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RESEARCH ARTICLE

Effect of dietary factors and time of day on iron absorption from oral iron supplements in iron deficient women

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Abstract

Guidelines generally recommend taking iron supplements in the morning away from meals and with ascorbic acid (AA) to increase iron absorption. However, there is little direct evidence on the effects of dietary factors and time of day on absorption from iron supplements. In iron-depleted women ($n = 34$; median serum ferritin 19.4 μg/L), we administered 100 mg iron doses labeled with ^{54}Fe, ^{57}Fe, or ^{58}Fe in each of six different conditions with: (1) water (reference) in the morning; (2) 80 mg AA; (3) 500 mg AA; (4) coffee; (5) breakfast including coffee and orange juice (containing ~90 mg AA); and (6) water in the afternoon. Fractional iron absorption (FIA) from these $n = 204$ doses was calculated based on erythrocyte incorporation of multiple isotopic labels. Compared to the reference: 80 mg AA increased FIA by 30% ($p < .001$) but 500 mg AA did not further increase FIA ($p = .226$); coffee decreased FIA by 54% ($p = .004$); coffee with breakfast decreased FIA by 66% ($p < .001$) despite the presence of ~90 mg of AA. Serum hepcidin was higher ($p < .001$) and FIA was 37% lower ($p = .059$) in the afternoon compared to the morning. Our data suggest that to maximize efficacy, ferrous iron supplements should be consumed in the morning, away from meals or coffee, and with an AA-rich food or beverage. Compared to consuming a 100 mg iron dose in the morning with coffee or breakfast, consuming it with orange juice alone results in a ~ 4-fold increase in iron absorption, and provides ~20 more mg of absorbed iron per dose. The trial was registered at Clinicaltrials.gov(NCT04074707).

1 | INTRODUCTION

Oral iron supplementation is considered the first line of treatment of iron deficiency in most women,[1] and guidelines generally recommend doses of 60–120 mg ferrous iron per day.[2] In an effort to increase absorption, it is often recommended to take iron in the morning away from meals and with ascorbic acid (AA).[3,4] However, these recommendations are based mainly on isotopic studies that assessed the effects of dietary inhibitors and enhancers on iron absorption from foods and/or iron fortificants added to foods.[5] In these studies, absorption of low doses of ferric and ferrous iron were reduced by phytic acid in many breakfast foods and by polyphenols present in tea and coffee, and increased by AA.[6] Phytic acid:iron and AA:iron molar ratios that inhibit/promote iron absorption in foods are well characterized,[5] but these may not apply to higher doses of ferrous iron used in oral supplements. In current guidelines, it is often assumed that these effects are similar for oral iron supplements, but there is little direct supporting evidence.

Clinicians often recommend taking a 500 mg AA supplement or an AA-rich beverage such as orange juice along with oral iron to improve absorption. However, there is little evidence to support this,

and it remains unclear what dose of AA is effective.[3,4] Older radioiron studies assessed the effects of different doses of AA on iron absorption from supplements, and some found modest enhancing effects.[7,8] However, a randomized trial of 35 mg oral iron with or without 200 mg of AA given 3 times per day to anemic adults reported comparable increases in hemoglobin (Hb) and serum ferritin at 8 weeks.[9] Coffee, like other polyphenol containing beverages, reduces absorption of food iron and iron fortificants,[10] but whether it affects iron absorption from oral iron supplements has not been tested. Many studies report lower iron absorption from oral iron doses given with mixed meals, compared to fasting.[11,12] Adding AA to meals can overcome much or all of the inhibiting effect of the meal on iron absorption from foods and iron fortificants,[6,13] but whether this is true for iron supplements taken with meals is unknown.

It is usually recommended that iron supplements be consumed in the morning.[14] Hepcidin, the major regulator of iron absorption,[15,16] shows clear diurnality,[17,18] but there is little direct evidence that this effects iron absorption. The diurnal increase in hepcidin is accentuated by a morning iron dose, which reduces iron absorption from a second dose given in the afternoon.[16] However, we are aware of no studies using iron stable isotopes that measured whether iron absorption from iron supplements given once daily varies by time of day.

