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Oral iron supplementation in iron-deficient women: How much and how often?

Nicole U. Stoffel[a], Hanna K. von Siebenthal[a], Diego Moretti[a,b], Michael B. Zimmermann[a,*]

[a] ETH Zurich, Laboratory of Human Nutrition, Institute of Food Nutrition and Health, Schmelzbergstrasse 7, CH-8092, Zurich, Switzerland
[b] Swiss Distance University of Applied Sciences, Nutrition Group, Health Department, CH-8105, Regensdorf, Switzerland

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ABSTRACT

Iron deficiency and iron deficiency anemia (IDA) are major public health problems worldwide, especially in young women. Oral iron supplementation can be an effective strategy to treat and prevent IDA, but guidelines vary. Some experts recommend doses of 150–200 mg elemental iron per day, with the dose split through the day. However, recent studies suggest this may not be an optimal regimen. The fraction of iron absorbed from high doses of oral iron is low, and unabsorbed iron can cause gut irritation, inflammation and dysbiosis, and these reduce compliance. In recent studies using serum hepcidin profiles and stable iron isotopes to quantify iron absorption in young women, we have shown that: (a) oral iron doses ≥60 mg in iron-deficient women, and doses ≥100 mg in women with IDA, stimulate an acute increase in hepcidin that persists 24 h after the dose, but subsides by 48 h; (b) therefore, to maximize fractional iron absorption, oral doses ≥60 mg should be given on alternate days; (c) the circadian increase in plasma hepcidin is augmented by a morning iron dose; therefore, iron doses should not be given in the afternoon or evening after a morning dose. If rate of Hb response is important, a pooled analysis of our data done for this review indicates that total iron absorption is also higher if twice the target daily iron dose is given on alternate days. In summary, these studies suggest changing from daily to alternate-day schedules and from divided to morning single doses increases iron absorption and may reduce side effects. Thus, providing morning doses of 60–120 mg iron as a ferrous salt given with ascorbic acid on alternate days may be an optimal oral dosing regimen for women with iron-deficiency and mild IDA.

1. Introduction

It is estimated that anemia affects one third of the global population, with approximately half of the cases resulting from iron deficiency; thus, over 1.2 billion individuals suffer from iron deficiency anemia (IDA) (Camaschella, 2019). Globally, IDA is 1 of the 5 leading causes of years lived with disability burden and is the first cause in women (Camaschella, 2019). Although oral iron supplementation is considered the first line of treatment of iron deficiency in most women (Brittenham, 2018), there is little consensus on recommended dose or dosing frequency.

2. Choice of iron compound

Iron preparations available on the market vary widely in dosage, compound, cost and bioavailability. Bioavailability of an iron supplement is defined as the proportion of iron present in an oral supplement that is absorbed and incorporated into erythrocytes (WHO, 2006; BNF, 2017). However, the terms 'bioavailability' and 'absorption' are often used interchangeably, and we use them both in this review. Ferrous iron is the preferred form because of its high bioavailability (Table 1). (Brittenham, 2018; WHO, 2006; BNF, 2017) Commonly-used ferrous iron compounds include the salts ferrous fumarate, ferrous sulfate, ferrous gluconate, and the amino acid chelate, ferrous bisglycinate. In general, the bioavailability of ferrous iron compounds is similar (WHO, 2006; BNF, 2017) and their side effect profile and efficacy to regenerate hemoglobin (Hb) are also comparable, as long as sufficient iron is given (BNF, 2017). However, they vary in their elemental iron content. Therefore, choice of ferrous iron salt is usually based on the amount of elemental iron and the cost.

Ferric iron has very low solubility at near-neutral or alkaline pH and must be reduced to ferrous iron prior to uptake by enterocytes (Sangkhae and Nemeth, 2017). Iron bioavailability from ferric iron preparations is typically 3 to 4 times lower than that of ferrous sulfate

* Corresponding author. Laboratory of Human Nutrition, Schmelzbergstrasse 7, 8092, Zürich, Switzerland.
E-mail addresses: nicole.stoffel@hest.ethz.ch (N.U. Stoffel), hanna.vonsiebenthal@hest.ethz.ch (H.K. von Siebenthal), diego.moretti@ffhs.ch (D. Moretti), michael.zimmermann@hest.ethz.ch (M.B. Zimmermann).

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Table 1
Iron content and relative bioavailability[a] (RBV) to ferrous sulfate of commonly used oral iron preparations.

Iron salt	Elemental iron content (%)	RBV to ferrous sulfate (%)
Ferrous sulfate	20	Reference
Ferrous sulfate (dried)	32.5	100
Ferrous fumarate	33	100
Ferrous gluconate	12	89
Amino acid chelates (e.g. ferrous biglycinate)	20	100
Carbonyl iron	99	5–20
Sugars (saccharides), usually contain ferric iron	Variable	Variable

[a] It should be noted that estimates of bioavailability of iron compounds are mainly derived from their comparisons at low doses as food fortificants rather than as supplements. (WHO, 2006) Bioavailability data of iron fortificants may not be entirely applicable to oral iron preparations.

(Brittenham, 2018; WHO, 2006; BNF, 2017; Santiago, 2012). Moreover, compared to ferric iron, ferrous iron is generally more effective in replenishing Hb in patients with IDA (Berber et al., 2014). Although carbonyl iron can be effective at high doses (Gordeuk et al., 1987), the absorption of H-reduced and carbonyl iron (two forms of elemental iron) is low and their use is generally not recommended. Several ferric iron-polysaccharide complexes are available and they are often marketed as having better taste and lower side effects, but there is no consistent evidence to support these claims. In a trial in patients with inflammatory bowel disease, ferric maltol was effective in correcting IDA (Gasche et al., 2015). In contrast, another study found supplementation with ferrous sulfate increased Hb concentrations more effectively than an iron polysaccharide complex, and there were more reports of diarrhea with the latter compound (Powers et al., 2017).

