

# A 1-h time interval between a meal containing iron and consumption of tea attenuates the inhibitory effects on iron absorption: a controlled trial in a cohort of healthy UK women using a stable iron isotope

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## ABSTRACT

**Background:** Tea has been shown to be a potent inhibitor of non-heme iron absorption, but it remains unclear whether the timing of tea consumption relative to a meal influences iron bioavailability.

**Objective:** The aim of the study was to investigate the effect of a 1-h time interval of tea consumption on nonheme iron absorption in an iron-containing meal in a cohort of iron-replete, nonanemic female subjects with the use of a stable isotope (<sup>57</sup>Fe).

**Design:** Twelve women (mean  $\pm$  SD age: 24.8  $\pm$  6.9 y) were administered a standardized porridge meal extrinsically labeled with 4 mg <sup>57</sup>Fe as FeSO<sub>4</sub> on 3 separate occasions, with a 14-d time interval between each test meal (TM). The TM was administered with water (TM-1), with tea administered simultaneously (TM-2), and with tea administered 1 h postmeal (TM-3). Fasted venous blood samples were collected for iron isotopic analysis and measurement of iron status biomarkers. Fractional iron absorption was estimated by the erythrocyte iron incorporation method.

**Results:** Iron absorption was 5.7%  $\pm$  8.5% (TM-1), 3.6%  $\pm$  4.2% (TM-2), and 5.7%  $\pm$  5.4% (TM-3). Mean fractional iron absorption was found to be significantly higher (2.2%) when tea was administered 1 h postmeal (TM-3) than when tea was administered simultaneously with the meal (TM-2) ( $P = 0.046$ ). An **w**50% reduction in the inhibitory effect of tea (relative to water) was observed, from 37.2% (TM-2) to 18.1% (TM-3).

**Conclusions:** This study shows that tea consumed simultaneously with an iron-containing porridge meal leads to decreased nonheme iron absorption and that a 1-h time interval between a meal and tea consumption attenuates the inhibitory effect, resulting in increased nonheme iron absorption. These findings are not only important in relation to the management of iron deficiency but should also inform dietary advice, especially that given to those at risk of deficiency. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT02365103. *Am J Clin Nutr* 2017;106:1413–21.

**Keywords:** iron bioavailability, iron deficiency, hepcidin, polyphenols, tea

## INTRODUCTION

Iron deficiency is a condition defined as a lack of iron stores that leads to an enduring negative iron balance (1), which

commonly affects women of childbearing age and children worldwide (2). Iron remains a marginal nutrient in the dietary intake of the UK population (3), leading to concerns of an increased risk of iron deficiency. The UK National Diet and Nutrition Survey shows that women aged 19–64 y do not meet the recommended level of dietary iron intake, with a mean  $\pm$  SD daily intake of 9.6  $\pm$  3 mg, which equates to 78% of the Reference Nutrient Intake (4). In countries where iron deficiency is not commonly attributable to iron metabolism-related diseases, it is suggested that insufficiency in absorbable iron to meet the iron requirement is one of the factors that may contribute to iron deficiency (5). The presence of inhibitors and enhancers in the diet will affect iron bioavailability more than the chemical forms of iron itself, and polyphenols have been shown to be potent inhibitors of iron absorption (6). Polyphenols can be found in plant-based sources, including tea, wine, and coffee (7). British adults (aged 19–64) consume an average of 1114 g tea, coffee, and water daily, and this amount is higher in women (1132 g/d) than in men (1097 g/d) on the basis of the recent National Diet and Nutrition Survey data (4).

To the best of our knowledge, few human studies to date have investigated the inhibitory effect of tea consumption on iron absorption with the use of an isotope-labeled test meal (TM). Hurrell et al. (8) found that the inhibitory effect of black tea on

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Supplemental Tables 1 and 2 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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Abbreviations used: CRP, C-reactive protein; ICP-MS/MS, inductively coupled plasma–tandem mass spectrometry; TM, test meal.

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iron absorption ranged between 79% and 94% in a study carried out by using a simple bread roll TM and different types of phenolic-containing beverages. Significant reductions in iron absorption were also observed ( $P < 0.001$ ) when the TM was given with tea than when given with water in a study by Thankachan et al. (9), which used a rice-based TM. A study by Disler et al. (10) carried out in 10 male Sprague-Dawley rats reported that the minimum time interval for the inhibitory effect to be established was 30 min, with 1.8-fold higher iron absorption compared with no time interval (27.6%–50.7%;  $P < 0.005$ ).

To date, it is apparent that limited data are available, especially from controlled intervention studies that investigated the inhibitory effect on iron absorption of tea components, and no studies in humans that addressed iron absorption, particularly time interval, have been carried out in the United Kingdom. Because tea is not only widely consumed in the United Kingdom (11) but extensively consumed worldwide, the relevance of this study has global impact. Therefore, we aimed to assess the effect of the time interval of tea consumption relative to a meal on nonheme iron absorption from a typical Western breakfast with the use of a stable iron isotope ( $^{57}\text{Fe}$ ) in nonpregnant women and to assess the potential link between hepcidin concentration and iron absorption.

## METHODS

### Subjects

Twelve premenopausal women aged 19–40 y were enrolled in the study with the use of posters and e-mails as recruitment media. All of the subjects were healthy, without any known history of gastrointestinal or metabolic disorders, nonpregnant, and nonlactating. The subjects were excluded if they had donated blood in the past 6 mo and regularly consumed nutritional supplements. Subjects were provided with a participant information sheet and were briefed on the study protocol before providing written consent prior to partaking in the study.