In this study in iron deficient women, our objective was to measure the effects of dietary inhibitors and enhancers, and time of day, on iron absorption from a high oral iron dose (100 mg iron as ferrous fumarate). We used a crossover design and multiple iron stable isotopes to assess the effects of two different doses of AA (80 mg, the amount in an AA-rich food or beverage, and 500 mg, a pharmacological dose), coffee, and a mixed breakfast meal containing coffee, semi-brown bread, yogurt, and orange juice (typically containing 80–90 mg AA), on iron absorption. In addition, we compared absorption from the supplement given in the morning and afternoon. Our hypotheses were: (1) iron absorption would be increased by both doses of AA, but the 500 mg dose would increase absorption more than the 80 mg dose; (2) iron absorption would be decreased by coffee; (3) iron absorption from the breakfast would not be decreased because of the presence of AA-rich orange juice in the meal; and (4) iron absorption would be lower in the afternoon than in the morning.

2 | METHODS

2.1 | Study design and participants

This study was conducted between June and December 2022 at the ETH Zurich, Switzerland. We recruited healthy, nonanemic, iron deficient women from universities in Zurich. Inclusion criteria were: female; aged between 18 and 45 years; plasma ferritin (PF) concentration ≤ 30 μg/L; Hb concentration > 12 g/dL; C-reactive protein (CRP) concentration <5 mg/L; body-mass index 18.5–26.5 kg/m^2; body weight < 70 kg; no major chronic diseases; not pregnant or lactating; non-smoking; no intake of vitamin and mineral supplements in the 2 weeks before study start and during the study; no blood transfusion, blood donation, or significant blood loss over the past 4 months. Exclusion criteria once enrolled were inability to follow study procedures and major illness. The Ethics Committee of the Canton Zurich approved the study (2022-00533) and all subjects provided written informed consent.

2.2 | Randomization and masking

All participants went through two blocks of iron absorption studies in which they received a labeled oral iron supplement under six different conditions on six different days (Figure 1). Participants were individually randomly assigned to a condition sequence, using a computer-generated list (Excel, Microsoft Office 2016). Assignment was not masked. The sequences were generated so that each isotope was used only once in each block and the reference condition was given on the first day of one block and the afternoon condition on the third day of the other block.

2.3 | Procedures

The study was a controlled, open-label, randomized cross-over trial. During the screening, 2–4 weeks before study start, we assessed 122 women for eligibility. We collected a venous blood sample for the determination of Hb, PF, CRP, and pregnancy status, and measured weight to the nearest 0.1 kg and height to the nearest 0.5 cm. Enrolled participants went through two blocks of iron absorption studies where they received labeled iron doses of 100 mg under six different conditions. The experimental phase lasted for 43 days. The iron doses were administered on days 1, 3, 5, 22, 24, and 26 (Figure 1) between 7:00 a.m. and 9:00 a.m. after an overnight fast and on day 5 or 26 between 4:00 p.m. and 6:00 p.m. after a 4 h fast (afternoon condition, see below). The participants consumed the iron under direct supervision of the investigators. After consuming the iron, participants were not allowed to eat or drink for 3 h, except for 500 mL of bottled water, which they drank 1–3 h after the test condition.

The iron supplements contained 100 mg elemental iron as ferrous fumarate (FeFum) (Ferrum Hausmann 100 mg Kaps, Vifor, Switzerland) and were labeled with 3 mg of ^{57}FeFum, ^{54}FeFum, or ^{58}FeFum. The labeled iron was consumed together with the iron supplement with a glass of nanopure water (200 mL) under the following six conditions: (1) with water only (reference); (2) with 80 mg AA; (3) with 500 mg AA; (4) with coffee; (5) with breakfast (described below); and (6) with water only, in the afternoon. We collected venous blood samples after an overnight fast on days 1 (baseline), 22, and 43 (endpoint) and in the afternoon of day 5 or 26. On the day of the afternoon iron administration, participants consumed a standardized lunch of vegetarian lasagna (400 g) 4 h before the visit. After that, the participants could drink 500 mL of bottled water, which they had to finish at least 1 h before the afternoon visit.

^{54}FeFum, ^{57}FeFum, and ^{58}FeFum were prepared by Dr. Paul Lohmann GmbH from ^{54}Fe-, ^{57}Fe-, and ^{58}Fe-enriched elemental iron (99.6%, 95.78%, and 99.89% isotopic enrichment, respectively). We prepared two stock solutions (80 mg/mL and 250 mg/mL) of food