Many multivitamin/mineral preparations usually contain low doses of iron that are insufficient to correct iron deficiency and may contain other minerals (such as zinc) that interfere with iron absorption (Olivares et al., 2012). Some oral iron preparations contain ascorbic acid, which is a potent enhancer of iron absorption (Teucher et al., 2004) and prebiotic galacto-oligosaccharides given with ferrous fumarate can increase iron absorption (Paganini et al., 2017). However, there is no good evidence that inclusion of other nutrients, such as the B-vitamins or vitamin A, improves iron bioavailability (BNF, 2017). Antacids and proton-pump inhibitors should not be taken with iron, because an increased gastric pH reduces iron dissolution and absorption. Controlled- or slow-release preparations of iron have no clear therapeutic advantage, are usually poorly absorbed and should not be used. They may cause less gastrointestinal side-effects but this is likely due to that fact that most of the iron is carried past the proximal duodenum (where most iron is absorbed) into the distal gut where absorption is negligible (UpToDate, 2020).

3. Effects of dietary factors on bioavailability

Oral iron should generally be taken on an empty stomach, at least an hour before meals, as many foods and drinks contain inhibitors of iron absorption. Fractional iron absorption from supplements varies from 2% to 13% when consumed with food versus 5%–28% when consumed fasting (Cook, 2005). In non-anemic women, mean fractional iron absorption from a 50 mg oral iron dose consumed while fasting was ≈10%, but was only 3% when consumed with food (Cook and Reddy, 1995). Iron released from supplements can form non-absorbable complexes with various food components in the gut lumen. For example, whole grains and pulses are rich in phytic acid, which is a strong inhibitor of iron absorption at even low levels (a molar ratio of phytate to iron of >1) (Lynch et al., 2018). Coffee, black tea, herbal teas, red wine and hot chocolate contain polyphenols, which also impair iron absorption (Lynch et al., 2018). Consuming a food or drink rich in ascorbic acid with oral iron can sharply increase absorption; ascorbic acid has a dose-dependent enhancing effect on iron absorption at a molar ratio ≥2:1 (e.g., 60 mg ascorbic acid: 10 mg iron) (Teucher et al., 2004). This enhancing effect is mainly through the luminal reduction of ferric to ferrous iron, but also due to its potential to chelate iron and keep it from binding to polyphenols and phytates (Teucher et al., 2004; Siegenberg et al., 1991). In many women, oral iron supplements taken on an empty stomach cause epigastric pain and nausea (Tolkien et al., 2015). If this occurs, the supplements can be taken with meals, which may reduce side effects but may also reduce absorption. If supplements are taken with meals, including a food or drink with the meal that is a good source of ascorbic acid (e.g. a US cup (240 ml) of orange juice typically contains 100 mg) can overcome the effect of dietary inhibitors, and allow supplemental iron to be well absorbed (Lynch et al., 2018).

4. Side effects of oral iron supplements

A recent meta-analysis of 20 trials showed an increased incidence of gastrointestinal side effects compared to placebo when oral ferrous sulfate was given (OR = 2.32, 95% CI 1.74–3.08, $p < 0.0001$) (Tolkien et al., 2015). Equal doses of iron as ferrous sulfate, ferrous fumarate and ferrous gluconate in healthy adults resulted in no significant differences in side effects (Hallberg et al., 1966). The most common adverse effects are epigastric pain, nausea, and constipation; these reduce compliance with therapy in 30%–70% of cases (Tolkien et al., 2015). It is uncertain whether iron-related side effects are dose-dependent; but side effects may be less common at doses ≤20 mg iron/day (Tolkien et al., 2015). Other data suggest oral doses ≤50 mg iron/day generate less side effects than higher doses. (Pena-Rosas and Viteri, 2006). Fractional iron absorption is higher from lower iron doses compared to higher doses, leaving less unabsorbed iron in the intestinal lumen (Moretti et al., 2015; Stoffel et al., 2017a). Unabsorbed iron may cause gut inflammation and dysbiosis, and enhance growth of enteropathogens (Paganini and Zimmermann, 2017). Several studies suggest intermittent dosing results in less gastrointestinal side effects compared to daily dosing (Pena-Rosas et al., 2015).

5. Expert guidelines for oral iron supplementation

Oral iron supplementation is considered first line treatment for iron deficiency and IDA in women (Camaschella, 2015) but guidelines vary. Traditionally, the recommended daily dose is 100–200 mg iron per day given as three or four divided doses of ferrous salts: e.g., a 325 mg ferrous sulfate tablet contains 65 mg of elemental iron; three tablets per day will provide 195 mg of iron (Brittenham, 2018). Expert groups generally recommend 80–200 mg of elemental iron per day for treatment of iron deficiency and IDA (Goddard et al., 2011; Gastroenterological Society of Australia, 2015; Pavord et al., 2012) but more recent guidelines suggest lower doses may be as effective and may produce less adverse effects (Camaschella, 2019; UpToDate, 2020); for example, recent British guidelines recommend 40–80 mg of elemental iron every morning as a ferrous iron salt (Pavord, 2020). Women of reproductive age in low-income countries are at high risk of IDA, and the World Health Organization (WHO) has guidelines for population-based iron dosing schedules to prevent anemia in menstruating women in settings where anemia prevalence is 20% or higher (WHO, 2011) or 40% or higher (Table 2) (WHO, 2016). WHO also recommends intermittent iron supplementation in young women in settings where daily supplementation is likely to be unsuccessful or not possible (Fernandez-Gaxiola and De-Regil, 2019). A systematic review concluded that, in comparison with daily supplementation, intermittent supplementation produces similar benefits on anemia but may be associated with less side effects (Fernandez-Gaxiola and De-Regil, 2019). Hb usually responds rapidly to effective oral iron therapy and an Hb increase of at

Table 2
WHO recommendations for iron supplementation in menstruating women and adolescent girls.

Prevention of iron deficiency anemia in menstruating women using weekly or daily oral iron supplementation		
Frequency	*One supplement per week*	*One supplement daily*
Supplement	Iron: 60 mg elemental iron Folic acid: 2800 μg	30–60 mg elemental iron
Duration	3 months of supplementation followed by 3 months of no supplementation after which the provision of supplements should restart	Three consecutive months in a year
Settings	Populations where the prevalence of anemia among non-pregnant women of reproductive age is 20% or higher	Where the prevalence of anemia in menstruating adult women and adolescent girls is 40% or higher

60 mg of elemental iron equals 300 mg of $FeSO_4$ heptahydrate, 180 mg of FeFum or 500 mg of ferrous gluconate. Modified from WHO Guidelines 2011 (WHO, 2011) and 2016 (WHO, 2016).

least 2 g/dL after 3 weeks of therapy indicates adequate therapeutic response (Brittenham, 2018). However, repletion of iron stores and normalization of serum ferritin may require 4–6 months of treatment (Brittenham, 2018).

