despite an administration of approximately one third of the cumulative iron dose in the first group. At the first time point analyzed (T14), some iron-status parameters showed a more

gradual response in the Product 1 group, particularly ferritin, transferrin, and TIBC, whereas serum iron and TSAT did not show relevant between-group differences. This early response profile of Product 1 may reflect differences in the underlying mechanisms of action. While higher-dose iron formulations may exert more immediate effects through direct iron delivery, the reduced iron strategy also relies on a simultaneous action on the intestinal ecosystem: modulation of the gut microbiota requires days to develop [29] and may therefore contribute to a gradual, rather than immediate, enhancement of iron absorption. Gut microbiota composition was not directly assessed in the present study. Therefore, we cannot determine whether the observed effects were associated with changes in microbial composition or whether BIOintestil® exerted microbiota-related effects in this specific population of women with iron deficiency. Previous studies on BIOintestil® were conducted in IBS and dysbiosis-related contexts [21, 22] and were used here only to support the biological rationale for including a microbiota-modulating component, rather than as direct evidence of microbiome modulation in the present cohort. In those previous studies, BIOintestil® was associated with changes in selected bacterial taxa, including a reduction in Clostridiaceae [22], a bacterial family previously discussed in relation to dysbiosis and altered iron metabolism [13]; however, whether similar effects occur in women with iron deficiency remains to be demonstrated.

The hematological findings should also be interpreted in the context of the baseline characteristics of the study population and the short duration of the intervention. Hemoglobin levels remained stable throughout the study, which is not unexpected because participants had iron deficiency but did not show marked anemia at baseline. In iron-deficient subjects without overt or severe anemia, restoration of iron stores and improvement in circulating iron parameters may precede detectable changes in hemoglobin concentration. Although RBC count showed a slight time-dependent increase, post hoc analyses did not identify significant differences between individual time points within either group. Therefore, this change should be interpreted cautiously and may reflect early erythropoietic adaptation or physiological variability rather than a clinically relevant increase in oxygen-carrying capacity.

The present findings should also be interpreted in relation to the enrolled population, which consisted of women with iron deficiency and low ferritin but without marked anemia at baseline. Patients with established iron deficiency anemia may show different hepcidin regulation because increased erythropoietic activity can suppress hepcidin and modify the response to oral iron dosing strategies. Dedicated studies in patients with IDA, ideally including serial hepcidin measurements, are therefore warranted.

This study supports the concept that optimizing intestinal iron handling, rather than increasing elemental iron exposure beyond physiological absorptive capacity, may represent a more rational and sustainable supplementation strategy. The use of a low-dose, alternate-day iron regimen is consistent with current evidence on hepcidin-mediated regulation of iron absorption and has been already evaluated in a double-blinded, randomized, placebo-controlled trial, on young women [30]. This strategy reduced iron deficiency at 6 months and triggered fewer gastrointestinal side effects. This alternate day strategy may also limit the accumulation of unabsorbed iron in the intestinal lumen, a factor known to contribute to gastrointestinal intolerance and microbiota imbalance [31]. In this context, based on previous evidence on BIOintestil[®], the continuous administration of a gut microbiota-modulating supplement may further support iron bioavailability by preserving intestinal homeostasis. Nevertheless, gut microbiome analysis was not included among the prespecified endpoints of the present study, since the scientific community is still investigating the mechanisms underlying the role of the gut microbiome in iron absorption [32]. Although the present study was not designed to evaluate primary prevention of anemia, these results suggest that this approach may be particularly suitable for the long-term management of chronic or recurrent iron deficiency, including mild iron deficiency anemia, where prolonged supplementation is often required. By combining preserved efficacy with reduced elemental iron exposure, this strategy may help maintain iron homeostasis over time while improving tolerability and adherence, which are critical factors in real-world clinical settings.

In a recent pilot double-blinded randomized control trial in iron-deficient young women [33], daily oral elemental iron (60 mg) for 14 days was more effective than equivalent amounts (120 mg) administered on alternate days in improving hematological parameters (mean corpuscular volume, mean corpuscular hemoglobin and reticulocyte counts). These results, which apparently contradict ours, should be interpreted in light of the short duration (only 14 days) and the very high doses of iron used. It is somewhat expected that a single dose of 120 mg would be only partially absorbed, especially given that ionic iron was used, without formulations capable of improving its absorption by enterocytes. Our study suggests that the administered iron dose can be reduced (from 60 to 21 mg every two days) without an apparent loss of effectiveness of supplementation. This reduction in iron dose may be desirable because lower luminal exposure to unabsorbed iron could theoretically limit iron-mediated oxidative and microbiota-related effects at the intestinal level, although oxidative stress biomarkers were not measured in the present study [31].