The sample size was estimated by using iron absorption data from a study by Derman et al. (12) carried out in Indian women aged between 21 and 71 y with the use of a maize meal labeled with radioisotope  $^{55}\text{Fe}$  or  $^{59}\text{Fe}$ . The study reported mean  $\pm$  SD iron absorption of 6.7%  $\pm$  6.2% when the TMs were administered with tea and 34.0%  $\pm$  23.0% when the TMs were administered without tea. With a Cohen's effect size ( $d$ ) of 1.32, the total sample size required in the present study was estimated to be 10 subjects (power = 0.95,  $\alpha$  error probability = 0.05). Incorporating a potential 20% drop out rate, the total sample size required to show a significant difference in iron absorption between the consumption of a meal with and without tea was estimated to be 12. The sample size was estimated by using G\*Power software, version 3.1.7 (Heinrich-Heine-Universität Düsseldorf) (13).

### Study design

A nonrandomized, controlled intervention study was carried out between November 2014 and March 2015 to investigate the effect of tea consumption on nonheme iron absorption with the use of  $^{57}\text{Fe}$  as a stable isotope (clinicaltrials.gov registration:

NCT02365103). Each subject attended the clinic on 5 separate occasions over a period of 56 d during which they were administered 3 standardized TMs and a reference iron dose in the following order: 1) a TM administered with water [TM-1 (control)], 2) a TM administered simultaneously with tea (TM-2), 3) a TM administered with tea 1 h postmeal (TM-3), and 4) a reference iron dose without a TM. The study protocol was approved by the National Research Ethics Service Committee North West–Greater Manchester East, United Kingdom (Research Ethics Committee reference: 14/NW/0310; Integrated Research Application System project 154775). All of the procedures were carried out in accordance with the Helsinki Declaration of 1975 as revised in 1983.

### Study protocol

Eligible volunteers provided informed consent at the beginning of the study. All of the subjects were required to fast for 10 h the night before each test dose of iron. The scheduled clinic visits at the clinical research laboratories in the Department of Clinical Sciences and Nutrition, University of Chester, were between 0800 and 1000. All of the subjects were asked to attend a total of 5 clinic sessions (days 0, 14, 28, 42, and 56), with a 14-d interval between clinic visits to allow the incorporation of the isotope into the erythrocytes. A standardized TM of porridge, extrinsically labeled with  $^{57}\text{FeSO}_4$ , was administered to the subjects with water or tea depending on the clinic, under supervision of the investigator. The subjects were not permitted to consume food or beverages for 3–4 h after the administration of the TM. All of the containers used to administer the TMs were washed with ultrapure water to ensure complete consumption of both TMs and the isotope label. The study protocol is summarized in Figure 1, and details of each clinic session are described in the following sections.

#### Clinic visit 1 (day 0)

Subjects' height and weight were measured, and a 30-mL fasted blood sample was collected in trace element-free EDTA blood collection tubes for isotopic analysis and iron status measurements. The whole-blood sample collected at each clinic was used for iron isotopic analysis, and the plasma sample was used for iron status measurements. The investigator conducted a 24-h food recall interview, and subjects were given a 3-d food diary to record their dietary intake at home. Subjects were then administered TM-1 (with water) to serve as a control.

#### Clinic visit 2 (day 14)

Subjects' weight was measured, and a 30-mL fasted blood sample was collected in trace element-free EDTA blood collection tubes. The investigator conducted a 24-h food recall interview, and subjects were then administered TM-2 (with tea).

#### Clinic visit 3 (day 28)

The third clinic visit followed the same procedures as in clinic visit 2, but subjects were administered TM-3 (with tea 1 h postmeal).

#### Clinic visit 4 (day 42)

The fourth clinic visit followed the same procedures as in clinic visit 2, but subjects were administered a reference iron dose

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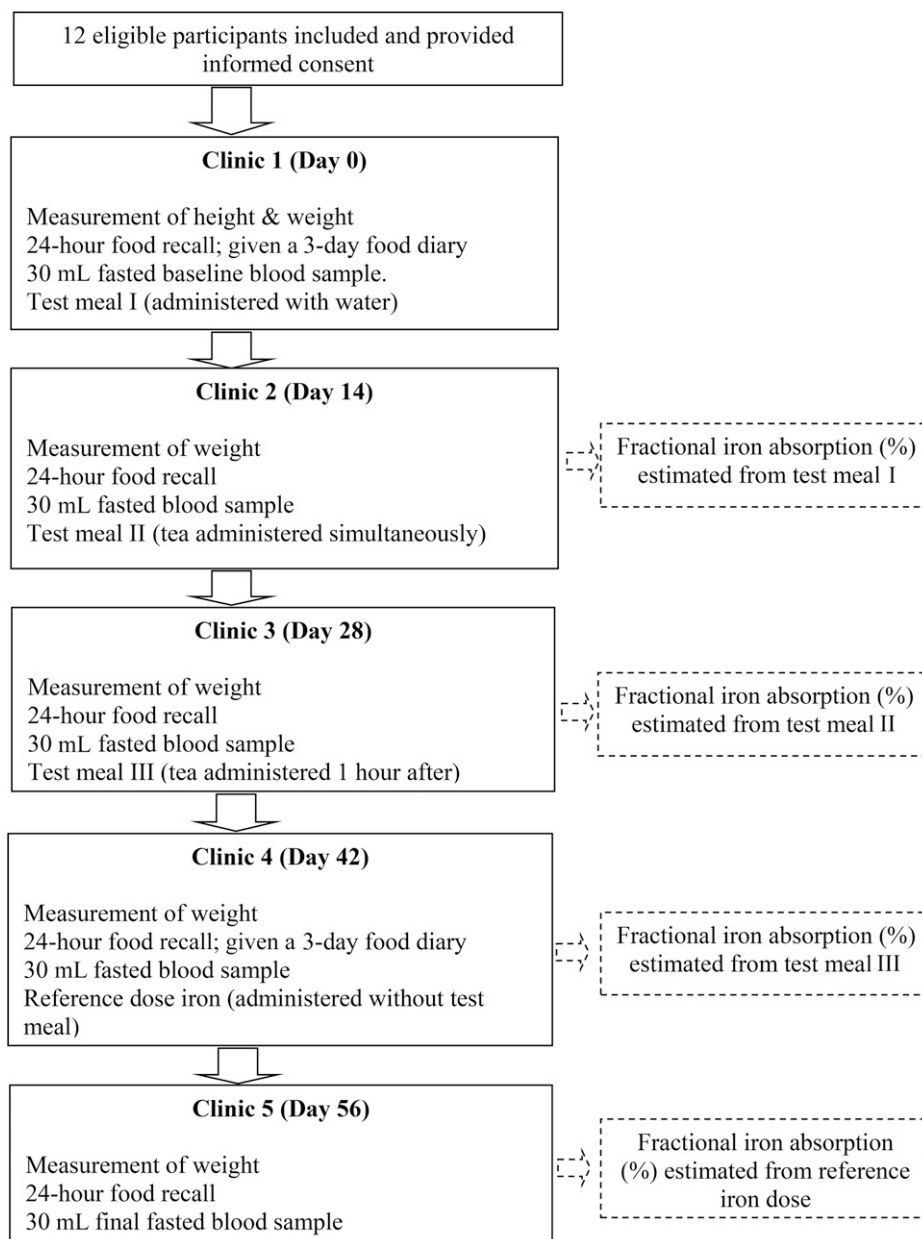