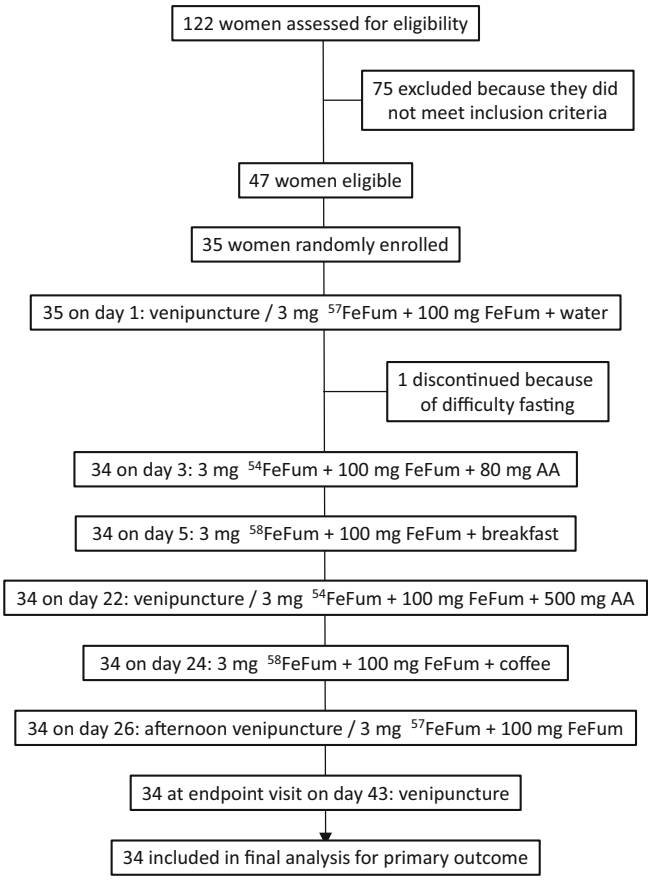

FIGURE 1 Schematic representation of the study design. In a randomized order, fasting subjects received on days 1, 3, 5, 22, 24, and 26, 100 mg FeFum given with 3 mg of an isotopic label in the morning between 7.00 a.m. and 9:00 a.m. with water only, 80 mg AA, breakfast, 500 mg AA and in the afternoon between 4:00 p.m. and 6:00 p.m. with water only. Subjects acted as their own controls. Iron and inflammation status markers and hepcidin were assessed on days 1 and 22 in the morning and on day 5 or 26 in the afternoon. Incorporation of iron stable isotopes was measured on days 22 and 34. AA, ascorbic acid; FeFum, ferrous fumarate.

grade L-AA (Sigma Aldrich) in the morning before the study visits, from which 1 or 2 mL, respectively, was pipetted into the nanopure water, which they consumed with the iron capsules and isotopes. The breakfast consisted of a 96 g semi-brown, wheat flour-based bread roll with 15 g butter and 25 g honey per serving, 180 g of plain whole milk yogurt, 250 mL of orange juice, 150 mL of black instant coffee made from 150 mL nanopure water containing 2 g of coffee powder (Nescafé Gold De Luxe, Nestlé). We bought the bread and orange juice in two batches and analyzed the composition of one sample in triplicate from each batch. The bread was frozen at −20°C and thawed the day before the test meal administration and the orange juice was stored in the refrigerator at 4°C. For the condition with only coffee, we used the same coffee preparation as in the breakfast.

We analyzed iron and calcium concentration in the bread (freeze dried and milled) and yogurt by inductively coupled plasma mass spectrometry (Q-ICP-MS iCap RQ, Thermo Scientific) after mineralization by microwave digestion (TurboWave, MLS) in HNO_3. The phytic acid concentration in bread was measured according to a modified method from Makower, using cerium for the extraction of phytic acid.[19] After hydrolyzation in sulfuric acid, inorganic phosphate was determined according to Van Veldhoven and Mannaerts[20] and converted into phytic acid concentrations. We measured the total polyphenol concentration in coffee with a modified Folin–Ciocalteu method[21] and expressed it as gallic acid equivalents. AA and dehydroascorbic acid in orange juice was first extracted in meta-phosphoric acid, then dehydroascorbic acid was reduced to AA with dithiothreitol as reducing agent. The AA in the extract was separated and quantified by HPLC (Acquity, Walters) on a C-18 reversed phase column.

