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Alternate day versus consecutive day oral iron supplementation in iron-depleted women: a randomized double-blind placebo-controlled study

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Summary

Background Guidelines to treat iron deficiency recommend daily provision of oral iron, but this may decrease fractional iron absorption and increase side effects. Our objective was to compare consecutive-day versus alternate-day iron supplementation.

Methods In a double-masked, randomized, placebo-controlled trial, young Swiss women (n = 150; serum ferritin ≤30 μg/L) were assigned to: daily 100 mg iron for 90 d, followed by daily placebo for another 90 d (consecutive-day group) or the same daily dose of iron and placebo on alternate days for 180 d (alternate-day group). The study period was 24/11/2021–10/8/2022. Co-primary outcomes, at equal total iron doses, were serum ferritin and gastrointestinal side effects; secondary outcomes were iron deficiency and serum hepcidin. Compliance and side effects were recorded daily using a mobile application. Data were analysed using mixed models and longitudinal prevalence ratios (LPR). The trial was registered at ClinicalTrials.gov (NCT05105438).

Findings 75 women were assigned to each group and included in the intention-to-treat analysis. Capsule adherence and side effect reporting was >97% in both groups. At equal total iron doses, comparing consecutive-day and alternate-day groups, median serum ferritin was 43.8 μg/L (31.7–58.2) versus 44.8 μg/L (33.8–53.6) (P = 0.98), the LPR for gastrointestinal side effects on days of iron intake was 1.56 (95% CI: 1.38, 1.77; P < 0.0001), and median serum hepcidin was 3.0 nM (IQR 2.0–5.0) versus 1.9 nM (1.4–2.9) (P < 0.0001). Iron deficiency prevalence after 3 months was 5.5% versus 4.3% (P = 0.74) and after 6 months was 11.4% and 3.0% (P = 0.049).

Interpretation At equal total iron doses, compared to consecutive day dosing of iron, alternate day dosing did not result in higher serum ferritin but reduced iron deficiency at 6 months and triggered fewer gastrointestinal side effects.

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Keywords: Iron; Supplementation; Deficiency; Gastrointestinal side effects; Hepcidin; Daily; Alternate day

Introduction

Oral iron supplementation with ferrous iron salts is the standard of care to treat iron deficiency in women, with daily iron doses of 60–200 mg usually being prescribed.[1] However, iron absorption from high-dose supplements is often low,[2] and unabsorbed iron in the gut can trigger gastrointestinal (GI) symptoms, gut irritation, metallic taste, inflammation, and dysbiosis,[3] resulting in lower adherence.[4] Common GI side effects during oral iron treatment are abdominal pain, nausea, constipation and diarrhea.[4]

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Research in context

Evidence before this study

We initially searched PubMed using the search terms "iron supplementation" OR "iron supplements" OR "iron deficiency" OR "alternate iron", with no language or date restrictions. The date of our first search was April 27, 2020, and our last search was on April 01, 2021. Many papers, reviews, guidelines and textbooks recommend daily oral iron supplementation for the treatment of iron deficiency and iron deficiency anemia. These generally recommended daily doses of 100–200 mg iron given as three or four divided doses. Stable iron isotope studies showed that iron absorption was lower from consecutive day versus alternate day dosing and that iron absorption was inversely correlated with serum hepcidin. Several small, nonblinded, and short-term studies that compared consecutive day with alternate day iron supplementation reported similar or greater increases in serum ferritin and/or hemoglobin with alternate day dosing. Several studies reported that intermittent dosing (weekly or alternate day) might trigger less side effects compared to daily dosing but reporting of side effects in these studies was done retrospectively. We could find no blinded studies with daily assessment of gastrointestinal side effects comparing alternate day versus consecutive day iron supplementation.

Added value of this study

To our knowledge, this is the first double-blind, randomized, placebo-controlled long-term intervention trial comparing alternate day versus consecutive day oral iron supplementation in iron depleted women. Also, we are aware of no previous studies using a specifically-designed mobile application to assess, on a daily basis, compliance and gastrointestinal side effects comparing alternate day versus consecutive day iron supplementation. Our design minimized potential bias from inflammation and recent oral iron intake on the treatment effect on serum ferritin. We show that in iron-depleted women, compared to consecutive day dosing of iron, alternate day dosing providing the same total dose of oral iron had comparable efficacy but triggered fewer gastrointestinal side effects.

Implications of all the available evidence

In general agreement with previous studies, our findings confirm that providing oral iron on alternate days rather than on consecutive days does not result in higher serum ferritin but reduced iron deficiency and is associated with lower incidence of gastrointestinal side effects. In most women with iron deficiency, speed of response is not critical but maintaining good compliance with oral iron supplements is difficult. Alternate day iron dosing may be better tolerated by many women, and this will likely increase compliance and efficacy.

Hepcidin is the key systemic regulator of iron absorption and homeostasis in humans[5] and elevated serum hepcidin reduces dietary iron absorption.[6] After the intake of oral iron supplements (≥60 mg), serum hepcidin peaks at 8 h, remains elevated at 24 h but not at 48 h, and therefore sharply lowers fractional iron absorption (FIA) from consecutive, but not alternate day dosing.[6–8] Two previous short-term stable iron isotope studies reported that iron absorption from oral supplements (60–200 mg iron) was increased by ~35–50% when given on the alternate day, compared to when given on the consecutive day.[7,8] Recent studies that investigated whether alternate day dosing of oral iron results in higher iron stores and/or hemoglobin (Hb) were small, nonblinded, and/or had short treatment periods of 3–8 weeks.[9–13]

Intermittent oral iron supplementation may be associated with fewer GI side effects, but most studies have been nonblinded and accurate reporting of side effects is difficult.[11,14] Adverse side effects of oral iron are usually recorded retrospectively with questionnaires,[15] but this method is subject to both recall bias and inaccurate recall.[16] Used prospectively, mobile applications that allow frequent self-reporting of side effects from medications have many advantages including higher recall accuracy.[17] To underpin side effects reporting, fecal calprotectin, a marker of gut inflammation,[18] and serum intestinal fatty acid binding protein (I-FABP), a marker of enterocyte damage,[19] can be measured to assess adverse effects of iron on the gut.[3]

Therefore, our study aim was to compare the effects of consecutive day versus alternate day oral iron supplementation (100 mg) on serum ferritin and GI side effects. For this study, we specifically developed a user-friendly mobile application, which allowed subjects to record GI side effects daily using simple forced-choice questions. Comparing consecutive day dosing for 3 months to alternate day dosing for 6 months, our main hypotheses were that, at an equal total iron dose, alternate day dosing would result in: (1) lower serum hepcidin and GI side effects; and (2) higher iron absorption and serum ferritin.