6. The 'mucosal block' and intermittent versus daily iron dosing

In 1943, an absorption study in dogs showed a reduction in iron absorption when iron was given after a preceding iron dose (Hahn et al., 1943) and the concept of the "mucosal block" was born. Subsequent investigations confirmed the blocking effect of high doses of iron on iron absorption from a subsequent dose (Fairweather-Tait and Wright, 1984; Fairweather-Tait et al., 1985). Daily administration of high iron doses to iron-sufficient rats resulted in a decrease in iron absorption, whereas the iron administered every 3rd day resulted in a constant absorption rate (Viteri et al., 1995). In a radioiron study in humans, iron absorption from a labeled 50 mg iron dose was compared after administration of a single dose or given after adults had received 50 mg iron daily for 6 days (Cook and Reddy, 1995). Total iron absorption was 9.8% from the single iron dose (50 mg) compared to 8.5% from the six doses (300 mg) administered over six consecutive days (Cook and Reddy, 1995). A similar study compared absorption from a 60 mg iron dose given before and after a daily supplementation with 60 mg of iron for 6 days; preceding iron intake did not affect absorption (Olivares et al., 1999). Moreover, comparing mean iron absorption from 240 mg of iron administered in 4 consecutive daily doses of 60 mg to mean iron absorption of 240 mg iron administered in doses of 120 mg per week showed no significant difference in absorption: 7.7% for daily and 10.9% for weekly administration (Olivares et al., 1999). Therefore, although animal studies suggest a blocking effect of a preceding iron dose on absorption from a subsequent dose, human studies have shown contradictory results (Hallberg, 1998).

7. Circulating hepcidin predicts iron bioavailability

An important determinant of absorption from iron supplements is the iron status of the individual. In order to maintain iron homeostasis, iron absorption is regulated within a range of dietary iron intakes and iron requirements (Cook, 1990). The primary regulator of body iron homeostasis is hepcidin. During iron deficiency, iron stores are exhausted and the hepatic BMP-SMAD signaling pathway that increases hepcidin expression is switched off through multiple mechanisms (Sangkhae and Nemeth, 2017). Thus, circulating hepcidin levels fall, ferroportin and the divalent metal transporter (DMT)-1 are fully expressed on enterocytes, and intestinal iron absorption increases (Sangkhae and Nemeth, 2017). In young women, serum hepcidin and iron absorption from an oral iron dose are negatively correlated (Young et al., 2009). In young men, plasma hepcidin predicts 36% of the variation in iron absorption in a multiple regression model (Roe et al., 2009). In young women, plasma hepcidin was a more modest predictor of dietary iron bioavailability, explaining 28% of the variance in iron absorption (Zimmermann et al., 2009).

In conjunction with hepcidin, local enterocyte regulation also plays a key role in iron absorption. Intestinal hypoxia-inducible factor (HIF)-2α is sensitive to enterocyte iron and oxygen levels and regulates apical and basolateral iron transporters (Schwartz et al., 2019). In enterocytes, increased stability and activity of HIF-2α mediates the adaptive increase in iron absorption during iron deficiency, under the control of the hepcidin/ferroportin axis (Schwartz et al., 2019).

8. Oral iron doses acutely increase plasma hepcidin

Plasma hepcidin not only responds to changes in body iron stores, its synthesis is also stimulated by high doses of oral iron (Zimmermann et al., 2009; Nemeth et al., 2004). In murine hepatocytes, there was an increase in hepcidin mRNA in response to holo-transferrin (TF) but not to apo-TF; holo-TF was shown to regulate hepcidin through the HJV/BMP2/6-dependent pathway (Lin et al., 2007). Thus, the acute increase in TF-bound iron after an oral iron dose stimulates hepcidin expression via the BMP-SMAD pathway (Sangkhae and Nemeth, 2017). In adults, urinary hepcidin excretion increased within 24 h after an iron dose and was proportional to the peak transferrin saturation, reflecting iron absorption (Nemeth et al., 2004; Lin et al., 2007). In iron-sufficient men, a 60 mg iron dose increased circulating absorbed iron at 2 h and produced a ≈30% increase in mean plasma hepcidin at 6 h; a 3.8 mg iron dose did not increase hepcidin (Zimmermann et al., 2009). Plasma hepcidin follows a circadian rhythm and typically increases over the day (Kroot et al., 2009; Schaap et al., 2013). Whether a morning iron dose would augment this circadian hepcidin increase and whether this would affect iron absorption from a later afternoon and/or a next morning dose was unclear. To answer this question, and to devise a supplementation schedule that would maximize fractional iron absorption, we performed a series of studies in young women. In these studies, we measured hepcidin profiles after varying oral iron doses of ferrous sulfate that were labeled with stable iron isotopes; this allowed us to quantify the effect of changes in serum hepcidin on iron bioavailability by measuring erythrocyte incorporation of the labels 14 days after dosing, as described below.

9. Using hepcidin profiles and iron stable isotopes to define optimal iron dosing regimens

In our first studies, we investigated whether the acute iron-induced increase in hepcidin influences iron absorption of successive daily iron doses and twice-daily iron doses (Moretti et al., 2015). We conducted 3 separate studies with the aim of measuring the acute iron-induced increase in hepcidin caused by ferrous sulfate supplements while quantifying iron absorption. We recruited 54 nonanemic young women (median (IQR) age study 1: 27 (23–32) y; study 2: 23 (21–25) y; study 3: 22 (21–25) y) with plasma ferritin ≤20 μg/L. In all studies, subjects acted as their own controls. In these studies and the ones described below, all 8.00 a.m. measurements were done after an overnight fast. We monitored plasma hepcidin and iron status markers before administration and up to 48 h post-administration at 8.00 a.m., 12.00 p.m.,