FIGURE 1 Flowchart of study protocol (5 clinic visits with 14-d intervals).

(3 mg  $^{57}\text{Fe}$  as  $\text{FeSO}_4$  and 35 mg ascorbic acid) without administration of any TM and were given a further 3-d food diary to record their dietary intakes.

### Clinic visit 5 (day 56)

Clinic visit 5 followed the same procedures as in clinic visit 2, with the collection of a final whole-blood sample without the administration of a TM.

### Stable isotope labels

A single isotope technique was used in the present study with the use of a stable iron isotope ( $^{57}\text{Fe}$ , 95.93% enriched; Trace Sciences) in metal form (300 mg), which was converted into ferrous sulfate solution ( $^{57}\text{FeSO}_4$ ), sterilized, and tested for

endotoxin (Anazao Health Corporation) and declared safe for human consumption. The enriched stable iron isotopes were transferred and kept in individual vials, flushed with nitrogen before being sealed, and stored at  $220^\circ\text{C}$  until TM administration. The specified dosages for TMs and the reference iron dose used in the study were 4 and 3 mg, respectively. The reference iron dose of 3 mg  $^{57}\text{Fe}$  with 35 mg ascorbic acid administered during a fasted condition was used as a measure of iron absorption under optimal state (14).

The dissolution process to produce a clear  $^{57}\text{FeSO}_4$  solution, which was added extrinsically to the TMs, was carried out by the Anazao Health Corporation on the basis of the company's standard procedure. After complete dissolution of the elemental  $^{57}\text{Fe}$  with sulfuric acid, which resulted in a clear and colorless solution, the solution was filtered over a 0.5- $\mu\text{m}$  membrane. The

prepared  $^{57}\text{FeSO}_4$  solution was shipped to the clinical research laboratory at the University of Chester in 1 vial containing a 75-mL solution, which was then transferred into individual sterile, borosilicate, glass, clear sample vials with fitted caps (Fisher Scientific UK Ltd.), labeled with dosage and concentration. The labeled vials were flushed with nitrogen gas, sealed, and stored at  $220\text{°C}$  until the day of TM administration. A total volume of 75 mL  $^{57}\text{FeSO}_4$  provides 4 mg  $^{57}\text{Fe}/\text{mL}$ .

## TMs

### TM preparation and composition

The TM meal used in the present study consisted of instant oat porridge (Sainsbury's UK Ltd.). TMs were freshly prepared on the day of each clinic by adding 200 mL boiling ultrapure water (TraceSELECT Ultra, Fluka Analytic; Sigma-Aldrich UK Ltd.) to 55 g porridge. This TM was used at all clinics and was prepared according to the same standardized method. The nutritional content of the porridge is summarized in Table 1. To each TM, a 4-mg dose of extrinsically labeled  $^{57}\text{Fe}$  was added before consumption. The reference iron dose was extrinsically labeled with a 3-mg dose of  $^{57}\text{Fe}$  with 35 mg ascorbic acid and administered in ultrapure water without any TMs (clinic visit 5).

### Preparation of tea

Black tea (Yorkshire Tea; Bettys and Taylors Group Ltd.) was prepared with the use of a standardized method at each clinic, by adding 200 mL boiling ultrapure water (TraceSELECT Ultra, Fluka Analytic) to 1 tea bag (3 g), and the mixture was steeped and infused for 3 min before straining for consumption. In each cup of tea, 12 mL homogenized semi-skim milk (Lakeland Dairies Co-op Society UK Ltd.) was added, and subjects were offered white granulated sugar (Fairtrade International) with the tea according to their preference. The tea was prepared on the day of the clinic and kept warm in a flask before consumption. The tea was administered at specific times either simultaneously with the porridge meal or 1 h postmeal.

