

Original Article

Bioavailability and incorporation of nonheme iron from staple Chinese diet in young urban Chinese women

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Background and Objectives: This study assessed the bioavailability and biological incorporation of nonheme iron from staple food diets in healthy young urban Chinese women and determined the relevant effects of typical regional patterns of staple foods in South and North China. **Methods and Study Design:** Twenty-two young urban Chinese women aged 20–23 years were enrolled and randomly allocated to two groups, with rice (rice group) and steamed buns (steamed buns group) as the staple food, respectively. Each participant received three meals daily containing approximately 3.25 mg of stable ⁵⁷FeSO₄ for 2 consecutive days, along with daily intravenous injection of approximately 2.0 mg of ⁵⁸FeSO₄. Nonheme iron absorption and infused iron incorporation rates were assayed. **Results:** In all participants, the rice group, and the buns group, nonheme iron intake was 7.2±1.6, 5.9±0.6, and 8.4±1.2 mg, respectively; mean ⁵⁷FeSO₄ absorption rate was 22.2%±9.6%, 22.2%±10.6%, and 22.2%±8.9%, respectively; and the mean infused ⁵⁸FeSO₄ incorporation rate was 91.6%±8.2%, 93%±7.3%, and 90%±9.1%, respectively. No substantial differences existed in the nonheme iron intakes and the ⁵⁷FeSO₄ absorption and ⁵⁸FeSO₄ incorporation rates between the rice and buns groups (all *p*>0.05). **Conclusions:** The bioavailability and incorporation rates of nonheme iron from representative comprehensive Chinese diets in healthy young urban Chinese women were evaluated. Our results can facilitate the establishment of dietary reference intake for iron in Chinese women.

Key Words: nonheme iron, absorption, incorporation, Chinese, women

INTRODUCTION

Iron deficiency-related anemia (mainly resulting from low iron intake, absorption, or incorporation) affects approximately 25% of the world population¹ and 29%–38% of childbearing women.² Our recent study based on a 2010–2012 China Nutrition and Health Survey showed that in China, childbearing women have a higher prevalence of iron deficiency anemia than do men and women in other age groups.^{3,4} Therefore, appropriately planning the iron intake in women's diets is vital.

In China, dietary reference intake (DRI) values may facilitate better dietary planning and nutrient status evaluation.⁵ However, iron DRIs, based on domestic research results, mainly the 2000 Chinese Total Diet Study (data unpublished), have been established only for young Chinese men; by contrast, for other Chinese populations (such as women), the DRIs have been based on the data from young Chinese men or foreigners. Primarily because of physiological, dietary, and other differences, iron bioavailability and incorporation, vital for accurately iron DRI assessment, for women should differ from those for men. Thus, women likely have different iron DRIs than do

men.^{5–8} Dietary iron consists of heme and nonheme iron. In contrast to heme iron (mainly from animal tissues), nonheme iron (mainly from plant foods) generally has a much lower bioavailability,⁹ which indicates that the estimation of nonheme iron absorption and incorporation is essential for determining iron DRIs.

Iron absorption may obviously vary with various diet- and host-related factors, such as nutrient content, cooking and processing methods, iron absorption enhancers or inhibitors, and nutritional and health status.^{10,11} An international consensus on nonheme iron bioavailability has not been reached,^{12–15} indicating that for setting iron

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DRI, domestic data rather than foreign-related data is preferable.

Currently, the double-stable isotope-labeling technique is the most applicable for estimating dietary mineral absorption and incorporation. It is performed by administering two isotopes (i.e., one taken orally and the other intravenously).¹⁶⁻¹⁸ We previously employed this technique to estimate the mean nonheme iron bioavailability and incorporation rates (11% and 85%, respectively) in typical comprehensive Chinese diets, including typical Chinese staple foods (i.e., rice- and wheat-based foods) and nonstaple foods (i.e., soup and various foods made from vegetables, meat, and fish) for healthy young urban Chinese men;¹⁹ however, similar rates in healthy young Chinese women have been demonstrated thus far. Here, we determined the relevant results in healthy young urban Chinese women by using a double-labeled stable isotope technique. In addition, in China, which contributes nearly 20% of the world population, the dietary patterns vary greatly across regions; thus, this study also investigated if nonheme dietary iron absorption and incorporation is influenced by the regional staple food patterns in South and North China. This study can provide results that facilitate the establishment of iron DRIs for the Chinese female population.

METHODS

Participants

Twenty-two eligible healthy young urban Chinese women aged 20–23 years were enrolled between January 2010 and March 2011 from Bethune Military Medical College, Shijiazhuang, Hebei, China. The participants were non-smokers, had a normal iron nutritional status, and were free of inflammation. The participants with acute or chronic diseases, who regularly took medication can influence iron absorption, with an abnormal iron nutritional status (e.g., early iron deficiency, iron deficiency, and iron deficiency anemia), or with inflammation were excluded through accurate and rigorous examinations.

In China, rice- and wheat-based steamed buns are major staple foods. Based on their conventional dietary hab-

its, the 22 eligible participants were randomly assigned to a rice and wheat-based steamed buns group (n=11 per group), with rice and the steamed buns as the staple food, respectively. The trial was registered at the Chinese Clinical Trial Registry (No.: ChiCTR-TRC-09000580) and approved by the Ethics Committee of the National Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention (Beijing, China). Written informed consent was obtained from each participant prior to this study.

Study design

The study was designed as previously described.¹⁹ The participants were required to uniformly eat the experimental food and stay on the study premises. According to the local food material supply and the participants' dietary habits, a 2-day experimental diet was designed: steamed buns or rice was served for breakfast, lunch, and supper each day as the staple foods. On Days 1 and 2, for breakfast, the nonstaple foods comprised respectively milk, tomato and egg soup, green pepper with shredded potato, seaweed strip salad, and tofu salad and milk, egg and cucumber soup, stir-fried cabbage, seaweed strip salad, and celery salad for breakfast; for lunch, they were respectively stir-fried chicken breast, stir-fried shredded pork tenderloin, vegetarian stir-fried green beans, vegetarian stir-fried cabbage, and seaweed and bok choy soup and stir-fried squid, stir-fried shredded pork tenderloin, braised eggplant, stir-fried green pepper, and seaweed and bok choy soup; and finally, for supper, they were braised ribbonfish, braised beef, stir-fried mushroom, stir-fried cabbage with vinegar sauce, seaweed and spinach soup, and apple and stir-fried shrimp, stir-fried tofu, stir-fried cauliflower, seaweed and spinach soup, and pear (Table 1).