During our study, none of the iron supplements tested caused alterations in inflammatory parameters, such as CRP or leukocyte count. Within the limits of this short-term clinical safety assessment, both formulations appeared well tolerated, with less than 20% of subjects reporting transient symptoms, predominantly intestinal, in both study arms. These mild AEs, which were considered to be plausibly related to the food supplement intake, resolved spontaneously. One treatment discontinuation due to gastrointestinal intolerance occurred in each study group. Although adherence was very high during the 42-day controlled study, these findings reflect treatment efficacy under supervised conditions and may not fully predict long-term effectiveness in routine practice. In the investigational group, participants took one capsule daily, while only the iron content alternated between days, thereby limiting the complexity usually associated with alternate-day dosing. Nevertheless, longer pragmatic studies in larger populations are needed to evaluate persistence, acceptability, and adherence during prolonged use. The smaller early change observed between T0 and T14 should therefore not be interpreted as evidence of poor compliance, but more likely reflects the lower cumulative iron exposure and the gradual time course of iron-store repletion.

The limitations of this study include its single-center design and relatively short duration (6 weeks), which do not allow for long-term conclusions regarding tolerability, sustainability of the supplementation strategy, or persistence of the effects after treatment discontinuation. In particular, no post-intervention follow-up was performed after cessation of supplementation; therefore, whether the improvements in ferritin and other iron-status parameters are maintained over time remains to be determined. Although baseline body weight, height, and BMI were available, detailed body composition parameters, such as fat mass and lean mass, were not assessed. Dietary intake was not quantitatively assessed using standardized dietary records, dietary recalls, or food-frequency questionnaires; therefore, habitual daily dietary iron intake could not be determined and potential differences in dietary iron exposure between groups or diet-treatment interactions cannot be excluded. Participants were instructed not to change their usual daily routine or lifestyle during the intervention, and the randomized longitudinal design helps reduce the likelihood that stable individual dietary characteristics alone account for the observed between-group pattern of longitudinal changes. Nevertheless, dietary intake may vary over time and was not formally monitored; therefore, residual dietary confounding remains possible. Accordingly, the present findings should be interpreted as a comparison of the effects of the two supplementation strategies on iron-status parameters under non-standardized dietary conditions,

rather than as a direct assessment of intestinal iron absorption independent of dietary iron intake. Menstrual blood loss represents a relevant background determinant of iron deficiency in this cohort. Nevertheless, menstrual status was recorded at enrolment and did not show clinically relevant imbalances between groups. However, menstrual blood loss may still have contributed to inter-individual variability in baseline iron status and response to supplementation. A further limitation of the study is that erythrocyte indices such as MCV, MCH, and MCHC were not available in the original dataset. Although these parameters could have provided additional information on microcytosis and hypochromia, particularly in subjects with overt iron-deficiency anemia, their absence limited the morphological characterization of the hematological response. Hepcidin concentrations were not measured in the present study. A reliable evaluation of the hepcidin response would have required a specifically designed mechanistic protocol, including standardized fasting blood collection at consistent times of day and at predefined intervals before and after iron administration. Such procedures would have increased protocol complexity and participant burden beyond the pragmatic clinical proof-of-concept design of the present study. Therefore, the proposed hepcidin-mediated rationale for alternate-day dosing remains based on previous evidence and cannot be directly confirmed in this cohort. The study also did not include specific biomarkers of oxidative stress or circulating inflammatory cytokines. Therefore, although the reduced-dose regimen was designed to limit the potential biological effects associated with unabsorbed iron, the present data do not directly demonstrate a reduction in systemic oxidative stress or cytokine-mediated inflammation. Gut microbiome composition was not assessed at baseline or after treatment. Therefore, although BIOintestil[®] was included on the basis of its previously reported microbiota-modulating properties, the present findings do not demonstrate that it modified the gut microbiota in women with iron deficiency or that microbiota modulation mediated the observed response.

Future mechanistic studies should include repeated standardized serum or plasma hepcidin-25 measurements, standardized redox biomarkers, selected inflammatory mediators, longitudinal gut microbiome profiling, and appropriate functional and metabolic analyses. Future clinical studies should also include longer treatment periods and post-intervention follow-up visits to determine whether the benefits of reduced-dose alternate-day iron supplementation are maintained after treatment cessation and whether they remain comparable to those achieved with standard daily supplementation.

Conclusions

In this randomized controlled study, a reduced-dose, alternate-day regimen of micronized, dispersible ferric pyrophosphate combined with a gut microbiota modulator achieved improvements in ferritin and other iron-status parameters comparable to those observed with standard daily sucrosomial iron, despite lower cumulative iron exposure. Both regimens were generally well tolerated. Larger and longer-term studies are needed to confirm whether this strategy may offer a sustainable alternative for the management of iron deficiency.

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Data availability The data presented in this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest Gianluigi Magri is a member of the Board of Directors of Diadema Farmaceutici Srl. Enzo Spisni and Maria Chiara Valerii are founders and shareholders of TGD Srl, the owner of the BIOintestil[®] patent. They no longer hold active roles in the company. The remaining authors declare no conflict of interest.

Informed consent Informed consent was obtained from all subjects involved in the study.

Institutional review board statement The study was conducted in accordance with the Declaration of Helsinki and approved by the International Ethics and Integrity Committee (approval No. NT0001616/24; approved on 17 September 2025).

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