Methods

Study design and participants

This randomized double-blind placebo-controlled study was conducted at the ETH Zurich in Switzerland. We recruited healthy women from the students and staff of the ETH Zurich and the University of Zurich by email announcement and advertisement on social media. Inclusion criteria were: female; age 18–45 years; serum

ferritin 30 μg/L or less (depleted iron stores); C-reactive protein (CRP) less than 5 mg/L (to exclude those with inflammation); hemoglobin (Hb) more than 11 g/dL (to exclude those with moderate-to-severe anemia); no major chronic disease; not pregnant or lactating; further inclusion criteria are in the Supplementary Material.

Randomization and masking

We randomly assigned participants to the two study groups using a computer-generated randomization list, which was generated independently by the Cantonal Pharmacy of Zurich. Randomization was done in blocks of six, which resulted in balanced numbers of participants between groups throughout enrollment. Group assignment was done by the study investigator using study IDs given to the participants when enrolled. Sealed envelopes with study group allocation were kept by an independent study monitor. Group allocation remained concealed to the participants and investigators until study end and completion of the data analyses.

Procedures

Participants in the consecutive-day group received daily 100 mg iron as $FeSO_4$ for 90 days, followed by daily placebo for another 90 days (Fig. 1). Participants in the alternate-day group received 100 mg iron as $FeSO_4$ and placebo on alternate days for 180 days. Participants in both groups received a designated kit packed by the Cantonal Pharmacy with two labelled containers (A and B) containing 50 capsules each at baseline (day 0) and at

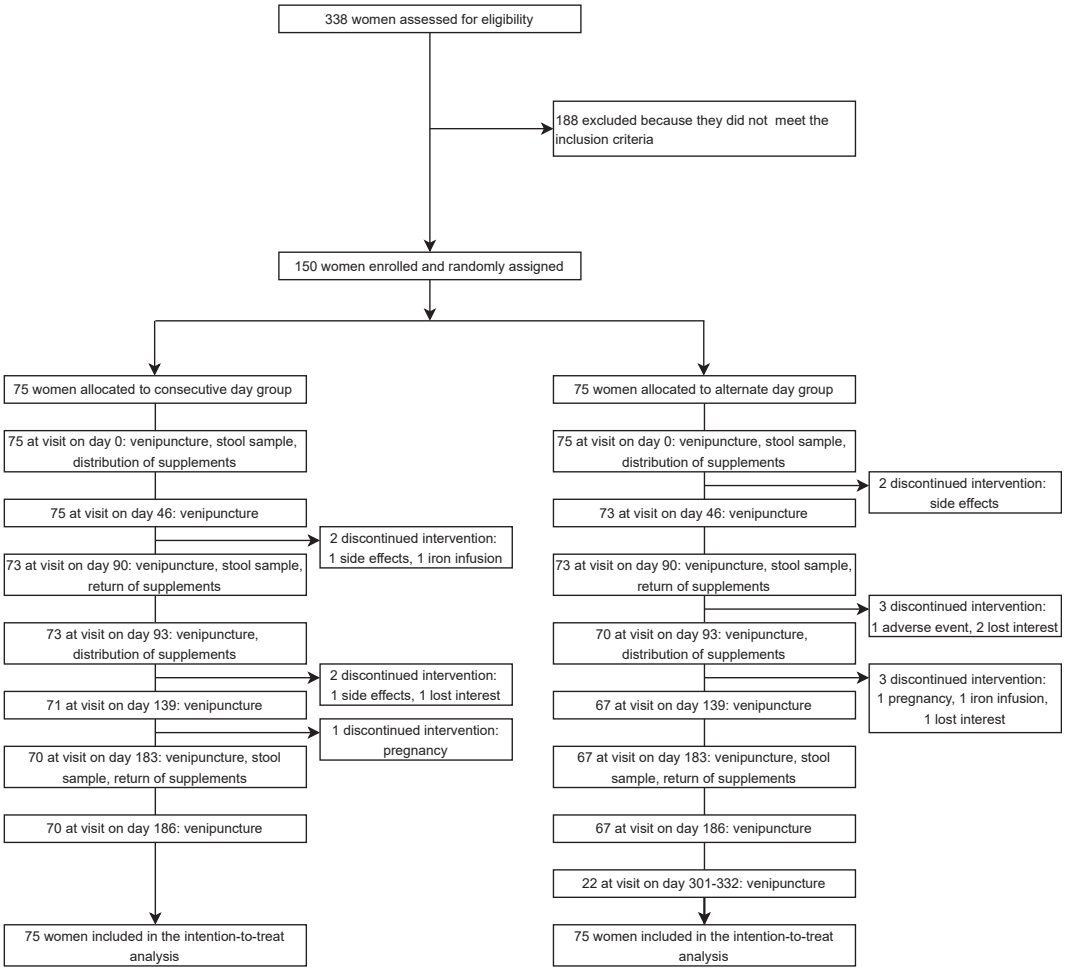

***Fig. 1:* Schematic representation of the study design**. Enrolled women were randomly assigned to either the consecutive- or alternate-day study group. Participants in the consecutive-day group received daily 100 mg iron as $FeSO_4$ for 90 days, followed by daily placebo for another 90 days. Participants in the alternate-day group received 100 mg iron as $FeSO_4$ and placebo on alternate days for 180 days. Participants came fasting to the ETH for a morning study visit every ~6 weeks for 6 months (days 0, 46, 90, 139, and 183), and for two additional visits 3 days following the midpoint and endpoint study visits (days 93 and 186). Participants from the alternate day group also came for a follow-up visit ~4 months after the study endpoint visit.

study mid-point (day 93). The consecutive-day group received two containers with iron capsules at baseline and two containers with placebo capsules at midpoint. The alternate-day group received one iron and one placebo capsule container, both at baseline and midpoint. Participants were asked to consume one capsule per day, alternating between containers A and B, in both groups, following an individual study calendar indicating from which container the capsule should be taken from each day. We recommended the subjects consume the capsules in the morning at least 1 h before breakfast with a dietary source of ascorbic acid, such as citrus fruit juice.