First, the participants started a 2-day adaptation period for the experimental diet, during which the participants were checked to see if they could accept the designed diet involving the related processing methods. On Day 0 (baseline), venous blood samples were collected from participants after an overnight fast to measure the serum

Table 1. Menu for the experimental period

		Day 1	Day 2
Breakfast	Staple food	Steamed buns/ rice	Steamed buns/ rice
	Non-staple food	Green pepper with shredded potato Tomato and egg soup Milk Seaweed strip salad Tofu salad	Stir-fried cabbage Egg and cucumber soup Milk Seaweed strip salad Celery salad
Lunch	Staple food	Steamed buns/ rice	Steamed buns/ rice
	Non-staple food	Stir-fried chicken breast Stir-fried shredded pork tenderloin Vegetarian stir-fried cabbage Seaweed and bok choy soup	Stir-fried squid Stir-fried shredded pork tenderloin Stir-fried green pepper Seaweed and bok choy soup
Dinner	Staple food	Steamed buns/ rice	Steamed buns/ rice
	Non-staple food	Braised ribbonfish Braised beef Stir-fried mushroom Stir-fried cabbage with vinegar sauce Seaweed and spinach soup Apple	Stir-fried shrimp Stir-fried cauliflower Stir-fried tofu Seaweed and spinach soup Pear

levels of C-reactive protein (CRP), hemoglobin (Hb), and some iron status indexes including unsaturated iron-binding capacity (UIBC), soluble transferrin receptor (sTfR), serum iron (SI), and serum ferritin (SF). During the 2-day experimental diet period, the participants received six test meals of two breakfasts, two lunches, and two suppers, providing the staple foods labeled with approximately 3.25 mg $^{57}\text{FeSO}_4$ per day (with the isotope ratio of ^{57}Fe in breakfast, lunch, and supper being 1:2:2) and nonstaple complementary foods, such as milk and fruits, under standardized conditions and site supervision.

In addition, 30–60 min after dinner, a $^{58}\text{FeSO}_4$ aqueous solution was intravenously injected into the participants at approximately 2.0 mg/day. After the 2-day experimental period, the participants recovered to their usual diets. Fourteen days later, venous blood samples were collected again from the participants after overnight fasting.

The dietary nonheme iron absorption and incorporation (utilization) were accordingly estimated. The experimental flow is illustrated in Figure 1.

Preparation of isotopic iron labels

^{57}Fe or ^{58}Fe elemental iron used in this study (Isoflex USA, San Francisco, CA, USA) was dissolved in 3M H_2SO_4 and then diluted with ultrapure water as appropriate. The exact isotopic iron compositions of the isotope labels (^{54}Fe , ^{56}Fe , ^{57}Fe , and ^{58}Fe) were assessed through multiple-collector inductively coupled plasma mass spectrometry (MC-ICP-MS, GV instrument, United Kingdom) and the ultimate concentrations of $^{57}\text{FeSO}_4$ and $^{58}\text{FeSO}_4$ were determined through atomic absorption spectroscopy (PinAAcle 900 T, PE, Shanghai, China). For every 1 mg

of stable iron isotope (^{57}Fe) in the solution, 10 mg of ascorbic acid, placed in ampoules, was added as an enhancer of dietary iron absorption.^{15,20} The isotopic ^{58}Fe preparation was sterilized and then intravenously infused, as measured through accurate assaying of the weight variation of the ampoules before and after the addition of the $^{58}\text{FeSO}_4$ solution.

Test meal preparation

The test meals were prepared using the method of Yang et al.¹⁹ The types and quantities of the components in the experimental diet were determined according to the local representative diet, based on the latest China National Nutritional Survey²¹ and the corresponding cluster analysis, and the balanced percentages of energy supplies from protein, fat, and carbohydrate. Eight types of foods, namely grains, beans and bean products, fruits, vegetables, seafood, meat, dairy products, and eggs, were used. All food materials from a local market were accurately weighed and uniformly prepared on the basis of the principle of maintaining the balance of nutrients and energy.

Steamed buns containing $^{57}\text{FeSO}_4$ (approximately 3.25 mg/day/participant) were prepared by steaming. Each bun contained 50 g of flour. The prepared buns were packed, labeled, and stored at -20°C until use. For each meal, the labeled buns were distributed to the participants in the buns group, with one bun being given for breakfast and two being given for lunch and two for supper to every participant. All the $^{57}\text{FeSO}_4$ -labeled buns were fully consumed.

Similarly, early in the morning on each experimental day, 200 g of rice with approximately 3.25 mg of