We measured Hb using a Sysmex XN-3000 analyzer (Sysmex Corporation) immediately after venipuncture from EDTA whole-blood and then prepared whole-blood aliquots which were stored at −20°C. The blood collected with serum tubes was centrifuged at 3000 rpm for 10 min, and the serum stored at −20°C. We measured serum ferritin, soluble transferrin receptor (sTfR), α-1-acid glycoprotein (AGP), and CRP in serum using a multiplex immunoassay[22] and serum iron and total iron binding capacity (TIBC) using colorimetry. Transferrin saturation (%TS) was calculated using the formula: (serum iron/TIBC) × 100. We measured serum hepcidin using the DRG Hepcidin 25 (bioactive) HS enzyme immunoassay (DRG Instruments GmbH, Marburg, Germany). Anemia was defined as Hb <120 g/L.[23] Iron deficiency was defined as serum ferritin <45 μg/L[24,25] and/or elevated sTfR >8.3 μg/mL and iron deficiency anemia was defined as serum ferritin <45 μg/L[24,25] and/or sTfR >8.3 mg/L and Hb <120 g/L.[23] We defined CRP >5 mg/L and/or AGP >1 g/L as indicating inflammation.

Whole blood samples were mineralized in duplicate using nitric acid and microwave digestion (TurboWave, MLS) followed by separation of the iron from the blood matrix by anion-exchange chromatography and a subsequent precipitation step with ammonium hydroxide.[26] We measured iron isotope ratios by using a multi-collector inductively coupled plasma mass spectrometry (Neptune, Thermo Fisher Scientific, Germany).[27] The amounts of ^{54}Fe, ^{57}Fe, and ^{58}Fe isotopic tracers in whole blood collected 17 days after completion of each of the two iron absorption studies (days 22 and 43) were calculated on the basis of the shift in iron isotope ratios and on the estimated amount of iron circulating in the body. Circulating iron was calculated on the basis of blood volume and mean Hb concentrations for each participant.[28] The calculations for fractional iron absorption (FIA) were based on the principles of isotope dilution,[29] taking into account that iron isotopic labels are not monoisotopic,[30] and assuming 80% incorporation of the absorbed iron into the erythrocytes.[31] Total iron absorption (TIA) was calculated based on FIA and the total amount of iron consumed per condition.

2.4 | Outcomes

The primary outcomes were FIA and TIA for each condition of iron administration, calculated as described above. Secondary measures

were iron status (serum ferritin, Hb, sTfR, serum iron, TIBC, TS), hepcidin, and inflammation markers (CRP, AGP).

2.5 | Statistical analysis

We powered the study to detect a 25% within-subject difference in FIA, based on the SD of the difference in FIA in young women from 5 mg iron given with and without AA (molar ratio of AA:iron = 2:1) in a previous study conducted at the ETH Zurich.[32] Using the log SD of 0.1694, a power of 80% and a level of significance of 0.05 we estimated a sample size of 26. Anticipating a drop-out rate of 20% and adding 3 more subjects due to uncertainty about effect sizes, we enrolled 35 participants in the study.

The SPSS statistical programming environment (IBM SPSS Software, Version 28) and Microsoft Office EXCEL 2016 (Microsoft, Redmond, WA) were used for the data analysis. Per protocol analysis was used to analyze primary outcomes. Data were examined for normality by use of the Shapiro–Wilk test. Normally distributed data were reported as mean (SD), and non-normally distributed data were reported as median (IQR). For CRP values below the lowest level of detection, we imputed random variables between 0 and LOD ($n = 27$). Morning versus afternoon iron and inflammation status markers were compared with paired samples *t*-tests or nonparametric tests. Morning versus afternoon FIA and serum hepcidin were compared with a one-tailed paired samples *t*-test, as we expected FIA to be lower and hepcidin to be higher in the afternoon. Linear mixed effects models were used to assess the effect of the different iron administration conditions on iron absorption. Logarithmically transformed data were used when model assumptions were not met. FIA was the dependent variable, the iron administration condition and serum ferritin were added to the models as fixed factors, and subject ID was added as a random factor (intercept). The reference condition (with water in the morning) and two enhancing conditions (with 80 mg and 500 mg AA) were compared in one model, and the reference condition and two inhibiting conditions (with coffee and breakfast) were compared in a second model. We did not correct post hoc tests for multiple comparisons, as the comparisons were prespecified. We performed multiple linear regression analyses to determine predictors of FIA and hepcidin for both morning and afternoon iron administration and did not include predictors which correlated >0.7 in the spearman's rho correlation matrix in the same model. The trial was registered at clinicaltrials.gov as NCT04074707.