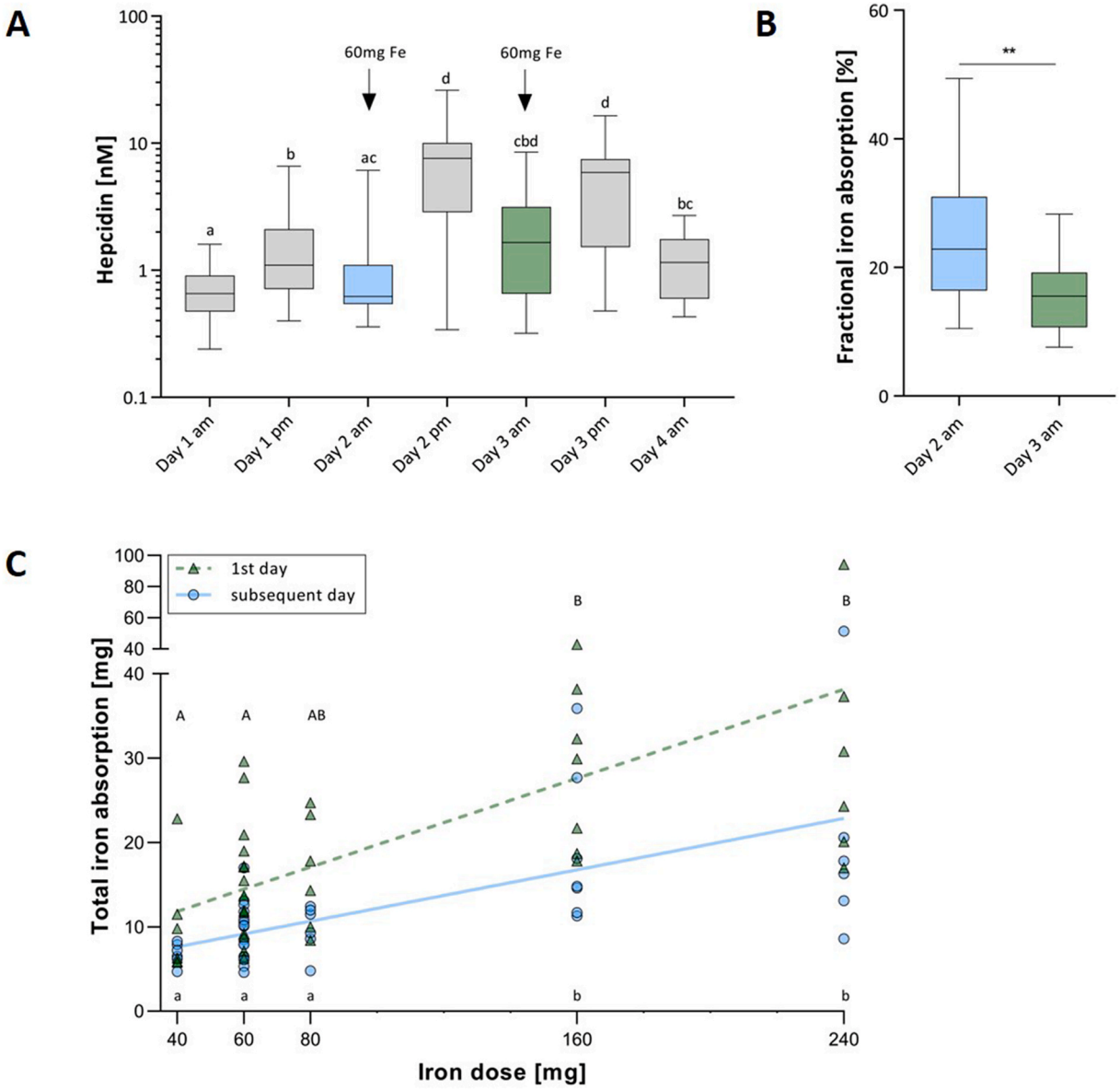

Fig. 1. In iron-deficient women (n = 16), an oral iron dose of 60 mg results in an increase in hepcidin after 24 h and in a decreased iron absorption from the consecutive dose. Doses are given both at 8.00 a.m. on consecutive days 2 and 3 and compared with day 1 (control day). **(A)** Hepcidin profiles during the observation period; boxes with different subscript letter differ significantly ($p < 0.05$). Boxes indicate median and interquartile ranges, whiskers describe the range of the data (min to max). **(B)** Fractional iron absorption measured on days 2 and 3 from the 60-mg Fe dose. **$p < 0.01$. **(C)** In iron-deficient women (n = 41), iron absorption, standardized to a plasma ferritin level of 15 μg/L, in relation to the dose administered on the first day (broken line, Δ) and on the second day (continuous line, o). At doses of 60 mg and higher, the first and second dose absorptions differed significantly ($p < 0.01$). Data with different superscripts differ significantly (capitals: first dose; minuscule: second dose).

and 5.00 p.m. In study 1, using a crossover design, we administered two iron challenges either as a single dose or as two doses given on consecutive days. Subjects were randomly assigned to start the study with one of the two treatments. Iron was administered at 8.00 a.m. in 4 different iron concentrations (40, 80, 160, and 240 mg as elemental Fe). In study 2, we administered two single doses of 60 mg elemental iron at 8:00 a.m. on two consecutive days and similarly assessed hepcidin response until 48 h post-administration. In studies 1 and 2, 24 h after doses ≥60 mg, hepcidin was increased ($p < 0.01$) and fractional iron absorption was decreased by 35%–45% ($p < 0.01$) (Fig. 1A and B). With increasing dose, fractional absorption decreased, whereas absolute absorption increased: a six-fold increase in iron dose (40–240 mg) resulted in only a three-fold increase in iron absorbed (6.7–18.1 mg) (Fig. 1C). In study 3, we assessed the effect of administering 60 mg Fe twice daily during 24 h (three doses in total) on hepcidin and iron absorption. Total iron absorbed from three doses (two mornings at 10.00 a.m. and an afternoon at 5.00 p.m.) was not significantly greater than that from only two morning doses (Fig. 2). In summary, these short-term data suggested that, to maximize fractional absorption: (A) oral iron at doses ≥60 mg should be spaced by 48 h; and (B) twice-daily dosing should be avoided (Moretti et al., 2015).