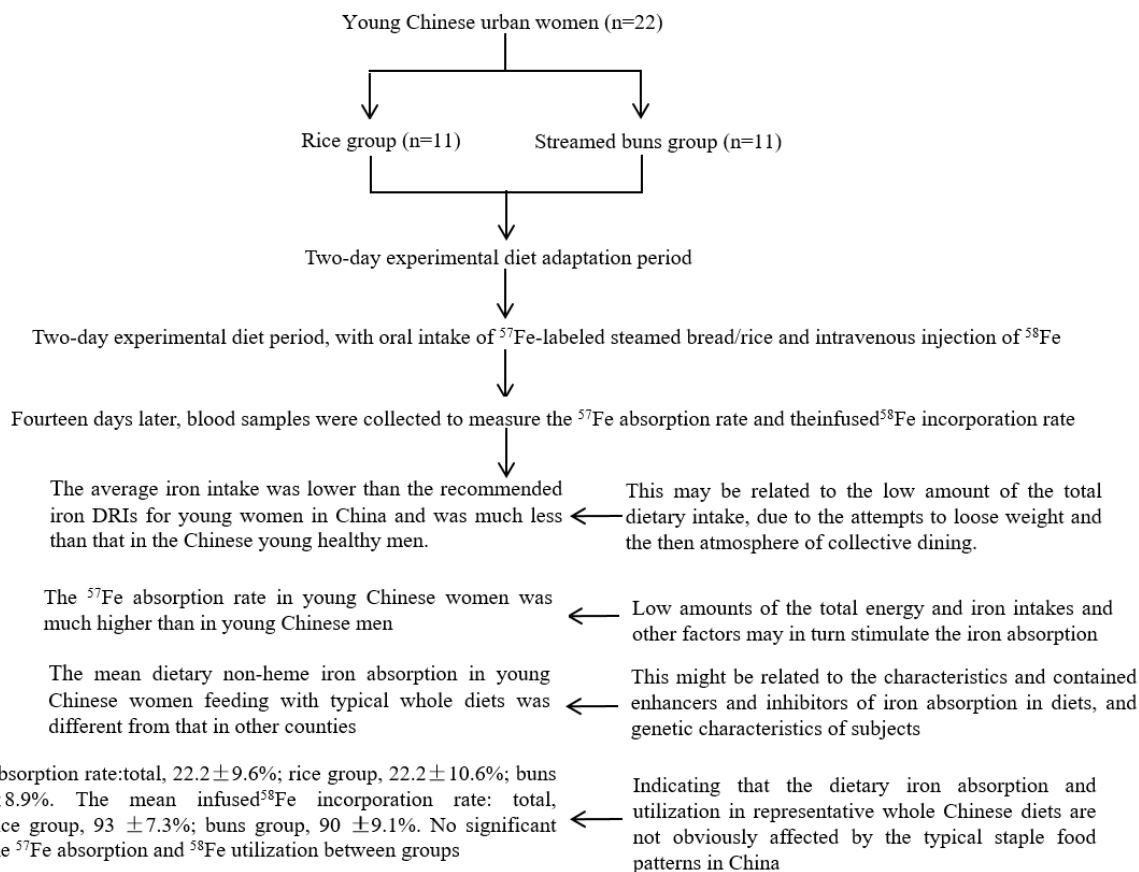


Figure 1. Conceptual diagram of this study.

$^{57}\text{FeSO}_4$ /day/participant was prepared by steaming then labeled, with the consumption ratios for breakfast, lunch, and dinner being 1:2:2. The prepared $^{57}\text{FeSO}_4$ -labeled rice was immediately served for breakfast and then reserved in a refrigerator and then reheated just before lunch and supper. All participants were asked to finish every rice grain.

In addition, the aforementioned nonstaple complementary foods were served for every participant at each meal. Other steamed buns or rice without measured isotopic content were also served if needed.

Dietary sample collection and detection

The sample collection and detection methods were performed according to the method of Yang et al.¹⁹ During the experimental diet, each participant's intake of every type of food component was accurately measured and recorded. Duplicate meal samples for each participant were collected, homogenized, packed, and stored at -20°C until use. Major dietary nutrients (including calories, carbohydrate, protein, fat, dietary fiber, and zinc), enhancers (i.e., ascorbic acid), and inhibitors (i.e., phytic acid and calcium) of iron absorption were assessed according to the respective national standard routine protocols of the People's Republic of China.^{15,20,22,23}

Blood analysis

Whole blood was collected and centrifuged, after which serum was separated and frozen at -20°C until use without freeze-thaw cycles. Hb, CRP, SF, sTfR, UIBC, and SI levels were assayed using an IMMULITE automatic system (Roche Diagnostics GmbH, Mannheim, Germany). Early iron deficiency was confirmed when sTfR >4.4 mg/L and iron deficiency was determined when SF <15 $\mu\text{g/L}$ on the basis of the reference range of the kit.⁵ Iron deficiency anemia was defined when SF <15 $\mu\text{g/L}$ and Hb <120 g/L. A CRP of <5 mg/L was considered normal, in line with the reference range of the kit.

Blood samples were mineralized using $\text{HNO}_3/\text{H}_2\text{O}_2$ treatment and microwave digestion with a microwave digestion system (EXCEL, Shanghai, China). The iron was separated from the blood matrix by using the ion exchange method. Iron isotopic analysis was performed through MC-ICP-MS according to the standard-sample bracketing method. The standard GBW04446 Isotopic

Abundances of Iron (National Institute of Metrology, Beijing, China), a type of Fe isotopic-certified reference material, was used to adjust the instrumental mass bias effect. All Fe isotopes were measured simultaneously in one sequence along with ^{60}Ni as a monitor to correct the isobaric interferences of ^{58}Ni on ^{58}Fe . An argon and hydrogen mixture was used as a collision gas to eliminate interference.²⁴ In optimized conditions, external precisions for $^{57}\text{Fe}/^{56}\text{Fe}$ and $^{58}\text{Fe}/^{56}\text{Fe}$ were in the order of 0.01%–0.03% relative standard deviation (RSD) and 0.1%–0.3% RSD, respectively. Enriched proportions of $^{57}\text{Fe}/^{56}\text{Fe}\%$ and $^{58}\text{Fe}/^{56}\text{Fe}\%$ at 2 weeks after the experimental period were measured accordingly.

Calculation of ^{57}Fe absorption and infused ^{58}Fe incorporation

Nonheme iron absorption and incorporation were measured using a stable isotope technique, which was based on the determination of the incorporation of an orally administered $^{57}\text{FeSO}_4$ dose and intravenously administered $^{58}\text{FeSO}_4$ dose into the erythrocytes of the participants.^{17,19} The formulas for total oral $^{57}\text{FeSO}_4$ absorption, iron mass, and circulating $^{56}\text{FeSO}_4$ mass were described previously.^{19,25,26}

Data analysis

Data were analyzed using Statistical Program for Social Science (SPSS Inc. Chicago, IL, USA). The data showed a normal distribution and were therefore presented as mean \pm standard deviation and were compared between the rice and steamed bun groups by using the Student's paired t test, for which $p < 0.05$ was considered statistically significant.

RESULTS

Basic participant characteristics

As listed in Table 2, the participants' mean age and BMI were 21.6 ± 1.5 years and 21.2 ± 2.4 kg/m^2 , respectively. No participants were obese or severely underweight. The participants had normal baseline iron nutritional statuses (with normal UIBC, SI, sTfR, SF, and Hb values); therefore, no participant had early iron deficiency, iron deficiency, or iron deficiency anemia. Moreover, none had inflammation as indicated by mean CRP levels being far below the kit's diagnostic criteria of >5 mg/L. At baseline,