Participants came fasting to the ETH for a morning study visit every ~6 weeks for 6 months (days 0, 46, 90, 139, and 183), and for two additional visits 3 days following the midpoint and endpoint study visits (days 93 and 186) (Fig. 1). Participants from the alternate day group also came for a follow-up visit ~4 months after the study endpoint visit. At baseline, height and weight were measured. We collected venipuncture blood samples (~8 mL) at all study visits for assessment of Hb, iron, and inflammation status. On days 0, 90 and 183, participants brought in a stool sample, which they had collected at home within 48 h of the visit. On days 90 and 183, participants returned the capsule containers for capsule counts as a measure of compliance and a blood sample was collected. From days 90–93 to 183–186, participants did not consume any capsules, and venipuncture was then done on days 93 and 186. This delay allowed us to use serum ferritin as an unbiased measure of body iron stores, as serum ferritin is acutely elevated, independent of body iron stores, for 2 days after a high dose of supplemental iron.[6] Along with serum ferritin, soluble transferrin receptor (sTfR), α-1-acid glycoprotein (AGP), CRP, serum iron and total iron binding capacity (TIBC) were measured on days 93 and 186. Hecidin, Hb, MCV (mean corpuscular volume), hematocrit and I-FABP were measured in the blood samples collected on days 90 and 183.

During the study, participants used a mobile application to record compliance and assess GI side effects daily. This application, entitled "FeStudy", working on Android and iOS systems, with a German or English version, was specifically designed for this study. Using the mobile application, on a daily basis, participants answered forced-choice questions concerning the supplement intake (yes, no), time of intake (morning, afternoon or evening), condition of intake (with or without a meal) and if they experienced any of four GI side effects (nausea, constipation, diarrhea and/or stomach pain) after the intake (yes, no) and if yes, their severity (mild, severe). Missed data entries could only be completed within one day after the missed entry. Data was stored on the personal device (mobile smartphone) and synchronized and stored on a server database at regular intervals. Stored data from the mobile application was monitored twice weekly by the study coordinator to examine compliance and side effects. Participants were prompted to take their capsules daily through the mobile application. Participants were contacted by email if, for at least three days, capsules were not consumed, application entries were missing and/or severe GI side effects were reported.

Iron capsules consisted of 100 mg elemental iron as pharmaceutical grade (P.Eur.7.2) anhydrous $FeSO_4$ (Dr. Paul Lohmann GmbH & Co. KGaA, Lüneburg, Germany) and the excipients mannitol and syloid 244 in hydroxypropyl methylcellulose (HPMC) capsules. Placebo capsules contained only the excipients and were identical in colour, size, weight and shape. Soho Flordis International Switzerland SA (Bioggio, Switzerland) manufactured the iron and the placebo capsules packed in containers of 50 capsules, and sent them to the Cantonal Pharmacy, where the containers were relabelled and blinded.

We measured Hb immediately after venipuncture, then centrifuged the blood at 3000 rpm at 20 °C for 10 min and stored the serum at –20 °C until the day of analysis. In serum, we measured ferritin, sTfR, AGP, CRP, serum iron and TIBC, hepcidin, and I-FABP. Calprotectin was measured in stool samples. Details of the analytical methods and reference ranges can be found in the Supplementary Material.

Outcomes

The prespecified co-primary outcomes were serum ferritin and longitudinal prevalence of GI side effects, measured after three months in the consecutive-day group versus after six months in the alternate-day group, at equal total doses. Secondary outcomes were prevalence of iron deficiency, serum hepcidin, markers of iron status, CRP, AGP, I-FABP and calprotectin. Additional secondary outcomes were serum ferritin and longitudinal prevalence of GI side effects after three months in the consecutive-day group versus the alternate-day group, and after six months in the consecutive-day group versus the alternate-day group.

Statistical analysis

Our power calculation assumed a SD of 15 μg/L on ferritin based on data from previous iron supplementation studies in our laboratory, a type I error rate of 5% and 90% power. We estimated a sample size of 40 participants per group would allow us to detect a clinically-relevant difference of 10 μg/L in serum ferritin after consecutive-day and alternate-day supplementation for three and six months, respectively. We anticipated a drop-out rate of 25%, resulting in a sample size of 53 participants per group. Because of the uncertainty about effect sizes and drop-outs, we increased our final sample size to 75 participants per group, or 150 participants in total. We confirmed this sample size by an effect-size calculation for LPR: given the sample size calculated

for SF (n = 150) and the mean (SD) longitudinal prevalence from our previous study comparing consecutive day versus alternate day dosing, we were able to discriminate an LPR = 1.32.[7]

Statistical analysis was done using the SPSS statistical programming environment (IBM SPSS Software, Version 28) and Microsoft Office EXCEL 2016 (Microsoft, Redmond, WA). Primary and safety analyses were performed on an intention-to-treat basis. Normally distributed data were reported as mean (SD) and non-normally distributed data were reported as median (IQR). To assess the effects of consecutive and alternate day dosing and time on variables, we fitted linear mixed models; details are found in the Supplementary Material. We did not impute missing data. Post-hoc tests were corrected for multiple comparisons using Bonferroni step-down (Holm) correction. We estimated total iron absorption assuming 1 µg/L serum ferritin corresponding to 8–10 mg liver iron stores[20] and calculated median total iron intake during the study in the two groups assuming median obligatory iron losses of 1.34 mg/day[21] as described in the Supplementary Material. Longitudinal prevalence ratios (LPRs) for GI side effects were calculated and time-by-group effect on the occurrence of GI side effects was assessed, as described in the Supplementary Material. P values < 0.05 were considered statistically significant. The trial was registered at ClinicalTrials.gov (NCT05105438).

Ethics statement

The Canton of Zurich Ethics Committee approved the study and all subjects provided informed written consent.

Role of the funding source

All authors had access to the dataset. The funders of the study had no access to the dataset, had no role in study design, the collection, analysis, or interpretation of the data, or the decision to submit for publication and writing of the report. HKS, NUS and MBZ accept responsibility to submit for publication.

Results

Between Nov 24, 2021, and Jan 21, 2022, 75 women were enrolled into each of the two groups. Five participants in the consecutive-day group and eight in the alternate-day group discontinued participation during the study; two in each group discontinued due to side effects (Fig. 1). In both groups, 75 women were included in the intention-to-treat analysis. The study was completed on December 12, 2022. Median capsule adherence was 98.3% (IQR 95.3–100.0) in the consecutive-day group and 98.9% (96.0%–100.0) in the alternate-day group (P = 0.46). In the consecutive-day group, 95.2% (IQR 80.8–99.3) of the capsules were consumed in the morning and 92.4% (72.4–97.8) without a meal; in the alternate-day group, the corresponding values were 94.9% (IQR 75.6–98.9) and 93.5% (85.8–98.3), respectively.