## Anthropometric measurements

Both height (centimeters) and weight (kilograms) were measured by using electronic scales (model 875; Seca) and a wall-mounted digital stadiometer (model 264; Seca) at baseline

TABLE 1  
Nutritional composition of porridge test meals

Nutrient	Value per serving <sup>1</sup>
Energy, MJ	8.63
Total fat, g	3.4
SFAs, g	0.6
MUFAs, g	1.3
PUFAs, g	1.3
Carbohydrate, g	32.8
Sugars, g	8.7
Starch, g	24.0
Fiber, g	3.9
Protein, g	8.7
Salt, g	0.14

<sup>1</sup> One serving = 55 g.

before any of the TMs were administered. The instruments used were calibrated before every measurement. BMI was then calculated by using the equation  $\text{BMI} = \text{weight (kg)}/\text{height (m)}^2$  and categorized by using cutoff values from the WHO (15).

## Blood sample handling

Both whole blood and plasma obtained from venipuncture were used in the study for analysis. At each clinic, 30-mL venous blood samples were collected with the use of trace element-free blood collection tubes containing EDTA. A total of 6 mL whole blood was placed into micro-centrifuge tubes and stored at  $280\text{°C}$  before isotopic iron analyses and was also used to measure full blood count indexes on the day of each clinic. The remaining blood samples were centrifuged at  $1600 \times g$  at  $48\text{°C}$  for 10 min by using a benchtop centrifuge to obtain plasma samples, which were placed into micro-centrifuge tubes and stored at  $280\text{°C}$  until analyses of iron status biomarkers. Whole-blood and plasma samples were used for the analysis of iron status biomarkers, including full blood counts (red blood cells, hemoglobin concentration, hematocrit percentage, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration), plasma ferritin, plasma C-reactive protein (CRP), and plasma hepcidin concentrations.

## Measurement of iron status biomarkers

Whole-blood samples were used to measure full blood count indexes by using an automated Ac.T diff Hematology Analyzer (Beckman Coulter Inc.). Plasma samples were used for the analysis of ferritin concentrations with an automated immunoanalyzer Mini VIDAS (Biomerieux), CRP concentrations were measured by using commercially available human ELISA kits (Quantikine Human CRP Immunoassay ELISA kit; R&D Systems Inc.), and hepcidin concentrations were measured by using a commercially available human ELISA kit (Human Hepcidin ELISA kit; Sincere Biotech Co. Ltd.).

## Isotopic analysis of blood samples

Isotope ratio measurements were carried out by using an Agilent 8800 triple quadrupole inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS) instrument (ICP-QQQ; Agilent Technologies). Samples and standards were introduced into the ICP-MS/MS with a MicroMist nebulizer and a Peltier-cooled ( $28\text{°C}$ ) Scott-type spray chamber. The Agilent 8800 contains an octopole-based collision-reaction cell, which is located between 2 quadrupole analyzers. A mixture of 10% ammonia in helium was introduced into the octopole cell as a reaction gas to remove the interferences on iron isotopes (Supplemental Table 1) and to attenuate the signal at the appropriate level for precise isotope ratio analysis. Iron isotopes were measured as iron-ammonia clusters  $[\text{mFe}(\text{NH}_3)_2]$  at masses of 88, 90, 91, and 92. Mass bias correction was performed by external standard correction with the use of iron (Fe). Pure Single-Element Standard (1000 mg/mL, 2%  $\text{HNO}_3$ ; Perkin Elmer) was used as a baseline reference. Frozen whole-blood samples were allowed to reach room temperature and then vortex mixed. One hundred microliters of the sample was mixed with 9.9 mL 0.005% tetramethylammonium hydroxide (vol:vol; BioXtra grade; Sigma-Aldrich).

### Estimation of fractional iron absorption

Fractional iron absorption (percentage) was estimated by the erythrocyte iron incorporation method, 14 d postdosing of the isotope, which involved the use of published equations to establish the percentage of iron absorption in each subject. Blood volume was used to calculate the circulating iron body pool (16) by using a published equation specific for females (17). On the basis of the estimation of blood volume and circulating body iron pool for each subject, iron incorporation into red blood cells was calculated by using another equation, which assumed that 80% of the absorbed iron was incorporated into erythrocytes (18, 19). The final fractional iron absorption was derived from mole fraction calculations for each iron source ("endogenous" iron and iron from the labeled oral dose) in the blood samples. This required knowledge (from ICP-MS/MS measurements) of the abundance of each iron isotope ( $^{56}\text{Fe}$ ,  $^{57}\text{Fe}$ ,  $^{58}\text{Fe}$ ) in the previous and present blood samples and the abundance of the iron isotopes in the oral isotope dosage (mainly  $^{57}\text{Fe}$ ). The erythrocytes became progressively more enriched with  $^{57}\text{Fe}$  as the subjects consumed more TMs because iron is not lost from erythrocytes until they break down after  $\sim 120$  d. This extra enrichment (above natural abundance) was taken into account when fractional iron absorption was estimated after each TM by using the previous blood sample's iron isotope abundances as the "endogenous" value. The calculated fractional iron absorption (percentage) was then normalized to a fixed reference value of 40% by using the reference iron dose (14) to take into account intersubject variability (20).

### Assessment of dietary intake

Two dietary assessment methods were used in the present study. Subjects were required to complete a 3-d food diary within the week after the baseline clinic visit and within the week before the postintervention clinic visit (to include 2 weekdays and 1 weekend day) to estimate their habitual dietary intake. To ensure that subjects had not consumed unusually high amounts of iron-rich foods immediately before each clinic visit, a 24-h recall interview was completed at each visit to assess dietary intake 1 d before the TM administration, during which subjects were asked to recall food consumption, including beverage consumption for the past 24 h in detail to the investigator. All of the dietary records were analyzed for nutritional content by using computerized dietary analysis software (Microdiet for Windows software, version 2.8.8; Downlee Systems UK Ltd.). The food items used for the analysis were derived from *McCance and Widdowson's The Composition of Foods, 6th summary edition* (21). Dietary reference values from the 1991 Committee on the Medical Aspects of Food Policy were used for comparison with intakes of energy, macronutrients, and micronutrients of the subjects (22).