Table 2. Basic participant characteristics

	Mean \pm SD	Rice group		Steamed buns group		p value
		Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	
Age (years)	21.6 \pm 1.50	21.9 \pm 1.30	21.2 \pm 1.60			0.16
Weight (kg)	56.0 \pm 7.0	55.4 \pm 5.60	56.5 \pm 8.40			0.73
Height (cm)	163 \pm 3.30	162 \pm 3.10	163 \pm 3.50			0.53
BMI (kg/m^2)	21.2 \pm 2.40	21.1 \pm 2.30	21.3 \pm 2.50			0.60
CRP (mg/L)	0.17 \pm 0.13	0.20 \pm 0.17	0.15 \pm 0.09			0.58
UIBC ($\mu\text{mol/L}$)	29.7 \pm 15.2	34.7 \pm 10.7	24.7 \pm 11.7			0.13
SI ($\mu\text{mol/L}$)	9.20 \pm 5.60	10.4 \pm 5.70	8.10 \pm 5.60			0.36
sTfR (mg/L)	3.03 \pm 1.00	2.62 \pm 0.61	3.44 \pm 1.17			0.054
SF ($\mu\text{g/L}$)	41.3 \pm 27.2	49.9 \pm 30.5	32.7 \pm 21.4			0.143
Hb (g/L)	130 \pm 13.4	134 \pm 14.8	127 \pm 11.8			0.395

SD: standard deviation; BMI: body mass index; CRP: C-reactive protein; UIBC: unsaturated iron-binding capacity; SI: serum iron; sTfR: soluble transferrin receptor; SF: serum ferritin; Hb: haemoglobin.

Table 3. Daily average intakes of nutrients

	Mean±SD	Rice group	Steamed buns group	<i>p</i> value
		Mean±SD	Mean±SD	
Calories (Kcal)	1361±91.8	1332±69.9	1389±105	0.156
Iron (mg)	7.20±1.60	5.90±0.60	8.40±1.20	<0.001*
Zinc (mg)	5.10±0.60	5.40±0.40	4.90±0.60	0.05
Calcium (mg)	692±154	599±75.3	784±159	0.002*
Carbohydrate (g)	168±12.2	178±6.10	157±3.80	<0.001*
Fat (g)	46.0±8.00	40.2±3.50	51.8±6.80	<0.001*
Protein (g)	63.7±7.80	54.1±5.90	68.0±7.30	<0.001*
Dietary fiber (g)	10.9±1.80	9.60±1.10	12.1±1.60	<0.001*
Ascorbic acid (mg)*	3.70±1.10	2.80±0.40	4.70±0.70	<0.001*
Phytic acid (mg)	4.50±1.40	3.20±0.30	5.70±0.70	<0.001*

SD: standard deviation.

*Represents significant differences ($p < 0.001$ or $p = 0.002$) between the rice and steamed buns groups.

Table 4. Iron isotopic intakes and iron absorption

	Mean±SD	Rice group	Steamed buns group	<i>p</i> value
		Mean±SD	Mean±SD	
Oral ⁵⁷ Fe dose (mg)	4.61±0.125	4.64±0.15	4.58±0.09	0.187
Oral ⁵⁸ Fe dose (mg) [†]	0.100±0.003	0.101±0.003	0.100±0.002	0.185
Dose ⁵⁸ Fe iv (mg)	3.60±0.174	3.64±0.04	3.55±0.239	0.224
Dose ⁵⁷ Fe iv (mg) [‡]	0.349±0.017	0.354±0.004	0.345±0.023	0.224
Baseline ⁵⁷ Fe: ⁵⁶ Fe	0.231±0.00001	0.0231±0.00001	0.0231±0.00001	0.678
⁵⁷ Fe: ⁵⁶ Fe at two weeks	0.0239±0.00030	0.0239±0.00032	0.0239±0.00028	0.825
Change in ⁵⁷ Fe: ⁵⁶ Fe	0.00085±0.00030	0.00086±0.00033	0.00084±0.00028	0.850
Baseline ⁵⁸ Fe: ⁵⁶ Fe	0.00307±0.00001	0.00306±0.00001	0.00307±0.00001	0.902
⁵⁸ Fe: ⁵⁶ Fe at two weeks	0.00524±0.00026	0.00529±0.0002	0.00518±0.0003	0.325
Change in ⁵⁸ Fe: ⁵⁶ Fe	0.00217±0.00027	0.00222±0.00022	0.00216±0.00029	0.326
⁵⁷ Fe absorption (mg)	0.239±0.103	0.239 ±0.115	0.239±0.110	0.995

Iv: intravenously administered; SD: standard deviation.

[†]Oral ⁵⁸Fe dose indicates the dose of the oral ⁵⁸Fe among the mixed oral ⁵⁷Fe preparation.

[‡]Dose ⁵⁷Fe iv indicates the dose of ⁵⁷Fe from the mixed iv ⁵⁸Fe preparation.

no significant differences in the basic characteristics (including age, weight, height, and BMI), serum inflammation levels (shown by CRP levels), and iron nutritional statuses (shown as serum levels of UIBC, SI, sTfR, SF, and Hb) between the rice and steamed buns groups were noted (all $p > 0.05$; Table 2).

Dietary sample test

The ⁵⁷Fe formulation comprised 96.73% ⁵⁷Fe, 2.11% ⁵⁸Fe, 1.16% ⁵⁶Fe, and 0.01% ⁵⁴Fe, whereas the ⁵⁸Fe formulation comprised 90.97% ⁵⁸Fe, 8.83% ⁵⁷Fe, 0.19% ⁵⁶Fe, and 0.01% ⁵⁴Fe. The participants' daily average intakes of main nutrients as well as enhancers (i.e., ascorbic acid) and inhibitors (i.e., phytic acid and calcium) of iron absorption were calculated (Table 3).

No participant ate extra nonlabeled buns or rice. The total mean calorie intake of these participants was approximately 1361 kcal—lower than the 2013 recommended dietary energy intake of 1800 kcal for young Chinese women with mild physical activity levels.⁵ The percentages of energy supplies from protein, fat, and carbohydrates were 18.7% ($63.7 \times 400/1361$), 30.4% ($46 \times 900/1361$), and 49% ($67.5 \times 400/1361$), respectively, which were generally consistent with the corresponding

recommended values (i.e., 10%–20%, 20%–30%, 50%–65% calories from protein, fat, and carbohydrate, respectively).⁵ This indicates that the diet was reasonably designed, with balanced percentages of energy supplies from protein, fat, and carbohydrate.

No significant difference was observed for calorie and zinc intakes between the rice and steamed buns groups. The iron, fat, protein, and dietary fiber content and iron absorption inhibitor (i.e., phytic acid and calcium) and iron absorption promoting factor (i.e., ascorbic acid) were significantly higher while the content of carbohydrates was significantly lower for the steamed buns group than for the rice group (all $p < 0.05$, Table 3).