3 | RESULTS

Between June 24, 2021, and September 29, 2022, 35 women were enrolled (Figure 1). One woman left the study after the first visit due to difficulties with fasting and 34 women completed the study, on December 9, 2022. Median age was 28 years (IQR 24–33). Mean (SD) weight was 56.7 (8.2) kg and median BMI was 20.3 kg/m^2 (19.1–23.2). Iron and inflammation status markers at baseline morning (day 1) and afternoon (day 5 or 26) are shown in Table 1. Median serum hepcidin was higher in the afternoon compared to the morning measurement (1.71 nmol/L (IQR 0.72–3.00) versus 0.75 nmol/L (0.42–1.49)), $p < .001$) (Table 1).

Nutrient composition of the breakfast, which included the coffee condition, is shown in Table 2. The bread roll (96 g) contained a mean (coefficient of variation [CV]) 512.4 mg (0.179) of phytic acid. The mean (CV) polyphenol content in coffee (150 mL) was 219.0 mg (0.01), the mean AA content in orange juice (250 mL) was 90.1 mg (0.54), and the mean (CV) calcium content in the bread roll bread and yogurt (180 g) was 29.3 mg (0.07) and 317.4 mg (12.5), respectively. In the breakfast, when the native food iron and supplemental iron were considered, the MR of phytic acid: iron was 0.42:1 and the MR of AA: iron was 0.27:1. In the conditions with 80 mg and 500 mg AA, the MRs of AA: iron were: 0.24:1 and 1.52:1, respectively.

From a total of $n = 204$ absorption values, median (IQR) fractional and total iron absorption for the six conditions are shown in Supplemental Table 1. Median FIA was higher with 80 mg AA (26.7% [IQR 19.1–32.6]) and 500 mg AA (30.5% [24.9–49.5]) compared to the reference (20.6% [12.3–25.5], $p < .001$ for both) (Figure 2A). There was no difference in iron absorption from the two AA conditions ($p = 0.226$). Compared to the reference, median FIA was lower with coffee (9.5% [IQR 6.2–16.3], $p = 0.001$) or breakfast (6.9% [4.1–13.0], $p < .001$) (Figure 2A). Iron absorption was lower from the breakfast compared to coffee ($p = .008$). Serum hepcidin was significantly higher in the afternoon (Figure 2B) and absorption from the afternoon dose (12.9% [9.9–21.6]) was lower than from the reference morning dose (Figure 2C), resulting in an FIA ratio of 0.63, this difference was only borderline significant ($p = .059$).

In the multiple linear regression analysis with morning FIA as the dependent variable, serum ferritin ($\beta = -0.556$) in one model, and hepcidin ($\beta = -0.438$) in the other model were significant predictors, explaining 13.7% and 12.2% of the variance (Supplemental Table 2). With afternoon FIA as the dependent variable, serum ferritin ($\beta = -0.619$) in one model, and hepcidin ($\beta = -0.737$) in the other model were significant predictors, and explained 42.8% and 55.3% of the variance, respectively. With morning hepcidin as the dependent variable, serum ferritin ($\beta = 0.687$) was a significant predictor, and explained 51.1% of the variance, while sTfR ($\beta = 0.149$) and CRP ($\beta = 0.294$) were not. With afternoon hepcidin as the dependent variable, serum ferritin ($\beta = 0.670$) was a significant predictor, and explained 60.3% of the variance, while CRP ($\beta = 0.218$) and sTfR ($\beta = -0.036$) were not (Supplemental Table 2).

4 | DISCUSSION

Our main findings are, in iron deficient women ingesting a dose of 100 mg oral iron, compared to the reference dose given only with water: (1) 80 mg AA increased iron absorption by 30%, but increasing the AA dose to 500 mg did not further increase absorption; (2) consumption with coffee decreased iron absorption by 54%; (3) consumption with breakfast including coffee decreased iron absorption further,

TABLE 1 Iron status and inflammation markers at baseline in the morning and in the afternoon.

	Baseline morning (day 1)	Afternoon (day 5 or 26)	*p*-value
n	35	34	
Hb, g/dL	12.9 (12.5–13.9)	13.2 (12.5–13.6)	.602
Serum ferritin, μg/L	19.4 (12.6–25.8)	27.6 (17.7–38.6)	<.001
sTfR, mg/L	4.4 (3.6–5.0)	4.4 (3.8–5.4)	.886
Serum iron, μg/mL	14.9 (10.3–18.0)	10.9 (8.1–13.5)	.001
TIBC, μg/mL	54.3 (7.6)	57.0 (6.7)	.049
TS, %	26.9 (9.9)	19.3 (7.2)	<.001
Serum hepcidin, nmol/L	0.75 (0.42–1.49)	1.71 (0.72–3.00)	<.001
CRP, mg/L	0.54 (0.23–1.60)	0.44 (0.14–0.96)	.043

Note: Data are mean (SD) or medians (IQR). *p*-Values were estimated using paired *t*-test or Wilcoxon matched-pair signed rank test as appropriate. Abbreviations: CRP, C-reactive protein; Hb, hemoglobin. sTfR, soluble transferrin receptor; TIBC, total iron binding capacity; TS, transferrin saturation.