Baseline characteristics of the participants in the consecutive-day and alternate-day groups are shown in Table 1. There were no group differences in any of the baseline variables. Baseline prevalence of iron deficiency and anemia was 48.0% and 4.0% in the consecutive-day group and 46.7% and 5.3% in the alternate-day group. Hematological and iron status parameters during the study are shown in Table 2. At the end of treatment (90 days for the consecutive-day group and 180 days for the alternate-day group), median serum ferritin was 43.8 µg/L (IQR 31.7–58.2) in the consecutive-day group versus 44.8 µg/L (33.8–53.6) in the alternate-day group (P = 0.98) (Table 2, Fig. 2A) and iron deficiency

	Consecutive-day (n = 75)	Alternate-day (n = 75)
Age, y	26 (21–29)	24 (21–28)
BMI, kg/m^2	21.7 (2.3)	21.3 (2.1)
Vegetarian, n (%)	24 (32.0)	33 (44.0)
Hemoglobin, g/dL	13.3 (0.8)	13.3 (0.8)
MCV, fl	90.1 (4.1)	90.8 (4.0)
Hematocrit, %	41.3 (2.3)	41.6 (2.3)
Serum iron, µmol	17.2 (11.2–21.1)	14.7 (10.4–17.8)
TS, %	23.7 (16.2–29.9)	20.4 (14.8–25.0)
Serum ferritin, µg/L	15.9 (9.0–22.5)	15.8 (10.1–21.9)
sTfR, mg/L	4.9 (4.4–5.9)	5.1 (4.4–5.8)
Body iron stores, mg/kg body weight	2.9 (0.2–4.2)	2.6 (0.9–4.4)
Serum hepcidin, nM	0.6 (0.3–1.3)	0.7 (0.4–1.3)
Iron deficiency, n (%)	36 (48.0)	35 (46.7)

Data are mean (SD), median (IQR), or n (%). Serum ferritin adjusted for inflammation.[22] Baseline characteristics between groups were compared using independent samples t-test, non-parametric tests and Chi-Square tests. Iron deficiency defined as serum ferritin <15 µg/L and/or sTfR >8.3 µg/mL. MCV = mean corpuscular volume. sTfR = serum transferrin receptor. TS = transferrin saturation.

Table 1: **Baseline characteristics of the participants according to study group.**

	Day 0	Day 46	Day 93	Day 139	Day 186	Group effect P value	Time effect P value	Group-time effect P value
n								
Consecutive-day	75	73	73	69	70			
Alternate-day	75	70	70	64	67			
Hemoglobin, g/dL								
Consecutive-day	13.3 (0.8)	13.6 (0.7)	14.0 (0.7)*	13.6 (0.8)	13.4 (0.8)	0.21	<0.0001	0.022
Alternate-day	13.3 (0.8)	13.4 (0.8)	13.7 (0.7)	13.5 (0.8)	13.4 (0.8)			
MCV, fl								
Consecutive-day	90.1 (4.1)	91.3 (4.0)	91.8 (3.7)	92.2 (3.3)	92.0 (3.1)	0.79	<0.0001	0.79
Alternate-day	90.8 (4.0)	91.3 (4.2)	92.1 (4.1)	92.5 (4.2)	92.3 (4.4)			
Hematocrit, %								
Consecutive-day	41.3 (2.3)	42.3 (2.2)	42.9 (2.3)	41.4 (2.3)	40.8 (2.2)	0.26	<0.0001	0.087
Alternate-day	41.6 (2.3)	41.7 (2.5)	42.3 (2.2)	41.3 (2.4)	40.8 (2.4)			
Serum iron, µmol								
Consecutive-day	17.2 (11.2–21.1)	–	13.7 (11.4–18.5)	–	13.1 (10.1–16.9)	0.30	0.14	0.019
Alternate-day	14.7 (10.4–17.8)	–	12.2 (9.4–17.4)	–	14.9 (10.9–18.5)			
TS, %								
Consecutive-day	23.7 (16.2–29.9)	–	24.4 (18.9–30.4)	–	21.1 (16.4–28.2)	0.33	0.020	0.0068
Alternate-day	20.4 (14.8–25.0)	–	21.9 (16.5–27.7)	–	25.8 (19.5–32.1)			
Serum ferritin, µg/L								
Consecutive-day	15.9 (9.0–22.5)	32.4 (23.6–44.7)*	43.8 (31.7–58.2)*	34.4 (20.2–47.2)	27.0 (18.4–42.0)****	0.96	<0.0001	<0.0001
Alternate-day	15.8 (10.1–21.9)	24.5 (17.3–31.3)	31.3 (24.7–40.1)	34.7 (23.8–42.9)	44.8 (33.8–53.6)			
sTfR, mg/L								
Consecutive-day	4.9 (4.4–5.9)	4.3 (4.0–4.9)	4.1 (3.7–4.6)	4.2 (3.8–4.8)	4.6 (4.0–5.0)	0.918	<0.0001	<0.0001
Alternate-day	5.1 (4.4–5.8)	4.4 (3.9–5.1)	4.2 (3.7–4.8)	4.3 (3.8–4.8)	4.2 (3.8–4.9)			
Body iron stores, mg/kg body weight								
Consecutive-day	2.9 (0.2–4.2)	5.5 (4.3–7.2)**	7.1 (5.6–8.3)**	6.2 (3.7–7.2)	5.1 (3.6–6.7)****	0.772	<0.0001	<0.0001
Alternate-day	2.6 (0.9–4.4)	4.7 (3.5–5.8)	5.9 (4.9–6.7)	6.0 (4.6–6.9)	6.9 (5.8–7.9)			
Serum hepcidin, nM								
Consecutive-day	0.6 (0.3–1.3)	1.9 (1.3–3.1)****	3.0 (2.0–5.0)***	1.8 (1.1–2.5)	1.4 (0.8–2.6)*	0.154	<0.0001	<0.0001
Alternate-day	0.7 (0.4–1.3)	1.0 (0.8–1.7)	2.0 (1.6–2.8)	2.1 (1.4–2.6)	1.9 (1.4–2.9)[a]			
Iron deficiency, n (%)								
Consecutive-day	36 (48.0)	3 (4.1)	4 (5.5)	7 (10.1)	8 (11.4)	NA	NA	NA
Alternate-day	35 (46.7)	9 (13.0)	3 (4.3)	6 (9.4)	2 (3.0)			

Data are mean (SD), median (IQR), or n (%). Analysed by linear mixed models, with independent samples t-test and non-parametric tests for variables with significant group-time effect, between group comparisons on that day: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. [a]Comparing consecutive-day group on day 90 versus alternate-day group on day 186: P < 0.0001. Post-hoc tests were adjusted for multiple comparisons using Bonferroni step-down (Holm) correction. Serum hepcidin, haemoglobin, MCV and hematocrit measured at time of iron intake (days 0, 46, 90, 39 and 183). Serum ferritin adjusted for inflammation.[22] Iron deficiency defined as serum ferritin <15 µg/L and/or sTfR >8.3 µg/mL. MCV = mean corpuscular volume. sTfR = serum transferrin receptor. TS = transferrin saturation.