Nonheme iron absorption and incorporation

During the 2-day experimental period, an average total of 4.61 mg of ⁵⁷Fe (oral) and 3.60 mg of ⁵⁸Fe (intravenous) was administered to each participant (Table 4). No significant difference in the actual oral or intravenous doses of the meal-administered ⁵⁷Fe or ⁵⁸Fe, basal and 2-week post-experimental blood ⁵⁷Fe (⁵⁷Fe:⁵⁶Fe) and ⁵⁸Fe (⁵⁸Fe:⁵⁶Fe) levels, and ⁵⁷Fe bioavailability and infused ⁵⁸Fe incorporation after 2 weeks was observed between the two groups ($p > 0.05$).

Table 5. Participants' iron absorption and incorporation rates

	Mean±SD	Rice group	Steamed buns group	<i>p</i> value
		Mean±SD	Mean±SD	
⁵⁷ Fe (oral) absorption rate	22.2%±9.6%	22.2%±10.6%	22.2%±8.9%	0.992
⁵⁸ Fe (iv) incorporation rate	91.6%±8.2%	93%±7.3%	90%±9.1%	0.418

Iv: intravenously administered; SD: standard deviation.

After 2 weeks, the mean ⁵⁷Fe absorption rate of the total participants was 22.2%±9.6% with no significant difference between the rice and steamed buns groups (22.2%±10.6% vs 22.2%±8.9%, *p*=0.992). The mean erythrocyte iron incorporation rate of the infused ⁵⁸Fe was 91.6%±8.2%, and similarly, no significant difference existed between groups (rice, 93%±7.3% vs steamed buns, 90%±9.1%, *p*=0.418; Table 5).

DISCUSSION

Rice and wheat are the major sources of staple foods for populations in South and North China, respectively. To estimate the iron DRI in China, it is essential to determine the dietary iron absorption and incorporation in rice and wheat among the Chinese population. Thus far, only one English-language study reported the nonheme iron bioavailability and incorporation for healthy young Chinese men.¹⁶ These data for healthy young Chinese women were never been estimated. In this study, we presented data in relation to the nonheme iron bioavailability and incorporation rates in representative diets of healthy young Chinese women, which was used to represent the standard female population, which is not substantially affected by the typical staple food patterns unique to South and North China. To our knowledge, these results have not been previously reported.

In this study, to estimate approximate dietary iron absorption and incorporation, typical comprehensive Chinese diets, rather than single meals, were served to the participants. In addition, first, it was crucial to design a representative experimental Chinese diet, which could consider the factors that influence the iron absorption in the diets. Based on the latest Chinese National Nutrition Survey²¹ along with cluster analysis, as well as the balanced percentages of energy supplies from protein, fat and carbohydrates, we designed typical comprehensive Chinese diets in which the total energy and proportions of the consumed protein, carbohydrate, and fat were, all in line with the dietary intake recommendations for the Chinese population.⁵ Moreover, iron status influences iron absorption, which requires assessment, and simultaneously, inflammatory markers also require being analyzed.²⁷ We thus strictly measured the participants' iron nutritional statuses and ruled out both iron deficiency anemia and early iron deficiency.²⁸ Moreover, the participants were free of inflammation (CRP >5 mg/L). Thus, our participants had satisfactory iron nutritional statuses without inflammation, which ruled out the influence of abnormal iron status or inflammation on the iron absorption.

The experimental isotope dose and administration method used may have affected the iron absorption in participants. In the present study, the same dose of ⁵⁷FeSO₄ was added into rice or steamed buns, mixed well, and consumed in three meals for 2 consecutive days, all

in line with the typical energy distribution ratio. This present dietary strategy enabled the estimation of typical dietary iron absorption under routine dietary conditions.

This study was carefully designed. First, supplementation of 30%–60% of stable isotope iron among the total dietary iron intake does not disturb the normal iron metabolism;²⁹ therefore, the iron supplement (56.9% of the total dietary iron intake) in this study was within the normal range. Second, according to iron fortification levels (14–26 mg of iron per kg of rice or flour) in China,³⁰ all the participants in the present study consumed an average of 200 g of rice or 250 g of flour [i.e., 2.8–5.2 mg of iron from rice (14–26 mg of iron/kg of rice × 0.20 kg) or 3.5–6.5 mg of iron from flour (14–26 mg of iron/kg of flour × 0.25 kg)]. Therefore, the daily addition of total labeled iron of 4.10 mg [(4.61 mg ⁵⁷Fe + 3.60 mg ⁵⁸Fe)/2 = 4.10 mg] in this study was within the scope of the normal iron fortification levels in China, which would not affect normal iron metabolism. Third, we did not ask the participants to initially eat a low iron diet but rather distributed the stable iron isotope doses in staple foods at six meals over 2 consecutive experimental days, which assured that this strategy was closer to actual dietary situations so as to avoid overestimation of iron biological incorporation. In addition, the 2-day experimental menu contained similar staple foods (i.e., steamed bread and rice) and covered representative local nonstaple foods, meant to reflect the typical diets of the study population. Fourth, according to the differences in the dietary habits regarding staple foods of South and North China, the participants were randomly assigned to rice or steamed buns groups. Fifth, the diet was reasonably designed, with a balanced distribution of energy supplies from protein, fat, and carbohydrates. Sixth, iron availability and incorporation are closely associated with the physiological status of body. For instance, in pregnant women, because of the stop of menstruation and consequent termination of iron loss, the physiological need for iron decreases, which results in the reduction of iron absorption.²⁶ In general, the participants had normal iron nutritional statuses and were not pregnant. Thus, other factors (such as pregnancy) influencing iron bioavailability and incorporation can be excluded.

In contrast to the single-labeled isotope method that cannot directly obtain iron absorption and incorporation data,³¹ we administered two stable isotope labels orally and intravenously, respectively, to distinguish the absorbed dietary iron and systematically utilized iron and thus to directly assess the incorporation rate of the infused isotopic iron label into erythrocytes.^{16–19} Thus, this technique can assure the accuracy and precision of the assessment and thus accurately estimate both the absorption and incorporation of dietary iron in the human body.