### Statistical analysis

All of the statistical analyses were performed with IBM SPSS Statistic Data Editor Software (version 21). The Shapiro-Wilk test was used to ascertain the normal or non-normal distribution for each variable. The non-normally distributed data, including the fractional iron absorption (percentage) and total iron absorbed (milligrams) were log-transformed for the purpose of performing statistical analyses, and results were re-transformed

to be reported as means  $\pm$  SDs in Results. One-factor repeated-measures ANOVAs were performed to compare the iron absorption from each pair of meals in comparison to the control TM (water). Pearson's correlation coefficient tests were performed to investigate the association between different iron status biomarkers and iron absorption (fractional absorption and total absorbed in milligrams). Differences were considered significant with a  $P$  value  $\neq 0.05$ .

## RESULTS

### Baseline physical characteristics, iron status, and dietary intake of subjects

All 12 women who commenced the study completed all phases of study, with no reports of adverse events. The subjects were healthy women with a mean  $\pm$  SD age of 24.8  $\pm$  6.9 y and a BMI (in  $\text{kg}/\text{m}^2$ ) of 22.6  $\pm$  2.7, which is in the normal range (Table 2). At baseline, all of the subjects had plasma ferritin concentrations within the normal range for healthy women and no elevated plasma ferritin concentration due to acute-phase reactions, indicated by the normal plasma CRP concentrations. Seven of the 12 subjects were not iron deficient on the basis of plasma ferritin concentrations  $\geq 15$   $\text{mg}/\text{L}$ , and only 2 subjects were anemic (hemoglobin concentrations of 9.0 and 10.0  $\text{g}/\text{dL}$ ) at baseline. The mean  $\pm$  SD plasma hepcidin concentration was 96.0  $\pm$  119.6  $\text{ng}/\text{mL}$ , which was within the expected normal range, with the exception of 3 subjects who had plasma hepcidin concentrations  $\geq 150$   $\text{ng}/\text{mL}$ , which accounts for the large SD. There are no universal threshold values for normal hepcidin concentration; therefore, a reference value of between 17 and 286  $\text{ng}/\text{mL}$  from a study that reported the 5–95% normal range for healthy women, who were sampled in the United States and Italy (23), was used to compare the concentrations found in the present study.

After dietary analysis of the food diaries, the estimated mean  $\pm$  SD daily energy intake was 6.75  $\pm$  1.91 MJ, which is consistent

TABLE 2

Baseline subject characteristics, iron status, and nutritional intakes<sup>1</sup>

Variable	Mean $\pm$ SD
Age, y	24.8 $\pm$ 6.9
Physical characteristics	
Height, m	166.6 $\pm$ 6.5
Weight, kg	63.0 $\pm$ 10.8
BMI, $\text{kg}/\text{m}^2$	22.6 $\pm$ 2.7
Iron status biomarkers	
Hemoglobin, $\text{g}/\text{dL}$	12.33 $\pm$ 1.52
Hematocrit, %	35.97 $\pm$ 4.49
RBCs, $3 \times 10^{12}/\text{L}$	3.93 $\pm$ 0.55
Mean cell volume, fL	91.84 $\pm$ 6.93
Plasma ferritin, $\text{mg}/\text{L}$	38.8 $\pm$ 42.7
Plasma CRP, $\text{mg}/\text{L}$	0.62 $\pm$ 0.77
Plasma hepcidin, $\text{ng}/\text{mL}$	96.0 $\pm$ 119.6
Mean daily nutritional intake	
Energy, MJ	6.75 $\pm$ 1.91
Protein, g	66.1 $\pm$ 23
Iron, mg	6.7 $\pm$ 1.5
Tea intake/d, cups	2 (average infusion)

<sup>1</sup>  $n = 12$ . CRP, C-reactive protein; RBC, red blood cell.

with the UK population data of 6.78  $\pm$  1.9 MJ for women aged between 19 and 64 y (4). Mean  $\pm$  SD daily iron intake was 6.7  $\pm$  1.5 mg, nearly 50% of the Reference Nutrient Intake, and was lower than in the UK population for women aged 19–64 y (9.6  $\pm$  3.0 mg/d). Habitual iron intake (based on the 3-d food diary), however, was found to be higher (8.4  $\pm$  1.8 mg) at day 56 than at baseline (6.7  $\pm$  1.5 mg) ( $P = 0.035$ ). Mean  $\pm$  SD daily iron intake estimated from the 24-h recall was 9.7  $\pm$  5.5 mg, and the dietary analysis from each clinic showed no unusually high intakes of iron, calcium, or vitamin C, which may have confounded subjects' iron absorption.

There were also no changes observed in subjects' hemoglobin and plasma hepcidin concentrations between baseline and postintervention, except for plasma ferritin, which was significantly lower postintervention (29.4  $\pm$  33.4 mg/L) than at baseline (38.8  $\pm$  42.7 mg/L) ( $P = 0.026$ ). Despite the lower plasma ferritin concentration postintervention, it was within the range of replete iron stores ( $>15$  mg/L). The absorption observed from the reference iron dose of 25.4% suggests that absorption was not affected by the changes observed in subjects' iron stores.