TABLE 2 Nutrient composition of the breakfast.

	Per 100 g fresh weight (mg)	Per serving (mg)
Bread		
Phytic acid (PA)	534.4 (0.19)	512.4 (0.18)
Iron	1.31 (0.03)	1.26 (0.04)
Calcium	30.5 (0.09)	29.3 (0.07)
Molar ratio PA: iron	34.5:1	
Yogurt		
Calcium	176.3 (14.5)	317.4 (12.5)
Coffee		
Polyphenols	146.0 (0.01)[a]	219.0 (0.01)
Orange juice		
Ascorbic acid (AA)	36.0 (0.54)	90.1 (0.54)
Molar ratio AA:Fe	22.7:1	

Note: Data as mean (CV) or mean (%SD) for yogurt.
[a]Per 100 mL coffee.

by 66%, despite the presence of ~90 mg of AA in the meal; (4) consumed in the afternoon, iron absorption was lower by 37% compared to the morning reference dose; however, this difference only approached significance.

Although there was an increase in iron absorption from the supplement when given with 80 or 500 mg of AA, the percentage increase was much lower than in previous studies with iron fortificants in foods, where similar doses of AA increased iron absorption 2–12-fold.[5] AA likely enhances iron absorption through several mechanisms. In the gut lumen, AA can reduce ferric to ferrous iron to allow uptake by the divalent metal transporter-1.[33] For iron supplements given in the ferrous form, AA may increase iron absorption by limiting the oxidization of ferrous to ferric iron in the stomach.[33,34] This may explain the lower increase in iron absorption when AA is given with ferrous iron (as opposed to ferric iron in foods), since most of iron taken in this form would likely arrive at the absorptive surface of the duodenum as the ferrous form even in the absence of AA. AA may also facilitate iron absorption by forming a chelate with ferric or ferrous iron at acid pH that remains soluble at the alkaline pH of the duodenum,[35,36] but again these effects may be less relevant for ferrous iron. Notably, in our study, an AA dose of 80 mg at very low MR of AA:Fe (0.24:1) increased iron absorption by 30%, while a 6.25-fold increase in the dose to 500 mg (to achieve a MR of AA:Fe of 1.5:1) did not further increase absorption. In contrast, in fortified foods, there is a steep linear increase in iron absorption up to a MR of AA:Fe of 7.5:1, followed by a less pronounced response at higher ratios.[34] In a previous study in adults given 30 mg of iron as ferrous sulfate with AA, an increase AA:Fe MR from 0.5 to 3 improved iron absorption from ~8.5% to 13%.[34] In another study in iron depleted women, administering 100 mg FeFum with prebiotic galacto-oligosaccharides or fructo-oligosaccharides enhanced iron absorption by 45% and 51%, respectively, ($p < .001$ for both).[37] Our data indicate that ferrous iron absorption from a supplemental dose is enhanced at a dose of AA typically found in a serving of AA-rich foods, such as citrus fruits and many green vegetables, but that there is no additional benefit on iron absorption from a 500 mg pharmacological dose of AA.

The inhibitory effect of polyphenols on iron absorption is thought to be due to the formation of unabsorbable iron complexes in the gut lumen.[38,39] The inhibition of iron absorption by coffee is mainly determined by its content of chlorogenic acid.[40] Previous studies assessed the effects of polyphenol-rich beverages on iron absorption from food iron and iron fortificants.[10,39,41,42] One study reported that beverages containing 100–400 mg polyphenols (our cup of coffee contained 219 mg) lowered iron absorption from a bread meal by 60%–90%.[39] When a cup of instant coffee was ingested with a semi-purified meal, food iron absorption was reduced from 5.88% to 0.97%.[10] To our knowledge, no previous study examined the effect of coffee or its polyphenols on absorption from supplemental iron doses. Notably, the 54% reduction in iron absorption from a high iron dose with coffee in this study is comparable to the inhibitory effect of similar doses of polyphenols on much lower levels of iron as fortificants in foods.[39,41] These data suggest that polyphenols remain potent inhibitors of iron absorption from oral iron supplements, despite a much lower ratio of polyphenols to iron than in previous meal and iron fortificant studies.