Table 2: **Hematological and iron status parameters during the study in the consecutive-day and alternate-day oral iron supplementation groups.**

prevalence was 5.5% and 3.0% (P = 0.46), respectively (Table 2). At 90 days, iron deficiency prevalence in the alternate-day group (4.3%) did not differ from the consecutive-day group (P = 0.74). At 6 months, iron deficiency was 11.4% and 3.0% in the consecutive-day and alternate-day groups, respectively (P = 0.049). During the intervention, we found significant time–group interactions on Hb, serum ferritin, sTfR, body iron stores, serum hepcidin, serum iron and transferrin saturation (%TS) (Table 2). Serum ferritin was higher in the consecutive-day group than in the alternate-day group at 46 and 93 days (P < 0.01 for both); the reverse was true at 183 days (P < 0.0001) (Table 2). Median serum ferritin was also higher in the alternate-day group at 4 months post-treatment compared to the consecutive-day group at 3 months post-treatment (39.4 µg/L (IQR 23.6–58.7) and 27.0 µg/L (18.4–42.0), respectively; P = 0.030). Serum hepcidin was higher in the consecutive-day group than alternate-day group on days 46 and 90 (P < 0.0001 and P < 0.001) (Table 2). At the end of treatment, median serum hepcidin was higher in the consecutive-day group on day 90 than in the alternate-day group on day 183 (3.0 nM (IQR 2.0–5.0) and 1.9 nM (1.4–2.9), respectively; P < 0.0001) (Table 2). The estimated median FIA based on the measured increase in serum ferritin and assumed obligatory iron losses over

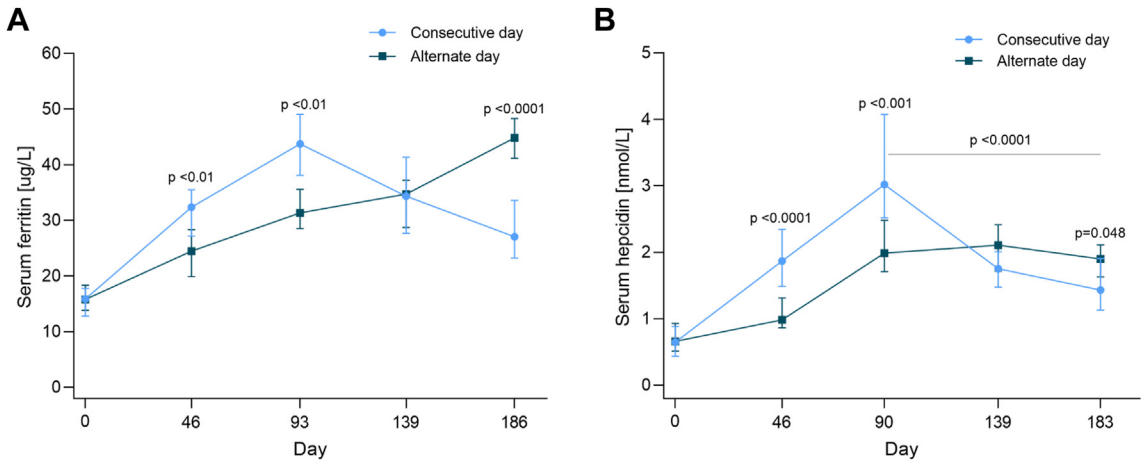

Fig. 2: **Serum ferritin and serum hepcidin concentrations during the study in the consecutive-day and alternate-day oral iron supplementation groups.** Serum ferritin (A) and serum hepcidin (B). The dots represent the medians, the error bars represent 95% confidence intervals, the horizontal line represents the comparison of the consecutive day group, day 90 versus alternate day group, day 183. Analysed by linear mixed models, with independent samples t-test and non-parametric tests for variables with significant group-time effects. Post-hoc tests were adjusted for multiple comparisons using Bonferroni step-down (Holm) correction. Serum ferritin adjusted for inflammation.[22]

90 days in the consecutive-day group and over 180 days in the alternate-day group was 4.1% (IQR 2.9–5.4) (n = 73) and 5.5% (4.7–6.5) (n = 67), equating to 36.0% higher absorption in the alternate-day group (P < 0.0001).

Inflammation markers at baseline and during the study are shown in Table 3. There were no group-time effects on CRP, AGP, I-FABP or fecal calprotectin. The prevalence of systemic inflammation was <8% in both groups throughout the study and did not differ between groups. Median (IQR) I-FABP was 24% higher in the consecutive-day group at 3 months compared to alternate-day group at 6 months; 712.8 (459.5–921.3) versus 573.3 (444.5–786.4) pg/mL (P = 0.22). There was a significant time effect on fecal calprotectin, which increased from baseline to 6 months in both groups (P = 0.011) (Table 3).

The LPR for all GI side effects at the end of treatment in the consecutive-day group (0–3 months) to the alternate-day group (0–6 months) was 1.91 (95% CI: 1.71, 2.13; P < 0.0001) (Table 4). The cumulative count of GI side effects over the 6 months in the study groups is shown in Fig. 3. A detailed description of the distribution of GI side effects among participants is shown in the Supplementary Table 1S. The LPR for nausea, diarrhea and stomach pain, but not constipation, was higher at the end of treatment in the consecutive-day group (0–3 months) to the alternate-day group (0–6 months) (for all, P < 0.0001) (Table 4). Stomach pain occurred much more frequently in the consecutive-day group compared to the alternate-day group (LPR 2.54 (95% CI: 2.05, 3.14); P < 0.0001). From 0 to 6 months, the LPR for side effects in the consecutive-day group compared to the alternate-day group was 1.20 (95% CI: 1.08, 1.33; P < 0.00080). From 0 to 3 months, the LPR for side effects in the consecutive-day group compared to the alternate-day group was 1.33 (95% CI: 1.19, 1.50; P < 0.0001). In contrast, from 4 to 6 months, there was no difference in the LPR for side effects between the groups (P = 0.070). Side effects decreased over time in the alternate-day group, with less side effects occurring from 4 to 6 months compared to 0–3 months (P < 0.001), with a significant group-time effect (P = 0.030). The LPR for side effects on days of iron intake only was 1.56 (95% CI: 1.38, 1.77; P < 0.0001) in the consecutive-day versus alternate-day group.