In a second series of studies, we did two prospective, open-label, randomized controlled trials assessing iron absorption in iron-depleted (SF ≤ 25 μg/L) young women (Stoffel et al., 2017a). In study 1, women (n = 20; median (IQR) age, 27 (24–30) y) were assigned to two groups: one group was given 120 mg iron at 8.00 a.m. and the other was given the dose split into two divided doses of 60 mg at 8.00 a.m. and 5.00 p.m. for three consecutive days. Fourteen days after the final dose, the groups were each crossed over to the other regimen. No significant differences were seen in fractional iron absorption (day 1–3 geometric

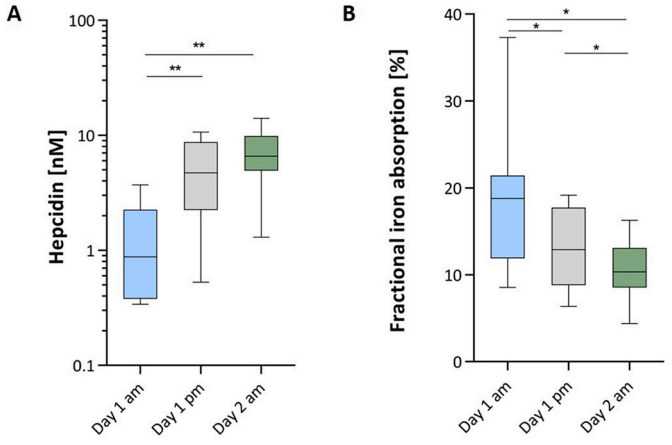

Fig. 2. In iron-deficient women (n = 13), twice-daily iron administration at 10.00 a.m. and at 5.00 p.m. results in increased hepcidin on the consecutive day and decreased iron absorption of the afternoon dose and the next morning's dose. **(A)** Hepcidin profiles. **(B)** Fractional iron absorption at the 3 time points of the 60-mg Fe dose. Boxes indicate median and interquartile ranges, whiskers describe the range of the data (min to max). *p < 0.05, **p < 0.01.

mean: 11.8% (7.1, 19.4) once daily vs 13.1% (8.2, 20.7) twice daily) or total iron absorption (day 1–3: 44.3 mg (29.4, 66.7) once daily vs 49.4 mg (35.2, 69.4) twice daily) between the two dosing regimens (Fig. 3A and B). Twice-daily divided doses resulted in a higher hepcidin concentration than once-daily dosing ($p < 0.05$). In study 2, women (n = 40; median (IQR) age, 22 (21–25) y) were randomly assigned (1:1) to two groups. One group was given 60 mg iron at 8.00 a.m. on consecutive days for 14 days, and the other group was given the same dose on alternate days for 28 days. At the end of treatment, geometric mean (-SD, +SD) cumulative fractional iron absorption was 16.3% (9.3, 28.8) in the consecutive-day group versus 21.8% (13.7, 34.6) in the alternate-day group ($p < 0.005$), and cumulative total iron absorption was 131.0 mg (71.4, 240.5) versus 175.3 mg (110.3, 278.5) ($p < 0.001$) (Fig. 4A and B). During the first 14 days of supplementation in both groups, serum hepcidin was higher in the consecutive-day group than the alternate-day group ($p < 0.005$). The total incidence of the two gastrointestinal side effects that were assessed (nausea and abdominal pain) was 33% higher with consecutive-day dosing than with alternate-day dosing. These data showed that in iron-depleted women, providing iron supplements on alternate days and in single doses optimizes iron absorption and might be a preferable dosing regimen.

We then tested these effects in women with IDA (median (IQR) age, 21 (20–24) y), in whom more severe iron depletion and hypoxia would be expected to more strongly upregulate iron absorption and fully suppress hepcidin (Mastrogiannaki et al., 2013). Our objective was to assess whether, in women with mild IDA, alternate-day administration of 100 and 200 mg iron increases iron absorption compared to consecutive-day iron administration (Stoffel et al., 2020). We performed a cross-over iron absorption study in women (n = 19; median Hb 11.5 mg/dl; mean SF 10 μg/L) who received either labeled 100 or 200 mg elemental iron at 8.00 a.m. on days 2, 3 and 5 and after a 16-day incorporation period, the other dose was given at 8.00 a.m. on days 23, 24 and 26 (days 2, 3 and 5 of the second period). Iron absorption on days 2 and 3 (consecutive) and day 5 (alternate) was assessed by measuring erythrocyte isotope incorporation. For both doses, serum hepcidin was higher on day 3 than on day 2 ($p < 0.001$) or day 5 ($p < 0.01$) but with no significant difference between days 2 and 5 (Fig. 5A and B). Similarly, for both doses, fractional iron absorption on days 2 and 5 was 40–50% higher than on day 3 ($p < 0.001$), while absorption on day 2 did not differ significantly from day 5. There was no significant difference in the incidence of gastrointestinal side effects comparing the two iron doses. In this study, in women with IDA, the mean serum hepcidin increase of ≈0.4 nM after doses of 100 and 200 mg was much smaller than the mean serum hepcidin increase of ≈1.85 nM (after correction for method comparison (Stoffel et al., 2017b)) after doses of 120 mg in non-anemic iron depleted women in our previous study (Stoffel et al., 2017a). These data show that even if in IDA hepatic hepcidin expression is strongly suppressed by iron deficiency and erythropoietic drive, the intake of oral iron supplements leads to a hepcidin increase persisting for 24 h and subsiding by 48 h. Therefore, alternate day dosing of oral iron supplements in women with IDA may be preferable because it sharply increases fractional iron absorption.