Consumption of the iron supplement with the breakfast test meal reduced iron absorption by 67% and was lower than with coffee only. A radioiron study in non-anemic women found that mean FIA from a 50 mg oral iron dose consumed while fasting was 9.8%, but was only 2.6% when consumed with a meal of beans vegetables and rice.[11] In iron sufficient adults ($n = 9$), mean FIA from 50 mg of ferrous iron was 2.6% with a wheat-based meal versus 7.9% when taken fasting.[12] Since the breakfast condition included orange juice, it appears that the enhancing effect of ~90 mg AA in orange juice does not counterbalance the inhibitory effects of a breakfast moderately high in polyphenols, phytic acid and calcium. In contrast, AA has been shown to prevent dose-dependent inhibitory effects of polyphenols and phytic acid on absorption from lower levels of iron in meals.[6,43,44] Previous studies reported that a glass of orange juice added to mixed meals

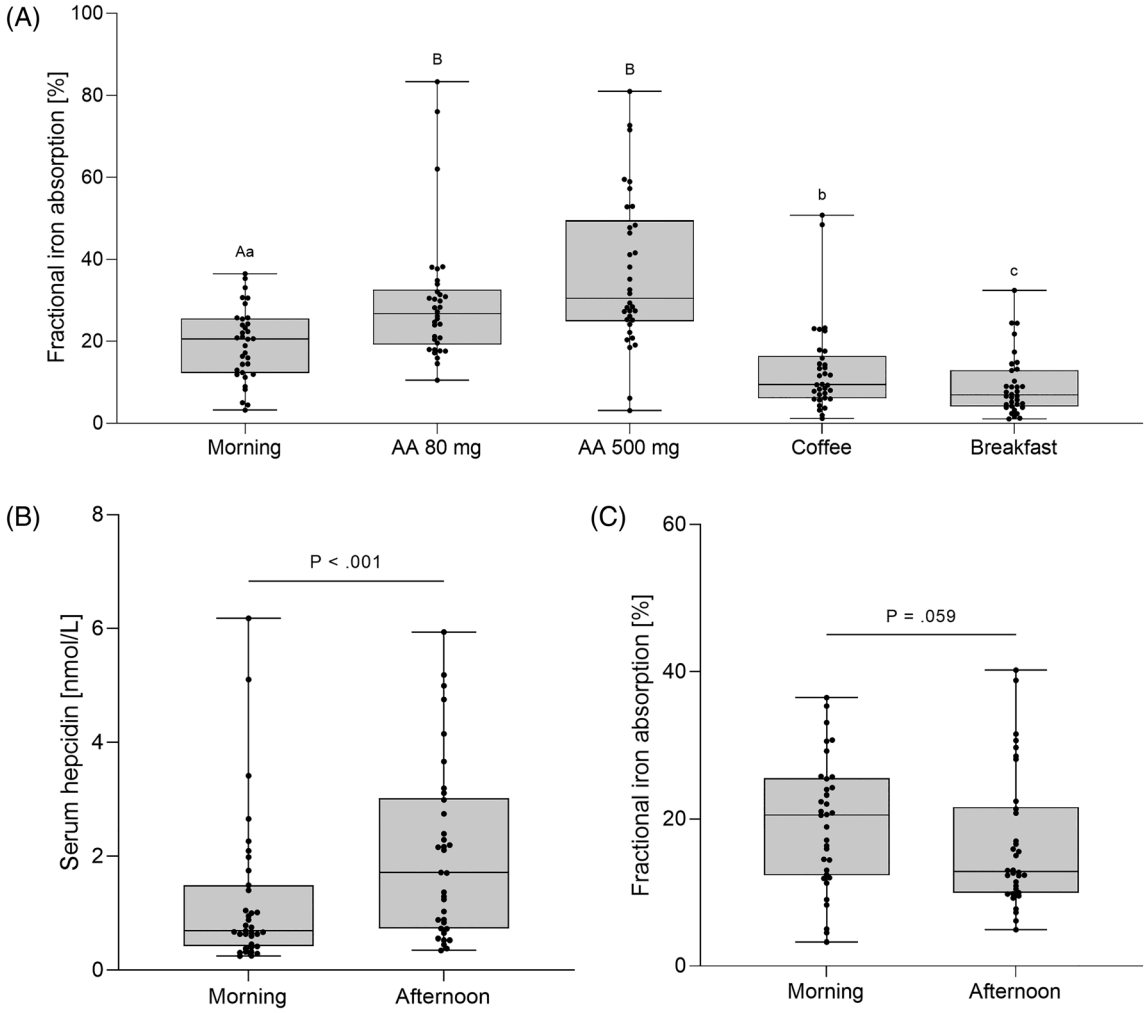

FIGURE 2 Fractional iron absorption (%) from all test conditions and serum hepcidin (nmol/L) concentrations in the morning and afternoon. (A) Fractional iron absorption from 100 mg FeFum with water in the morning, with 80 mg AA, with 500 mg AA, with 150 mL coffee or with a breakfast ($n = 34$). (B) Serum hepcidin in the morning (day 1) and afternoon (day 5 or 26). (C) Fractional iron absorption from 100 mg FeFum with water in the morning (day 1 or 22) or in the afternoon (day 5 or 26). The effect of the test conditions on iron absorption was assessed by mixed-effect model in a model with enhancers (morning, 80 mg AA and 500 mg AA) and inhibitors (morning, coffee and breakfast), followed by post hoc tests. Morning versus afternoon serum hepcidin concentration and fractional iron absorption were compared using one-tailed paired samples *t*-tests. Boxes show the median, 25th, and 75th percentiles; whiskers show the range; points show individual values. Boxes with different subscript letter differ significantly (capitals: model with enhancers, minuscule: model with inhibitors, $p < .05$). AA, ascorbic acid.

increased food iron absorption (~4 mg iron per meal) by 2–3-fold,[13,45] and entirely reversed the inhibitory effect from polyphenol-rich tea.[46] Unlike these studies, our findings indicate that including a dietary source of AA in an inhibitory meal taken with an oral iron supplement does not mitigate the sharp decrease in absorption due to the meal matrix.

Hepcidin is the major regulator of iron absorption, and serum hepcidin negatively correlates with iron absorption from meals and supplements.[15,16] Serum hepcidin shows clear diurnality in iron replete and iron deficient adults,[17,18] and hepcidin expression may be an indirect target for the core circadian clock.