### Iron absorption from TMs

The present study used 3 standardized TMs administered successively at 14-d intervals either with water (TM-1), simultaneously with tea (TM-2), or with tea administered 1 h after the test meal (TM-3). None of the TMs contained nonheme iron and each was labeled extrinsically with 4 mg  $^{57}\text{Fe}$  as  $\text{FeSO}_4$ , except for the reference iron dose, which was labeled with 3 mg  $^{57}\text{Fe}$  as  $^{57}\text{FeSO}_4$  and 35 mg ascorbic acid (to enhance the iron absorption of the reference dose). TM-1 served as the reference meal and was used as the basis for comparison and to calculate iron absorption ratio and tea inhibitory effect. Absorption data for the reference iron dose were used to correct the intervariability between subjects' iron status background, normalized to a fixed reference value of 40% (24) on the basis of a published equation (20). Table 3 shows the iron absorption (percentage), iron absorption ratio to the control TM (water), and total iron absorbed (milligrams). Each mean value for iron absorption comprised 12 values, 1 for each subject. For TM-1, 3 of the subjects had absorption values of "0%" because their enriched isotope ratios ( $^{57}\text{Fe}$ : $^{58}\text{Fe}$ ) were below the limit of quantification in comparison to the unenriched sample. In other words, the instrument (ICP-MS/MS) was unable to confidently distinguish a "signal" from the background "noise" in these samples. This was also the case for 3 subjects at TM-2 and 2 subjects at TM-3.

The mean iron absorption (percentage) was higher in TM-1 (meal + water) and in TM-3 (meal + tea + 1 h) than in TM-2

(meal + tea) (data shown in Table 3), as anticipated. A reduction of  $\sim 35\%$  in fractional iron absorption was observed when the TM was administered simultaneously with tea (TM-2) compared with the TM administered with water (TM-1), but this difference was not significant ( $P = 0.398$ ). The mean fractional iron absorption was  $\sim 2.2\%$  higher, in absolute terms, when tea was administered 1 h after the subjects were administered their TM (TM-3) ( $P = 0.046$ ) than when the TM was administered simultaneously with tea (TM-2). This was similar to the fractional iron absorption of the meal administered only with water (5.73% compared with 5.69%). Consistent with the percentage of iron absorbed, the mean total iron absorbed (milligrams) was also significantly higher by 0.05 mg in TM-3 (meal + tea + 1 h) compared with TM-2 (meal + tea) ( $P = 0.049$ ). There was no significant difference between TM-1 (meal + water) and TM-2 (meal + tea) with regard to the total iron absorbed ( $P = 0.530$ ). There were substantial variations in fractional iron absorption between subjects. There was a wide range of percentages of absorption, varying from undetectable to 31.1% (Supplemental Table 2). However, the fractional iron absorption reported in the present study was normalized by using a reference iron dose to account for intervariability between subjects, which enabled the findings to be compared against the previously published literature. The inhibitory effect was reduced by  $\sim 50\%$ , from 37.2% in TM-2 (meal + tea) to 18.1% in TM-3 (meal + tea + 1 h), indicating that allowing a 1-h time interval between a meal and tea consumption will lead to increased iron absorption.

No significant association was observed between both of the principal iron status biomarkers (plasma ferritin and plasma hepcidin concentrations) and iron absorption. However, positive associations were observed between plasma ferritin and plasma hepcidin concentrations when the TM was administered 1) with water ( $r = 0.918$ ,  $P = 0.0001$ ) (Figure 2A), 2) simultaneously with tea ( $r = 0.882$ ,  $P = 0.0001$ ) (Figure 2B), and 3) with tea 1 h postmeal ( $r = 0.841$ ,  $P = 0.001$ ) (Figure 2C), which indicates a very strong association between these 2 key iron biomarkers that play a substantial role in the regulation of iron absorption.

### DISCUSSION

The study investigated the effect of tea consumption on nonheme iron absorption from a porridge meal that used a  $^{57}\text{Fe}$  iron isotope, as well as ascertaining the impact of time interval of the potential inhibitory effect of consuming tea relative to consumption of an iron isotope-labeled porridge meal. In agreement with previous studies (8, 9, 12, 25–29), the present study shows that tea reduces nonheme iron absorption by  $\sim 37\%$  when compared with water used as a control beverage. The

TABLE 3  
Fractional iron absorption from TMs with specified beverages and iron absorption ratio (tea administration relative to water)<sup>1</sup>

	TM-1 (administered with water)	TM-2 (administered with tea simultaneously)	TM-3 (tea administered 1 h postmeal)
Fractional iron absorption, %	5.7 $\pm$ 8.5	3.6 $\pm$ 4.2	5.7 $\pm$ 5.4*
Iron absorption ratio	1	0.65 $\pm$ 0.67	1.18 $\pm$ 1.19
Total iron absorbed, mg	0.23 $\pm$ 0.51	0.14 $\pm$ 0.26	0.19 $\pm$ 0.28 <sup>†</sup>

<sup>1</sup> Values are means  $\pm$  SDs. A 1-factor repeated-measures ANOVA was performed to assess the differences in mean iron absorption between each test meal. \* $\dagger$ Different from TM-2: \* $P = 0.046$ ,  $\dagger P = 0.049$ . TM, test meal.