Discussion

The findings for our two co-primary outcomes are that, at equal total iron doses, comparing consecutive-day and alternate-day iron dosing in iron-depleted young women: 1) median serum ferritin was 43.8 µg/L (31.7–58.2) versus 44.8 µg/L (33.8–53.6) (P = 0.98); and 2) the LPR for GI side effects on days of iron intake was 1.56 (95% CI: 1.38, 1.77; P < 0.0001). For our secondary outcomes, at equal total iron doses, comparing consecutive-day and alternate-day groups: 1) median serum hepcidin was 3.0 nM (IQR 2.0–5.0) versus 1.9 nM (1.4–2.9) (P < 0.0001) and 2) iron deficiency prevalence after 3 months was 5.5% versus 4.3% (P = 0.74) and after 6 months was 11.4% and 3.0% (P = 0.049).

Our previous short-term stable iron isotope studies in iron-depleted women showed that FIA from oral iron doses ≥60 mg given 24 h after a preceding dose was decreased by 35–45%,[6] and that absorption was 34% higher when 60 mg of iron as $FeSO_4$ was given on alternate days for 28 days versus on consecutive days for

Variable	Day 0	Day 46	Day 93	Day 139	Day 186	Group effect P value	Time effect P value	Group-time effect P value
n								
Consecutive-day	75	73	73	69	70	NA	NA	NA
Alternate-day	75	70	70	64	67			
CRP, mg/L								
Consecutive-day	0.47 (0.20–1.10)	0.61 (0.27–1.44)	0.56 (0.26–1.19)	0.34 (0.16–1.25)	0.40 (0.21–0.74)	0.46	0.50	0.61
Alternate-day	0.42 (0.21–1.26)	0.42 (0.24–0.87)	0.36 (0.20–0.76)	0.34 (0.13–0.94)	0.33 (0.13–0.82)			
AGP, g/L								
Consecutive-day	0.66 (0.55–0.74)	0.62 (0.55–0.74)	0.64 (0.55–0.75)	0.62 (0.55–0.71)	0.62 (0.55–0.70)	0.25	0.16	0.47
Alternate-day	0.64 (0.52–0.78)	0.58 (0.52–0.68)	0.60 (0.51–0.70)	0.61 (0.55–0.71)	0.61 (0.52–0.69)			
Systemic inflammation, n (%)								
Consecutive-day	4 (5.3)	2 (2.7)	2 (2.7)	4 (5.8)	3 (4.3)	NA	NA	NA
Alternate-day	5 (6.7)	1 (1.4)	2 (2.9)	5 (7.8)	3 (4.5)			
I-FABP, pg/mL								
Consecutive-day	710.1 (449.8–1003.7)	–	712.8 (459.5–921.3)	–	612.5 (400.0–887.3)	0.76	0.055	0.57
Alternate-day	733.5 (537.6–873.7)	–	667.0 (465.3–946.7)	–	573.3 (444.5–786.4)			
Fecal calprotectin, µg/g								
Consecutive-day	5.9 (3.6–5.9)	–	7.7 (4.2–33.6)	–	8.4 (3.9–26.5)	0.16	0.011	0.63
Alternate-day	5.7 (3.3–13.8)	–	7.2 (4.0–13.3)	–	8.5 (3.6–16.6)			

Data are mean (SD), median (IQR), or n (%). Analysed by linear mixed models. Post-hoc tests were adjusted for multiple comparisons using Bonferroni step-down (Holm) correction. Inflammation defined as a CRP >5 mg/L or AGP >1 g/L.[23] I-FABP and faecal calprotectin measurement on days 0, 90, and 183. AGP = α-1-acid glycoprotein. CRP=C-reactive protein. I-FABP = intestinal fatty-acid binding protein.

Table 3: **Inflammation markers during the study in the consecutive-day and alternate-day oral iron supplementation groups.**

14 days.[7] Those absorption values are similar to our estimates of 36% greater absorption from alternate day dosing in the present study, derived from changes in serum ferritin. However, our data suggest that the increase in fractional absorption with alternate day dosing does not entirely offset the provision of half of the iron dose: serum ferritin was higher in the consecutive-day group compared to the alternate-day group at 3 months ($P < 0.01$). Also, providing the same total iron dose via alternate day dosing for 6 months did not result in a greater increase in serum ferritin than consecutive day dosing for 3 months. Possible explanations for this could include: (i) higher obligatory body iron losses (1.34 mg/day in menstruating women[21]) during 6 months versus 3 months, which blunted the effect of the same total iron dose given over twice the time; and/or (ii) the presumed gradual reduction in iron absorption as iron stores were replenished in both groups; in young women, dietary FIA plateaus at a serum ferritin of ~50 µg/L,[24] which is near the median serum ferritin achieved by both groups at the end of treatment. The significant decrease in serum ferritin from days 90–180 in the consecutive-day group no longer receiving iron supplementation indicates that the population was in negative iron balance without supplementation; that is, their dietary iron intakes were not covering their basal iron losses.

Recent randomized trials from India and Turkey reported greater or similar efficacy of alternate day versus consecutive day iron dosing in women with iron deficiency anemia.[9–11] But small numbers, short treatment periods (3–6 weeks) and nonblinded study designs make these trials difficult to interpret. In a recent randomized controlled double-blinded trial, anemic Indian men and women (n = 200) received either one tablet of iron as $FeSO_4$ (60 mg) daily or two tablets of $FeSO_4$ (120 mg) on alternate days as a single morning dose for 8 weeks.[13] There was no group difference in mean change of Hb at 8 weeks ($P = 0.47$). In a parallel group study, male and female endurance-trained runners (serum ferritin <50 µg/L) received 105 mg iron as $FeSO_4$ either on consecutive or on alternate days for 8 weeks[12]; although the alternate-day group received half the iron dose, there were no group differences in serum ferritin throughout the study.