[47] The diurnality in hepcidin is likely physiologically relevant, but there is little direct evidence that it affects iron absorption. We have previously shown that the diurnal increase in plasma hepcidin is accentuated by morning iron supplementation,[16] and this is associated with a reduction in iron absorption of 26% from supplemental iron doses given in the afternoon, compared to morning doses, in iron deficient women.[16] However, we are aware of no previous studies using iron stable isotopes that measured whether iron absorption from oral iron supplements given once daily varies by time of day. Our findings suggest that there is diurnal variation in supplemental iron absorption: median absorption from the afternoon dose (12.9%) was lower than from the reference morning dose (20.5%) (Figure 2C), but this difference was borderline significant and needs confirmation in future studies. Notably, hepcidin was more than 2-fold higher in the afternoon than in the morning (Table 1, Figure 2B), and hepcidin at time of supplement administration was a significant predictor of morning and afternoon absorption from the iron supplement (Supplemental Table 2).

This study has several strengths. Iron absorption was measured using erythrocyte incorporation of multiple stable iron isotopes. We studied iron deficient young women, one of the main target groups of iron supplementation. Our cross-over study design allowed for within-subject comparisons and allowed us to compare morning versus afternoon iron absorption, independent of a previous morning dose. This study also has limitations. The effect of enhancers and inhibitors might be overestimated, as subjects were fasted overnight and fasted again for 3 h after the test condition. The breakfast contained a variety of enhancers and inhibitors, but we did not test them separately, making it difficult to attribute effects to individual dietary components.

Our findings suggest practical ways to optimize current oral iron supplementation guidelines to increase absorption in iron deficient women. To maximize iron absorption, oral iron should be consumed in the morning, away from meals or coffee, and with an AA-rich food or beverage. Following these recommendations could have a large effect on the efficacy of iron supplements: compared to consuming a 100 mg iron dose in the morning with coffee or breakfast, consuming it alone with orange juice would result in a nearly 4-fold increase in iron absorption, and provide ~20 more mg of absorbed iron per dose.

AUTHOR CONTRIBUTIONS

Hanna K. von Siebenthal, Diego Moretti, Michael B. Zimmermann, and Nicole U. Stoffel conceived the studies and obtained funding; all authors contributed to the design of the trials; Hanna K. von Siebenthal, N.W., and Nicole U. Stoffel conducted the studies; Hanna K. von Siebenthal, Michael B. Zimmermann, and Nicole U. Stoffel analyzed the data and wrote the first draft of the manuscript; and all authors contributed to the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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