## INHIBITORY EFFECT OF TEA ON IRON ABSORPTION

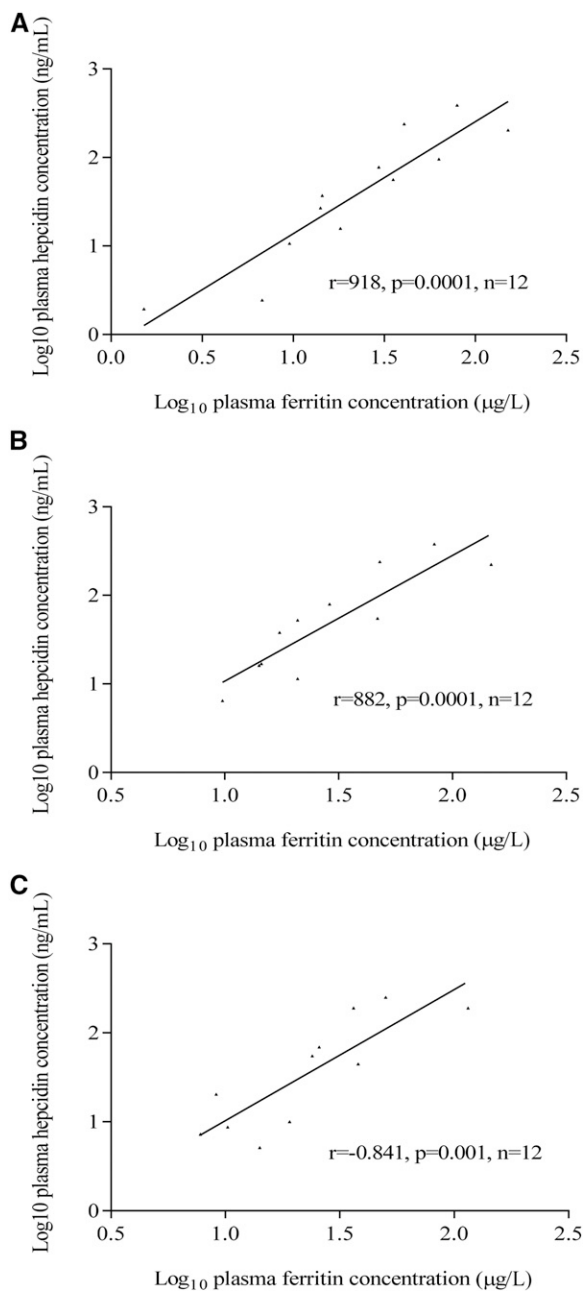


FIGURE 2 Associations between log<sub>10</sub> plasma ferritin and plasma hepcidin concentrations on day 14 (A), day 28 (B), and day 42 (C).  $n = 12$ .

study also shows that a 1-h time interval between tea consumption and a meal has a substantial impact in counteracting this inhibitory effect, by 1.6-fold.

In the present study, the overall mean  $\pm$  SD iron absorption from porridge, administered with either water (TM-1) or tea (TM-2 and TM-3), was 6%. Depending on the conditions and chemical forms of iron itself (heme or nonheme), the fractional iron absorbed by individuals is reported to be classically low, ranging from as low as 5% to as high as 35% (30). It can be presumed that the nature of the cereal-based meal is the main contributory factor that leads to the low iron absorption, because the TM used in the present study had a potentially low amount of iron with the presence of phytate as an iron inhibitor, which is

consistent with findings from previous studies that used a cereal-based TM in their study (26, 27). These studies reported a wide range of fractional iron absorption values, between 0.56% and 18.8% (31, 32).

Consistent with findings reported from previous studies, despite the difference in TM used, iron absorption was reduced when the tea was administered with a TM as opposed to when administered with water. Table 4 compares iron absorption reported in previous studies in which TMs were administered with either water (as a control) or tea to show the inhibitory effect. The magnitude of the inhibitory effect shown in previous studies ranged between 26% and 99% in comparison to the present study, which was 37%. Variability in tea brewing time, brands, and the total amount of tea used, which may influence the phenolic concentration, are probably among the factors that contribute to discrepancies in the findings (33). The iron status of the subjects may also dictate iron absorption and this has been shown in previous studies. A potential explanation for the lower inhibitory effect of tea observed in the present study could be due to the majority of the subjects not being anemic or iron deficient. This is supported by the low mean  $\pm$  SD absorption of the reference iron dose (25.4%  $\pm$  18.3%) compared with previous studies that reported a higher absorption of iron from both TMs and reference doses (Table 4). Thankachan et al. (9) compared iron absorption from a rice meal between subjects with iron deficiency anemia and normal subjects and showed that iron absorption was higher in the iron deficiency anemia group (7.1%) than in the control group (3.5%), which suggests that iron status has a significant impact on the amount of iron absorption and is based on physiologic requirements.

The finding that plasma ferritin and plasma hepcidin concentrations were positively associated in the present study is consistent with a limited number of previous studies that measured iron absorption concurrently with hepcidin and ferritin concentrations, despite the different TMs used (34–36). Hepcidin, a systemic iron regulator, directly binds ferroportin to cause its internalization and degradation to hinder iron efflux (37).

The inhibition of iron absorption by tea observed in this study could be due to the formation of insoluble iron-tannin complexes in the lumen of the gut (27). In support of the findings from previous studies that tea is a prominent inhibitor of iron absorption, the present study showed that a 1-h time interval has a substantial effect in reducing the inhibitory effect by 37%. There is limited evidence to support this finding on the time interval effect, specifically in human studies, because previous studies were carried out in rats or used other polyphenol-containing beverages. Disler et al. (10) showed that the time interval between the administration of tea and an iron-containing meal substantially reduced the inhibitory effect. However, tea was administered at several time points 3 h before iron solutions were administered, instead of after the TM as in the present study. A linear relation between the time interval and iron absorption was observed, and the absorption was 2-fold higher if the tea was administered 1 h before a meal, which is similar to the 1.6-fold magnitude increase (TM-2 compared with TM-3) observed in our study.