Some differences existed in the intakes of major nutrients between the rice and steamed buns groups because of

the different dietary restrictions and food intakes during the individual participants' free access to food; however, these did not directly affect the ^{57}Fe absorption and ^{58}Fe incorporation for the two groups. Compared with the healthy young men (consuming 2364-kcal energy) in the same experimental period,¹⁹ our female participants consumed less total dietary energy (1361 kcal). In addition, the mean iron intake of 10.3 mg/day in premenopausal British and Irish women³² and 14.0 mg/day in Chinese childbearing age women³³ has been recommended, whereas in the present study, the average iron intake was 7.2 mg/day—lower than the recommended iron DRIs for young Chinese women and much lower than the iron intakes of both the aforementioned female populations^{32,33} and of healthy young Chinese men.¹⁹ This may be related to the low amount of the total dietary intake of these young women in this study. During the experimental period, after eating the specified ^{57}Fe -labeled rice or buns, no participant added the additional nonlabeled staple foods. According to the following feedback, this is attributable to the attempts of these young women to lose weight and also to the atmosphere of collective dining (i.e., participants might not eat more when others around do not). This should be noted for future related studies involving women participants.

In addition, the iron intake for the steamed buns group participants was significantly higher than that for the rice group participants. This might be attributable to the iron from flour in the buns consumed by the former group participants being more than that by the latter group. However, because the isotopic ^{57}Fe label doses were equally distributed in the staple food that was required to be completely consumed by each participant, the difference in the total iron intake between the two groups did not affect the actual intake of the ^{57}Fe labels.

In this study, likely because of the difference of the individual participants' dietary habits and attempts to lose weight and of the atmosphere of collective dining, the nonstaple foods were not similarly consumed by the participants. Consequently, some types of foods with relatively rich ascorbic acid (i.e., high intake levels of ascorbic acid) were less eaten by the rice group participants than by the steamed buns group participants. Nevertheless, no significant difference with regard to nonheme iron absorption and incorporation was observed between the two groups. This suggests that nonheme iron absorption and incorporation depend on the comprehensive effects of multiple factors such as cooking means and iron enhancers (i.e., phytic acid and calcium) and inhibitors (i.e., ascorbic acid). Enhancers and inhibitors of iron absorption, such as zinc and calcium, are assessed in the next section to determine their influence on the noniron absorption and incorporation, which is in turn beneficial for appropriately setting the related DRIs for zinc, calcium, and other minerals later.

Eating iron-rich food or taking iron supplements does not necessarily increase iron absorption.^{34,35} Iron absorption represents the ratio of the dietary iron absorbed through the human gastrointestinal tract, which is determined by assessing the incorporation rate of oral ^{57}Fe into erythrocytes. The present study revealed a mean dietary nonheme iron absorption of $22.2\% \pm 9.6\%$ in total for

young Chinese women eating typical comprehensive diets, $22.2\% \pm 10.6\%$ for the rice group, and $22.2\% \pm 8.9\%$ for the steamed buns group. Interestingly, the mean dietary iron absorption in our study was higher than that of 17% for British and Irish women³⁶ and 3.2%–11.6% for young Swiss women.³⁴ Conversely, it is similar to the nonheme iron absorption of 20.3% for a British nonpregnant female population.²⁶ This might be related to differences in diets, contained enhancers and inhibitors of iron absorption, and genetic characteristics influencing the iron absorption and incorporation between Chinese and other female populations.

Iron incorporation indicates the ratio of the iron incorporated into the iron-containing biomacromolecules (mainly hemoglobin) among the absorbed dietary iron over a period of time. Iron incorporation is calculated as the incorporation rate of intravenously infused ^{58}Fe into erythrocytes. At 14 days after the ^{58}Fe infusion, the mean overall iron incorporation rate was $91.6\% \pm 8.2\%$ in total, $93\% \pm 7.3\%$ for the rice group, and $90\% \pm 9.1\%$ for the steamed buns group. These results are similar to what was reported for a British nonpregnant female population ($90.1\% \pm 6.0\%$).²⁶

Iron storage (i.e., SF level) is negatively correlated with iron absorption,^{19,36,37} therefore, the estimation of iron bioavailability requires a well-defined iron status. In general, the estimation of iron absorption should be performed for participants with low SF levels but who are not anemic. This can maximally stimulate iron absorption but simultaneously avoid disturbing normal physiological processes.³⁶ Specific SF cut-offs, which should be referred to when measuring the iron absorption rate, have been proposed in different countries such as 25 or 30 $\mu\text{g/L}$ for SF.^{38–40} In the present study, the efficacy of the nonheme absorption and incorporation was estimated on the condition of a mean SF level of $41.3 (\pm 27.2)$ $\mu\text{g/L}$, representing normal SF levels (by using a reference range of 15–150 $\mu\text{g/L}$) for adult women according to the used detection kit. Given that SF concentration is extensively relied on for both the used techniques and the detailed assay kit, we modulated the SF level (if necessary) when relying on this data to set iron DRIs.

No significant difference existed for dietary iron absorption and infused iron incorporation between the rice and steamed buns groups, indicating that the dietary iron absorption and incorporation in representative comprehensive Chinese diets were not affected by the regional staple food patterns in China. Given that this study was carefully designed and that the basic characteristics (such as age, weight, height, and BMI), inflammatory parameter (i.e., CRP), iron status (e.g., SF, SI, and sTfR), and oral ^{57}Fe and intravenously administered ^{58}Fe doses of the participants were nearly consistent between the two groups, the present result is reliable and not influenced by confounding factors.

A related report demonstrated no obvious difference in the dietary iron bioavailability between men and women.⁴¹ By contrast, we found a ^{57}Fe absorption rate of $22.2\% \pm 9.6\%$ in young Chinese women was much higher (about by one times) than in young Chinese men ($11\% \pm 7\%$) as determined in a parallel study conducted during the same experimental period as the present

study.¹⁹ This may be attributable to such factors as the low amounts of total energy and iron intakes in these young women (potentially caused by the various aforementioned factors), the ratio of the administered isotopic labels to the total iron intakes, and the unique physiological characteristics of females. In particular, low total iron intake and other factors may in turn stimulate iron absorption to meet the physiological requirements in women.

The main results are briefly summarized and interpreted in Figure 1. A comparison of the iron incorporation rates between males and females has not been reported previously. We showed that the infused ⁵⁸Fe incorporation rate was slightly higher for young women (91.6%±8.2%) than for young men (85%±8%).¹⁹ Further study is required to verify these results.