We previously showed that after the intake of oral iron doses ≥60 mg, serum hepcidin peaks at 8 h and remains elevated at 24 h, but not at 48 h. Also, serum hepcidin was increased during dosing of 60 mg given on consecutive days for 14 days versus on alternate days for 28 days.[7] In this study, we show that this effect is present over 3 months of consecutive day iron doses: serum hepcidin was higher in the consecutive-day compared to the alternate-day group at 46 and 90 days ($P < 0.0001$ and $P < 0.001$, respectively) (Fig. 2B). However, because serum hepcidin is upregulated by acute iron doses and increased circulating iron concentrations as well as increased liver iron stores, the higher serum hepcidin in the consecutive-day group may have been due to both the acute effect of consecutive day dosing and a greater increase in body iron stores in this group during the first 3 months.

	% LP	$N_{event}/N_{observation}$	% LP	$N_{event}/N_{observation}$	LPR	95% CI	P value[a]
	Days 1–90: Consecutive-day (n = 75)		Days 1–180: Alternate-day (n = 74)				
Side effects	9.53	616/6462	4.99	586/11,732	1.91	1.71, 2.13	<0.0001
Mild	8.20	530/6462	4.31	506/11,732	1.90	1.69, 2.14	<0.0001
Severe	1.28	83/6462	0.68	80/11,732	1.88	1.39, 2.56	<0.0001
Nausea	2.89	187/6462	1.53	180/11,732	1.89	1.54, 2.31	<0.0001
Constipation	1.16	75/6462	0.79	93/11,732	1.46	1.08, 1,98	0.013
Diarrhea	2.43	157/6462	1.47	172/11,732	1.66	1.34, 2.05	<0.0001
Stomach Pain	3.05	197/6462	1.20	141/11,732	2.54	2.05, 3.14	<0.0001
Days 1–180	**Consecutive-day (n = 75)**		**Alternate-day (n = 74)**				
Side effects	5.99	735/12,275	4.99	586/11,732	1.20	1.08, 1.33	0.00080
Mild	5.19	637/12,275	4.31	506/11,732	1.20	1.07, 1.35	0.0015
Severe	0.80	98/12,275	0.68	80/11,732	1.17	0.87, 1.57	0.30
Nausea	1.69	207/12,275	1.53	180/11,732	1.10	0.90, 1.34	0.36
Constipation	0.87	107/12,275	0.79	93/11,732	1.10	0.83, 1.45	0.51
Diarrhea	1.56	191/12,275	1.47	172/11,732	1.06	0.87, 1.30	0.58
Stomach pain	1.87	230/12,275	1.20	141/11,732	1.56	1.27, 1.92	<0.0001
Days 1–90	**Consecutive-day (n = 75)**		**Alternate-day (n = 74)**				
Side effects	9.53	616/6462	7.15	445/6220	1.33	1.19, 1.50	<0.0001
Nausea	2.89	187/6462	2.23	139/6220	1.29	1.04, 1.61	0.019
Constipation	1.16	75/6462	1.08	67/6220	1.08	0.78, 1.50	0.67
Diarrhea	2.43	157/6462	2.11	131/6220	1.15	0.92, 1.45	0.22
Stomach pain	3.05	197/6462	1.74	108/6220	1.76	1.39, 2.21	<0.0001
Days 91–180	**Consecutive-day (n = 72)**		**Alternate-day (n = 68)**				
Side effects	2.05	119/5813	2.56	141/5512	0.80	0.63, 1.02	0.070
Nausea	0.34	20/5813	0.74	41/5512	0.46	0.27, 0.79	0.0046
Constipation	0.55	32/5813	0.47	26/5512	1.17	0.70, 1.96	0.57
Diarrhea	0.58	34/5813	0.74	41/5512	0.79	0.50, 1.24	0.302
Stomach pain	0.57	33/5813	0.60	33/5512	0.95	0.59, 1.53	0.84

Data are LP (Nevent/Nobservation), LPR (95% CI). LP = Longitudinal prevalence. LPR = Longitudinal prevalence ratio. [a]Testing the null hypothesis that longitudinal prevalence is equal in both groups versus the alternative that longitudinal prevalence is not equal in both groups.

Table 4: **Gastrointestinal side effects during the study in the consecutive-day and alternate-day oral iron supplementation groups.**

Adverse effects on the GI tract are the most commonly reported side effects of oral iron supplementation and are often a reason for poor adherence.[4] A recent meta-analysis reported an increased risk of GI side effects with $FeSO_4$ supplementation compared to placebo or iv iron,[4] and the side effects were independent of dose but dependent on dosing regimen. Another meta-analysis of six studies reported that women taking iron supplements intermittently (one, two or three times a week, on non-consecutive days) were less likely to experience any side effects compared to women taking iron supplements on a daily basis (RR 0.41, 95% CI 0.21–0.82; 1166 participants).[25] Other studies comparing consecutive day and alternate day dosing have reported fewer GI side effects with alternate day dosing,[7,11] but not all studies agree.[9,10] However, many studies that reported GI side effects with oral iron supplementation were limited by lack of blinding,[9–12] and/or by long periods of recall.[9–11] In contrast, our study was carefully double-blinded, and using a novel, specifically-designed mobile application, we were able to assess side effects daily, in 'real time'. Thus, we feel our side effect data are accurate and confirm that alternate day dosing is associated with fewer GI side effects.

Notably, there was a significant between-group difference in GI side effects only during 0–3 months, but not from 4 to 6 months. Because the consecutive-day group received placebo from 4 to 6 months, this suggests alternate day dosing produces minimal GI side effects; the presence of GI side effects in the consecutive-day group during the 3-months placebo phase is a reminder that GI symptoms are common and occur for many reasons. To provide objective evidence of effects of iron on the gut, we measured serum I-FABP as a measure of enterocyte integrity and fecal calprotectin as a measure of gut inflammation. However, similar to the findings in our shorter iron absorption study comparing consecutive and alternate day dosing,[7] we did not find any significant between-group differences in these biomarkers. Although median I-FABP was 24% higher in