Two recent randomized trials have compared alternate day dosing to daily dosing of oral iron supplements in subjects with IDA (Kaundal et al., 2020; Mehta et al., 2020). In the first, anemic Indian adults (n = 62; baseline mean ± SD Hb, 8.9 ± 1.5 g/dl), received 120 mg of iron given on alternate days versus 60 mg iron given twice daily (Kaundal et al., 2020). In this trial, the median increase in Hb in the alternate day group after 6 weeks was not significantly different from that in the daily arm after 3 weeks and there was significantly more nausea in the daily group (Kaundal et al., 2020). In the second, anemic Indian adults (n = 40; baseline mean ± SD Hb, 10.1 ± 1.5 g/dl) received 60 mg iron given on alternate days versus 60 mg iron given on

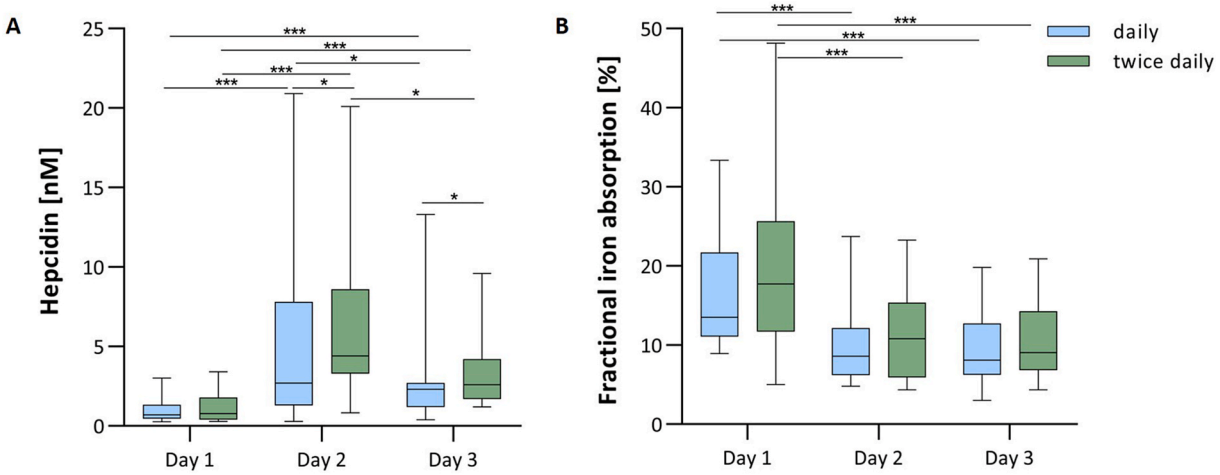

Fig. 3. In iron deficient women (n = 20) receiving single morning iron doses (120 mg) or split morning and afternoon doses (each 60 mg) for 3 days, there were no significant differences in iron absorption but twice-daily divided doses resulted in a higher serum hepcidin. **(A)** Hepcidin profiles. **(B)** Fractional iron absorption. Boxes indicate median and interquartile ranges, whiskers describe the range of the data (min to max). *p < 0.05, ***p < 0.001.

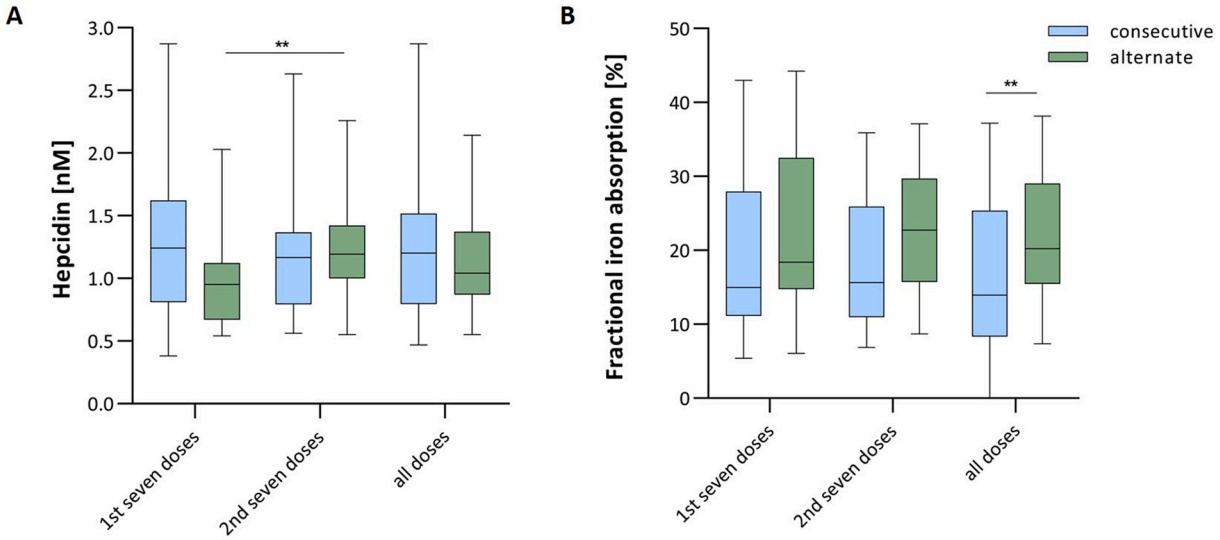

Fig. 4. In iron-deficient women (n = 40) given 14 daily or alternate day iron doses of 60 mg, cumulative fractional iron absorption was higher in the alternate-day group versus the consecutive-day group. **(A)** Serum hepcidin concentrations in samples taken during the first seven iron doses, the second seven doses, and during the entire supplementation phase, by group. During the first 14 days of supplementation in both groups, serum hepcidin was higher in the consecutive-day group than the alternate-day group. **(B)** Fractional absorption (%) during the first seven iron doses, the second seven doses, and during the entire supplementation phase, by group. Boxes indicate median and interquartile ranges, whiskers describe the range of the data (min to max). **p < 0.01.