It should be noted that the iron absorption amounts reported in the present study are relative to a specific meal. However, gastric emptying of solid foods can vary between individuals and can also be affected by variations in meal components and

TABLE 4

Inhibitory effect of tea consumption on iron absorption in comparison to water as a control beverage<sup>1</sup>

Study, country, year (ref)	Population (n); age, <sup>2</sup> y	Test meals	Absorption, <sup>3</sup> %		Inhibition, <sup>4</sup> %	Reference iron dose, <sup>3</sup> %
			Meal (water)	Meal (tea)		
Present study <sup>5</sup>	Healthy women (n = 12); 24.8 $\pm$ 6.9	Porridge + 200 mL tea	5.7 $\pm$ 8.5	3.6 $\pm$ 4.2	37	25.4 $\pm$ 18.3
Disler et al., India, 1975 (25) <sup>6,7</sup>	Healthy women (n = 8); 26–60	Bread + 200 mL black tea	10.4 $\pm$ 4.4	3.3 $\pm$ 3.0	68	35.8 $\pm$ 25.0
		Rice and soup + 200 mL black tea	10.8 $\pm$ 4.1	2.5 $\pm$ 1.6	77	34.7 $\pm$ 24.7
Derman et al., India, 1977 (12) <sup>7</sup>	Healthy women (n = 22); 21–71	Maize porridge + 150 mL black tea	3.8 $\pm$ 4.0	2.1 $\pm$ 2.5	45	50.5 $\pm$ 31.5
de Alarcon et al., Italy, 1979 (26) <sup>7</sup>	Thalassemia patients (n = 5); 11–23	Hamburgers + 240 mL tea	15.6 $\pm$ 12.8	5.2 $\pm$ 5.5	67	NA
Morck et al., USA, 1983 (27) <sup>7</sup>	Healthy adults (n = 37); 18–50	Beef hamburger + 200 mL tea	3.71 (2.94, 4.68)	1.32 (1.01, 1.71)	64	15.73 (13.22, 18.71)
Kaltwasser et al., Germany, 1998 (28) <sup>7</sup>	Hemochromatosis patients (n = 18); 47.4 $\pm$ 16.1	Homogenized rice and beef + 200 mL tea	22.1 $\pm$ 3.4	6.9 $\pm$ 1.4	69	NA
Hurrell et al., USA, 1999 (8) <sup>7</sup>	Healthy adults (n = 77); 19–40	Bread roll + 275 mL tea				NA
		275 mL tea (n = 9)	12.9 (10.7, 15.6)	0.74 (0.57, 0.95)	94	
		275 mL tea (n = 10)	5.63 (4.64, 6.84)	0.89 (0.68, 1.16)	84	
		275 mL tea (n = 10)	4.46 (3.47, 5.72)	0.92 (0.72, 1.18)	79	
		275 mL tea (n = 10)	8.64 (6.00, 12.4)	0.83 (0.56, 1.23)	90	
		100% strength (n = 9)	6.58 (4.76, 9.10)	0.59 (0.45, 0.96)	91	
		50% strength	4.33 (3.31, 5.67)	1.05 (0.72, 1.53)	84	
		25% strength	—	0.66 (0.45, 0.96)	99	
		25% strength (n = 9)	—	1.18 (0.84, 1.66)	73	
		10% strength	—	1.48 (0.99, 2.21)	66	
5% strength	—	1.47 (1.03, 2.12)	66			
Samman et al., Australia, 2001 (29) <sup>7</sup>	Healthy women (n = 10); 26 $\pm$ 4	Pasta + bread + 4 mL green tea extract	12.1 $\pm$ 4.5	8.9 $\pm$ 5.2	26	38.6 $\pm$ 18.1
Thankachan et al., India, 2008 (9) <sup>5</sup>	IDA and healthy women (n = 20); 18–35; IDA group; 22.6 $\pm$ 3.5; and iron-replete group; 24.3 $\pm$ 2.9	Tomato rice + tea (different amounts)				NA
		150 mL tea	18.2 (12.6, 26.4)	7.1 (4.3, 11.7)	61	
		300 mL tea	19.7 (13.5, 29.0)	5.6 (3.1, 10.1)	72	
		150 mL tea	7.5 (4.0, 14.2)	3.5 (2.1, 5.9)	53	
		300 mL tea	5.2 (2.6, 10.8)	1.6 (0.7, 3.8)	69	

<sup>1</sup> IDA, iron deficiency anemia; NA, not applicable; ref, reference.<sup>2</sup> Values are means  $\pm$  SDs or ranges.<sup>3</sup> Values are means  $\pm$  SDs or geometric means; ranges in parentheses.<sup>4</sup> Inhibitory effect (%) = [meal (water) – meal (tea)]  $\div$  100.<sup>5</sup> Meals were extrinsically labeled with stable iron isotopes.<sup>6</sup> A second substudy used an intrinsically labeled meal.<sup>7</sup> Meals were extrinsically labeled with iron radioisotopes.

consistency (38), which has been reported to range between 45 and 108 min in several studies in healthy subjects who consumed TMs of different compositions (39–41).

In conclusion, the present study shows that tea can be distinctly regarded as a potent inhibitor of nonheme iron absorption from a cereal-based breakfast. It is also evident from this study that not consuming tea simultaneously with a meal will have an impact on attenuating the inhibitory effect of tea on iron absorption. In addition to attenuating the effect of inhibition of iron absorption, it is pertinent to incorporate dietary advice that would increase iron absorption, such as incorporating ascorbate-containing fruit juice to a meal to

enhance iron absorption. The findings of the present study may have implications in the management of iron deficiency, especially in at-risk groups predisposed to iron deficiency. Translating the findings of the present study into dietary advice at a health care level would help maximize dietary iron absorption, because tea is not only widely consumed in the United Kingdom (11) but is extensively consumed worldwide.

The authors' responsibilities were as follows—SM: designed the research and had primary responsibility for the final content; SFAF: conducted the research and analyzed the data; and all authors: wrote, read, and approved the final manuscript. None of the authors reported a conflict of interest related to the study.



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