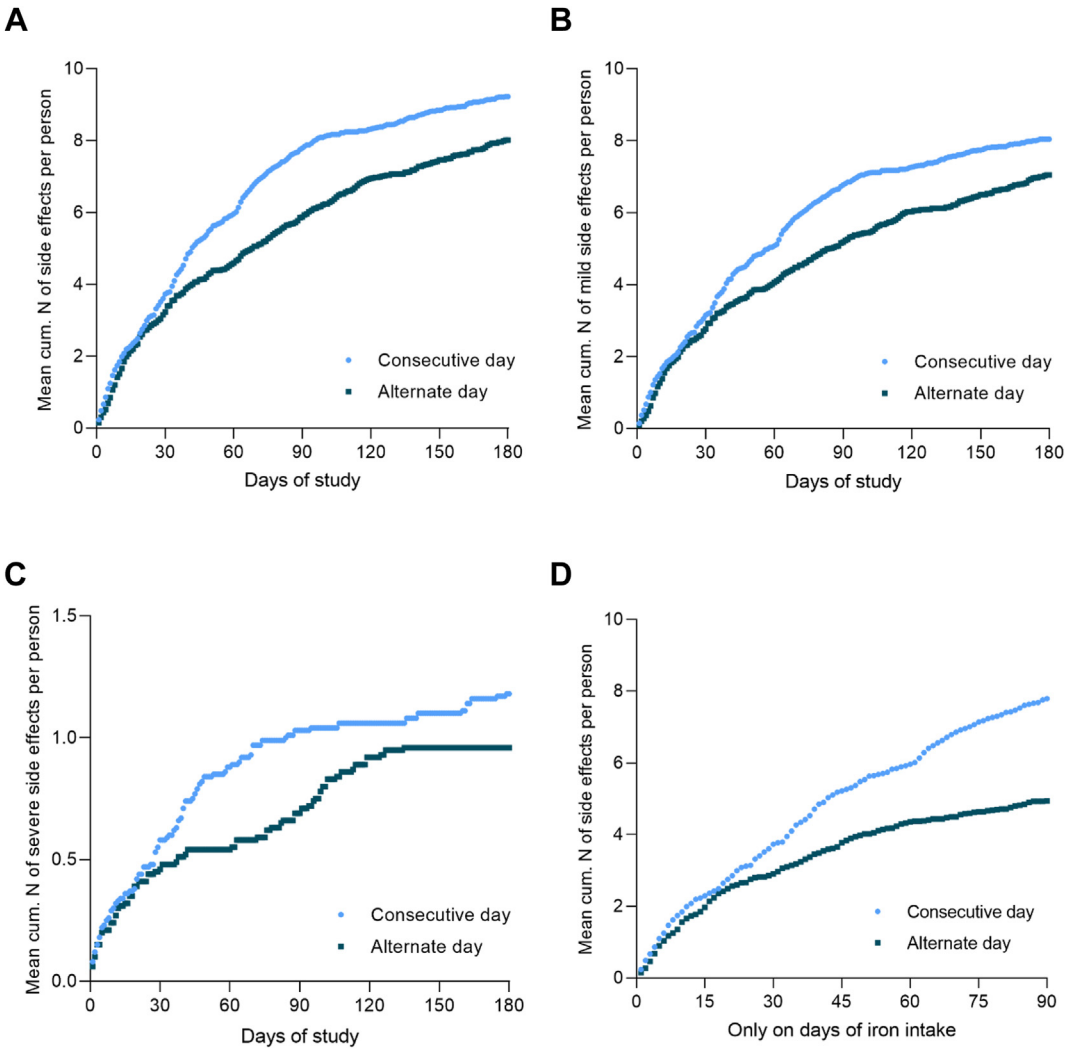

***Fig. 3:* Mean cumulative count of side effects during the study in the consecutive-day and alternate-day oral iron supplementation groups.** Mean cumulative number of side effects per person (A), mean cumulative number of mild side effects per person (B), mean cumulative number of severe side effects per person (C) and mean cumulative number of side effects per person only on the days of iron intake (D). The longitudinal prevalence ratio (LPR) for all side effects in the consecutive-day group compared to the alternate-day group was 1.20 (95% CI: 1.08, 1.33; p < 0.00080). The LPRs for mild and severe side in the consecutive-day group compared to the alternate-day group was 1.20 (95% CI: 1.07, 1.35; P = 0.002) and 1.17 (95% CI: 0.87, 1.57; P = 0.30), respectively. The LPR for side effects only on the days when iron was taken in the consecutive-day group compared to the alternate-day group was 1.56 (95% CI: 1.38, 1.77; P < 0.0001).

the consecutive-day group at 3 months compared to alternate-day group at 6 months, this difference was not statistically significant.

Comparative studies which attempt to retrospectively ascertain side effects through interview are subject to both recall bias and inaccurate recall. Recall accuracy is higher with shorter time intervals since occurrence and high subject motivation.[16,26] Our specifically-designed mobile application enabled daily contact with our subjects to assess compliance and side effects via simple forced-choice questions. Reception of the application was overwhelmingly positive. A recent review reported multiple benefits of mobile apps for side effect reporting[17] and the use of the mobile app likely improved adherence and reporting accuracy in our study.

This study has several strengths. The study was rigorously double blind and capsule adherence was very high. Our design allowed us to assess the intervention effect in two ways: comparing the same treatment length with different total iron doses, and the same total iron dose with different treatment lengths. However, it did not allow us to compare the same total iron dose given over the same treatment length. Future studies could be done to compare equal total doses given over

the same treatment length by doubling the dose given on the alternate day. This study also has limitations. Because the subjects were prompted by the mobile app to take their supplements, adherence was high in both groups; without these daily reminders, adherence may have varied. Only a small number of our participants were mildly anemic; we did not study women with moderate-to-severe anemia, who might respond differently to those with no anemia or mild anemia.

Our findings show that, compared to consecutive day dosing of iron, alternate day dosing did not result in higher serum ferritin but led to a reduction in iron deficiency at 6 months and triggered fewer GI side effects. It appears the increase in FIA with alternate day dosing does not fully offset the halving of the iron dose per unit time, so alternate day dosing reduces total iron absorption per unit time compared to consecutive day dosing. This may not be optimal in women with symptomatic iron deficiency anemia, in whom rapid repletion of body iron is needed. Nevertheless, in most women with iron deficiency, speed of response is not critical,[27] but maintaining good compliance with oral iron is difficult. Alternate day iron dosing will likely be better tolerated by many women, and this may increase compliance and efficacy.

Contributors

HKS, DM, MBZ and NUS conceived the studies and obtained funding; all authors contributed to the design of the trials; HKS, SG, UMK, JS, FV and NUS conducted the studies; HKS, MBZ and NUS analyzed the data and wrote the first draft of the manuscript; and all authors contributed to the final version of the manuscript.

Data sharing statement

Data described in the manuscript will be made available upon reasonable request.

Declaration of interests

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102286.

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