This study has some limitations. First, this study involved a relatively short experimental diet period involving six meals. Additional research with observations over a longer experimental period would contribute toward validating the present results. Second, because no data had existed for the dietary iron absorption of rice and wheat for female Chinese populations before this study, estimating the minimum sample size for this randomized controlled study was difficult. The estimation of the sample size in this study was performed in reference to previous studies using the double-labeled iron stable isotope method.^{13,43,44} Studies using a double-labeled stable isotopic technique used two types of stable isotopic labels, which is expensive and generally includes a rather limited sample size (typically 10–20 participants).^{13,41,44} Typically, in such studies, young healthy participants are tested as the reference population and the result is generalized for other populations. The participant number of 22 in our study, although apparently small, is actually comparable to the aforementioned related studies. Nevertheless, in the future, a randomized controlled study including more participants should be performed to verify these results. Third, the total energy and iron intakes from the experimental diet were relatively low, despite the ratio of the energy supplies from protein, fat, and carbohydrates being maintained at a reasonable level. This may have caused a higher level of iron bioavailability and incorporation for participants with low iron intake levels than for those with a normal level because of undetermined stimulation mechanisms in the human body. This requires amendment in future studies. Moreover, future studies should involve the investigation of the dietary iron absorption and incorporation for different female populations, particularly those prone to anemia (e.g., pregnant women and elderly women).

In conclusion, we carefully estimated the nonheme dietary iron absorption and incorporation in typical comprehensive Chinese diets among healthy young urban Chinese women. Our results were not significantly influenced by the typical regional staple food patterns in South and North China. Our study may be referred to and our results generalized from to set the iron DRIs for the Chinese female population.

AUTHOR DISCLOSURES

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REFERENCES

- McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutr.* 2009;12:444-54. doi: 10.1017/S1368980008002401.
- Pasricha SR, Drake-Smith H. Iron deficiency anemia: problems in diagnosis and prevention at the population level. *Hematol Oncol Clin North Am.* 2016;30:309-25. doi: 10.1016/j.hoc.2015.11.003.
- Hu Y, Chen J, Li M, Li W, Yang Y, Yang L, Wang R, Piao J. Study on the anemia status of Chinese urban residents in 2010-2012. *Zhonghua Yu Fang Yi Xue Za Zhi.* 2016;50:213-6. doi: 10.3760/cma.j.issn.0253-9624.2016.03.004. (In Chinese)
- Li M, Hu Y, Mao D, Wang R, Chen J, Li W, Yang X, Piao J, Yang L. Prevalence of anemia among Chinese rural residents. *Nutrients.* 2017;9:192. doi: 10.3390/nu9030192.
- Chinese Nutritional Society. *Chinese Dietary Reference Intakes.* Beijing: Science Press; 2013.
- Miura K, Ando K, Tsuchihashi T, Yoshita K, Watanabe Y, Kawarazaki H et al. [Scientific statement] Report of the Salt Reduction Committee of the Japanese Society of Hypertension(2) Goal and strategies of dietary salt reduction in the management of hypertension. *Hypertens Res.* 2013;36:1020-5. doi: 10.1038/hr.2013.105.
- Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. *Institute of Medicine of the National Academies Dietary reference intakes for calcium and vitamin D.* Washington, DC: The National Academies Press; 2011.
- Food and Nutrition Board, editor. *Institute of Medicine. Dietary reference intakes for energy, carbohydrate, fiber, fatty acids, cholesterol, protein, and amino acids (Macronutrients).* Washington, DC: National Academies Press; 2005.
- Gropper SS, Smith JL, Groff JL. *Advanced Nutrition and Human Metabolism.* Belmont, CA: Wadsworth; 2005.
- Fairweather-Tait SJ, Collings R. Estimating the bioavailability factors needed for setting dietary reference values. *Int J Vitam Nutr Res.* 2010;80:249-56. doi: 10.1024/0300-9831/a000031.
- Collings R, Harvey LJ, Hooper L, Hurst R, Brown TJ, Ansett J, King M, Fairweather-Tait SJ. The absorption of iron from whole diets: a systematic review. *Am J Clin Nutr.* 2013;98:65-81. doi: 10.3945/ajcn.112.050609.
- Hunt JR, Zeng H. Iron absorption by heterozygous carriers of the HFE C282Y mutation associated with hemochromatosis. *Am J Clin Nutr.* 2004;80:924-31. doi: 10.1093/ajcn/80.4.924.
- Reddy MB, Hurrell RF, Cook JD. Meat consumption in a varied diet marginally influences nonheme iron absorption in normal individuals. *J Nutr.* 2006;136:576-81. doi: 10.1093/jn/136.3.576.
- Bach Kristensen M, Hels O, Morberg C, Marving J, Bügel S, Tetens I. Pork meat increases iron absorption from a 5-day fully controlled diet when compared to a vegetarian diet with similar vitamin C and phytic acid content. *Br J Nutr.* 2005;94:78-83.
- Diaz M, Rosado JL, Allen LH, Abrams S, García OP. The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable