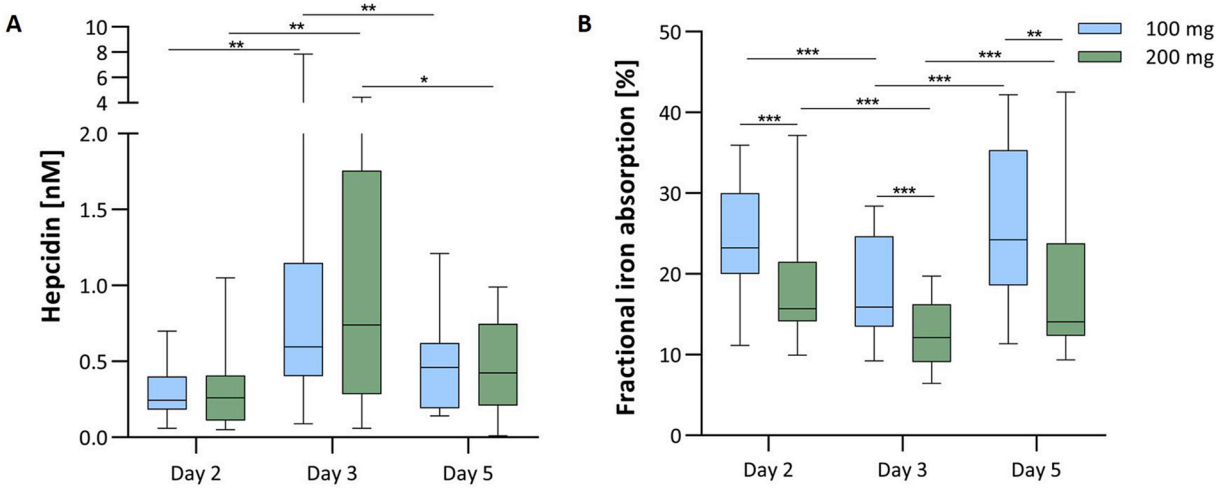

Fig. 5. In women with iron deficiency anemia (n = 19) who received oral iron doses of either 100 or 200 mg, fractional iron absorption was greater when the doses were given on alternate days then when given on consecutive days. **(A)** Serum hepcidin concentrations and **(B)** fractional iron absorption on days 2, 3 and 5. 100 and 200 mg doses result in an increase in hepcidin after 24 h but not after 48 h, and in a decrease in iron absorption from the consecutive day dose (day 3), but not the alternate day dose (day 5). Boxes indicate median and interquartile ranges, whiskers describe the range of the data (min to max). *p < 0.05, **p < 0.01, ***p < 0.001.

consecutive days. In this trial, the mean increase in Hb on day 21 in the alternate day iron group (+1.58 ± 0.53 g/dl) was greater than in the daily group (+0.41 ± 0.25 g/dl) (p < 0.05). (Mehta et al., 2020).

10. Giving twice the daily iron dose on alternate days: effects on iron absorption

The studies described above clearly show that alternate day dosing of oral iron supplements increases fractional iron absorption by 34–50% compared to the same dose given on consecutive days. Therefore, alternate dosing compared to consecutive dosing using the same iron dose will reduce the amount of unabsorbed iron in the gut lumen, potentially reducing gastrointestinal side effects and improving compliance. However, the 50% increase in fractional iron absorption 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 daily dosing. Thus, alternate dosing might also reduce the rate of Hb response. In women with severe symptomatic IDA, rapid repletion of Hb using intravenous iron supplementation may be indicated. However, in most patients with iron deficiency or mild IDA, the speed of Hb response may not be critical (BNF, 2017) and a potential option would be to provide twice the iron dose on alternate days; that is, 120 mg on alternate days instead of 60 mg on consecutive days. However, fractional iron absorption decreases significantly with increasing iron dose (Fig. 1C). Does the decrease in fractional iron absorption from a doubled dose offset the benefits of the increase in absorption from alternate day dosing?

To answer this question, in a new analysis for this review, we performed multiple linear regression analysis on pooled absorption data from our previous iron supplementation studies (Moretti et al., 2015; Stoffel et al., 2017a, 2020) to assess whether doubling the consecutive day iron dose and giving it on alternate days results in higher total iron

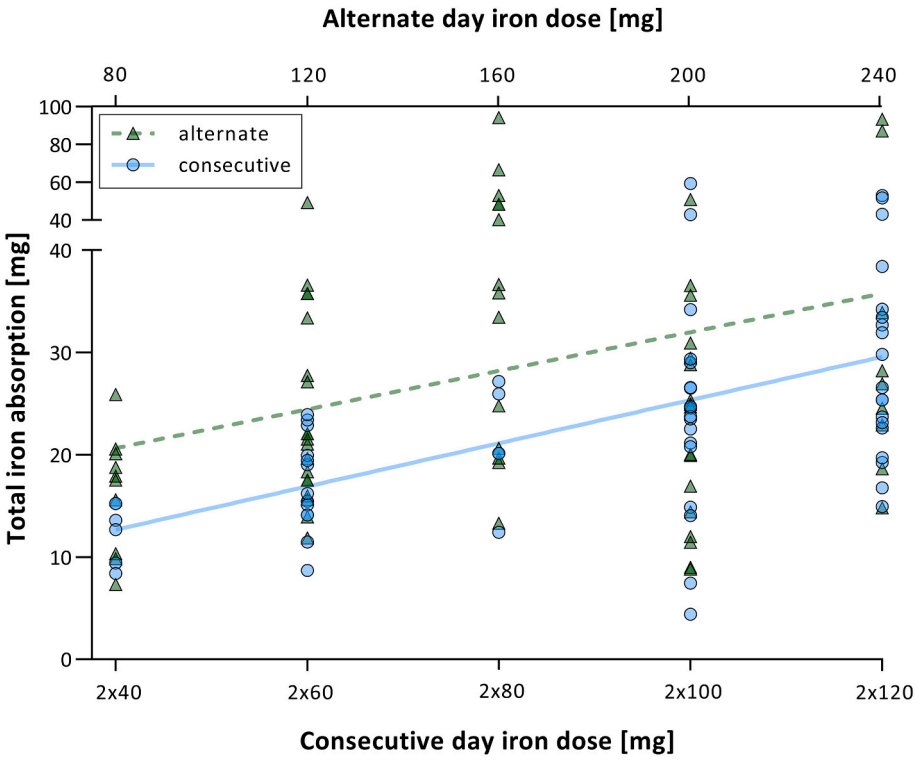

Fig. 6. In iron-deficient women and women with iron deficiency anemia (n = 64), total iron absorption from 80 mg, 120 mg, 160 mg, 200 mg and 240 mg given on alternate days (dashed line) is higher than total iron absorption from 2 × 40 mg, 2 × 60 mg, 2 × 80 mg, 2 × 100 mg and 2 × 120 mg, respectively, given on consecutive days. Overall, across all doses, alternate day dosing of twice the dose resulted in higher total iron absorption compared to consecutive day dosing. The two lines indicate linear regressions on total iron absorption from consecutive or alternate day dosing. Consecutive day dosing: R^2 = 28.4%, y = 0.1057x + 4.213; alternate day dosing: R^2 = 7.2%, y = 0.09446x + 13.09.

Table 3
In young women, comparisons of total iron absorption from different doses of elemental iron given on alternate days versus half the dose given on consecutive days. Estimates based on iron absorption data from references (Moretti et al., 2015; Stoffel et al., 2017a, 2020).

	Total iron absorption (mg)	p-value
2 × 40mg on consecutive days 1 × 80mg on alternate days	12.1 (9.4, 15.6) 15.4 (10.3, 22.8)	0.214
2 × 60mg on consecutive days 1 × 120mg on alternate days	16.6 (12.3, 22.3) 22.7 (15.5, 32.2)	0.018
2 × 80mg on consecutive days 1 × 160mg on alternate days	20.4 (15.0, 27.9) 34.6 (20.1, 59.5)	0.058
2 × 100mg on consecutive days 1 × 200mg on alternate days	21.8 (12.0, 39.4) 20.9 (12.6, 34.9)	0.496
2 × 120mg on consecutive days 1 × 240mg on alternate days	28.1 (19.7, 39.9) 31.7 (16.7, 60.1)	0.571

Comparison of 80, 120, 160 and 240 mg were done from data in iron-deficient non-anemic women using independent sample t-tests; comparison of 200 mg from data in women with iron deficiency anemia using a dependent sample t-test. Absorption in all studies was corrected for a SF of 15 μg/L (Cook et al., 1991).

absorption. We compared total iron absorption from 80 mg, 120 mg, 160 mg, 200 mg and 240 mg given on alternate days versus total iron absorption from 2 × 40 mg, 2 × 60 mg, 2 × 80 mg, 2 × 100 mg and 2 × 120 mg, respectively, given on consecutive days. In our previous studies (Moretti et al., 2015; Stoffel et al., 2017a, 2020), there was no difference in fractional iron absorption or total iron absorption comparing an iron dose given on the first (baseline) day and on the alternate day; therefore, for the purpose of this analysis, we used total iron absorption from the baseline day as representing absorption from alternate day dosing. We doubled the total iron absorption from the consecutive day dose to represent absorption from consecutive day dosing. Using these values, controlling for serum ferritin, linear mixed model analysis showed a significant dose ($p < 0.001$) and timing effect ($p < 0.001$) on total iron absorption and a significant timing*dose interaction ($p < 0.01$). Overall, across all doses, alternate day dosing of twice the dose resulted in higher total iron absorption compared to consecutive day dosing (Fig. 6). In post-hoc comparisons using independent sample t-tests, with absorption values corrected for a SF of 15 μg/L (Cook et al., 1991) (Table 3), alternate day dosing of 120 and 160 mg resulted in significantly higher total iron absorption than consecutive day dosing of 60 mg and 80 mg, respectively. In contrast, total iron absorption from alternate day dosing of 80 mg, 200 mg and 240 mg, although higher for the 80 mg and 240 mg doses, did not differ significantly from consecutive day dosing of 40 mg, 100 mg and 120 mg, respectively. The lack of difference at the lowest iron dose may be explained by the fact that in our original study (Moretti et al., 2015), a 40 mg iron dose did not result in a significant increase in serum hepcidin 24 h later. For the higher doses (200 and 240 mg) the lack of difference may be explained by the fact that fractional iron absorption decreases more sharply at higher doses (Fig. 1C), offsetting the increase in fractional iron absorption from alternate day dosing, and that variability in iron absorption is higher (Stoffel et al., 2017a, 2020). These data suggest that, if rate of Hb response is important when providing oral iron, a potential option is to provide twice the target daily iron dose on alternate days to increase the total amount of iron absorbed per unit time.

11. Conclusions

- The bioavailability and side effect profile of ferrous iron salts are similar, but their elemental iron content and cost varies; thus, choice should generally be based on the amount of iron and cost.
- Compared to ferric salts, ferrous salts are generally better absorbed.
- Absorption of oral iron, particularly when taken with meals, will be higher if given with ascorbic acid at a molar ratio of ≥2:1 to iron; that is, about 6 mg of ascorbic acid for each 1 mg of iron.
- Although WHO has recommended intermittent iron supplementation (WHO, 2011), proposing as the rationale a mucosal block in enterocytes lasting for 5–6 days, our data clearly indicate that 48 h, not 5 or 6 days, is sufficient time for iron absorption to return to baseline.
- Given as ferrous sulfate, oral iron doses ≥60 mg in non-anemic women with iron deficiency and ≥100 mg in women with IDA trigger an increase in circulating hepcidin that persists 24 h after the dose, but subsides by 48 h. It appears oral iron doses ≤40 mg do not trigger an acute increase in circulating hepcidin in iron-deficient subjects. This suggests the optimal dosing schedule to maximize fractional iron absorption in women with iron deficiency and mild IDA is to give oral doses ≤40 mg daily and give doses ≥60 mg on alternate days.
- There is a circadian increase in circulating hepcidin over the day and this is augmented by a morning iron dose; therefore, iron doses should not be given in the afternoon or evening after a morning dose.
- If rate of Hb response is important, twice the target daily iron dose can be given on alternate days. However, because fractional iron absorption decreases sharply with increasing iron doses, and unabsorbed luminal iron likely has adverse effects on the gut, lower doses may be better tolerated and improve compliance.

12. Future research priorities

- Variation in iron absorption is not fully explained by iron markers and plasma hepcidin: it is possible that a portion of the remaining variance is explained by effects modulated via the iron regulatory protein/iron-responsive elements system, HIF2α, or H-ferritin–related intra-enterocyte functions. Thus, the relative importance of extra- versus intra-enterocyte regulation of iron absorption during iron deficiency need to be clarified.
- In the above studies in women with IDA (Stoffel et al., 2020), the subjects were only mildly anemic; alternate day dosing should be assessed in women with more severe IDA.
- Hepcidin is strongly suppressed during pregnancy, particularly in women with IDA. Whether oral iron doses in pregnant women can stimulate increases in circulating hepcidin that reduce absorption from subsequent doses is uncertain. This should be clarified to inform better dosing schedules for this important target group.
- Alternate day iron dosing may optimize iron absorption and is possibly a better tolerated regimen, but larger prospective studies are needed comparing daily versus alternate day dosing to assess effects on repletion of Hb and serum ferritin, as well as gastrointestinal side effects.

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Declaration of competing interest

None.

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