- isotopes. *Am J Clin Nutr.* 2003;78:436-40. doi: 10.1093/ajcn/78.3.436.
16. Patterson KY, Veillon C. Stable isotopes of minerals as metabolic tracers in human nutrition research. *Exp Biol Med (Maywood).* 2001;226:271-82.
 17. Yang L, Yang X, Piao J, Tian Y, Li P, Wang Y, Wang J. Studies on zinc bioavailability from a representative diet in Chinese urban women of childbearing age using a doublelabel stable isotope technique. *J Trace Elem Med Biol.* 2005;19:159-64. doi: 10.1016/j.jtemb.2005.09.001.
 18. Cercamondi CI, Egli IM, Ahouandjinou E, Dossa R, Zeder C, Salami L et al. Afebrile plasmodium falciparum parasitemia decreases absorption of fortification iron but does not affect systemic iron utilization: a double stable-isotope study in young Beninese women. *Am J Clin Nutr.* 2010;92:1385-92. doi: 10.3945/ajcn.2010.30051.
 19. Yang L, Zhang Y, Wang J, Huang Z, Gou L, Wang Z, Ren T, Piao J, Yang X. Non-Heme Iron Absorption and Utilization from typical whole Chinese diets in young Chinese urban men measured by a double-labeled stable isotope technique. *PLoS One.* 2016; 11:e0153885. doi: 10.1371/journal.pone.0153885.
 20. Reddy MB, Hurrell RF, Cook JD. Estimation of nonheme-iron bioavailability from meal composition. *Am J Clin Nutr.* 2000;71:937-43. doi: 10.1093/ajcn/71.4.937.
 21. Zhang B, Zhai FY, Du SF, Popkin BM. The China Health and Nutrition Survey, 1989-2011. *Obes Rev.* 2014 15 Suppl 1:2-7. doi: 10.1111/obr.12119.
 22. Hurrell R, Egli I. Iron bioavailability and dietary reference values. *Am J Clin Nutr.* 2010;91:1461S-7S. doi: 10.3945/ajcn.2010.28674F.
 23. Petry N, Egli I, Gahutu JB, Tugirimana PL, Boy E, Hurrell R. Phytic acid concentration influences iron bioavailability from biofortified beans in Rwandese women with low iron status. *J Nutr.* 2014;144:1681-7. doi: 10.3945/jn.114.192989.
 24. Zhou T, Zhao M, Wang J, Lu H. Absolute measurements and certified reference material for iron isotopes using multiple-collector inductively coupled mass spectrometry. *Rapid Commun Mass Spectrom.* 2008;22:717-20. doi: 10.1002/rcm.3406.
 25. Barrett JF, Whittaker PG, Williams JG, Lind T. Absorption of non-haem iron in normal women measured by the incorporation of two stable isotopes into erythrocytes. *Clin Sci (Lond).* 1992;83:213-9.
 26. Whittaker PG, Barrett JF, Lind T. The erythrocyte incorporation of absorbed non-haem iron in pregnant women. *Br J Nutr.* 2001;86:323-9.
 27. Harvey LJ, Berti C, Casgrain A, Cetin I, Collings R, Gurinovic M et al. EURRECA- Estimating iron requirements for deriving dietary reference values. *Crit Rev Food Sci Nutr.* 2013;53:1064-76. doi: 10.1080/10408398.2012.742860.
 28. U.S. Centers for Disease Control and Prevention. Second national report on biochemical indicators of diet and nutrition in the U.S. population 2012. Atlanta (GA): National Center for Environmental Health. April 2012 [cited 2013/02/04]; Available from: <http://www.cdc.gov/nutritionreport>.
 29. Turnlund JR. Bioavailability of dietary minerals to humans: the stable isotope approach. *Crit Rev Food Sci Nutr.* 1991; 30:387-96. doi: 10.1080/10408399109527549.
 30. National Health and Family Planning Commission of the People's Republic of China. Standard for the use of nutritional fortification substances in foods GB 14880—2012, national food safety standard of state standard of the Peoples' Republic of China. 2012 [2012-03-31]; Available from: <http://www.moh.gov.cn/cmsresources/mohwsjdcmsrdocument/doc14526.pdf>.
 31. Chang S, Huang Z, Ma Y, Piao J, Yang X, Zeder C, Hurrell RF, Egli I. Mixture of ferric sodium ethylenediaminetetraacetate (NaFeEDTA) and ferrous sulfate: an effective iron fortificant for complementary foods for young Chinese children. *Food Nutr Bull.* 2012;33:111-6. doi: 10.1177/156482651203300204.
 32. Fairweather-Tait SJ, Jennings A, Harvey LJ, Berry R, Walton J, Dainty JR. Modeling tool for calculating dietary iron bioavailability in iron-sufficient adults. *Am J Clin Nutr.* 2017;105:1408-14. doi: 10.3945/ajcn.116.147389.
 33. Wang Z, Sun J, Wang L, Zong M, Chen Y, Lin Y et al. Effect of iron supplementation on iron deficiency anemia of childbearing age women in Shanghai. *Wei Sheng Yan Jiu.* 2012;41:51-5.
 34. Cercamondi CI, Icard-Vernière C, Egli IM, Vernay M, Hama F, Brouwer ID, Zeder C, Berger J, Hurrell RF, Mouquet-Rivier C. A higher proportion of iron-rich leafy vegetables in a typical Burkinabe maize meal does not increase the amount of iron absorbed in young women. *J Nutr.* 2014;144:1394-400. doi: 10.3945/jn.114.194670.
 35. Moretti D, Goede JS, Zeder C, Jiskra M, Chatzinakou V, Tjalsma H, Melse-Boonstra A, Brittenham G, Swinkels DW, Zimmermann MB. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood.* 2015;126:1981-9. doi: 10.1182/blood-2015-05-642223.
 36. Dainty JR, Berry R, Lynch SR, Harvey LJ, Fairweather-Tait SJ. Estimation of dietary iron bioavailability from food iron intake and iron status. *PLoS One.* 2014;9:e111824. doi: 10.1371/journal.pone.0111824.
 37. Navas-Carretero S, Pérez-Granados AM, Sarriá B, Carbajal A, Pedrosa MM, Roe MA, Fairweather-Tait SJ, Vaquero MP. Oily fish increases iron bioavailability of a phytate rich meal in young iron deficient women. *J Am Coll Nutr.* 2008; 27:96-101.
 38. Nelson R, Chawla M, Connolly P, LaPorte J. Ferritin as an index of bone marrow iron stores. *South Med J.* 1978;71: 1482-4.
 39. Sharma JC, Roy SN. Value of serum ferritin as an index of iron deficiency in elderly anaemic patients. *Age Ageing.* 1984;13:248-50.
 40. Yu D, Huo J, Xie L, Wang L. Meta-analysis of studies on cut-off value of serum ferritin for identifying iron deficiency. *Wei Sheng Yan Jiu.* 2013;42:228-35.
 41. Nair KM, Brahmam GN, Radhika MS, Dripta RC, Ravinder P, Balakrishna N, Chen Z, Hawthorne KM, Abrams SA. Inclusion of guava enhances non-heme iron bioavailability but not fractional zinc absorption from a rice-based meal in adolescents. *J Nutr.* 2013;143:852-8. doi: 10.3945/jn.112.171702.
 42. Pizarro F, Olivares M, Hertrampf E, Nuñez S, Tapia M, Cori H, Lopez de Romana D. Ascorbyl palmitate enhances iron bioavailability in iron-fortified bread. *Am J Clin Nutr.* 2006; 84:830-4. doi: 10.1093/ajcn/84.4.830.
 43. Davidsson L, Ziegler EE, Kastenmayer P, Hurrell RF. Erythrocyte incorporation of iron by infants: iron bioavailability from a low-iron infant formula and an evaluation of the usefulness of correcting erythrocyte incorporation values, using a reference dose or plasma ferritin concentrations. *Br J Nutr.* 2000;84:847-53.
 44. DellaValle DM, Glahn RP, Shaff JE, O'Brien KO. Iron Absorption from an intrinsically labeled lentil meal is low but upregulated in women with poor iron status. *J Nutr.* 2015;145:2253-7. doi: 10.3945/jn.115.217273.