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

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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.³⁴ 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evaluation.⁵ However, iron DRIs, based on domestic research results, mainly the 2000 Chinese Total Diet Study (data unpublished), have been established only for young Chinese men; by contrast, for other Chinese populations (such as women), the DRIs have been based on the data from young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.⁶⁷ 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,⁸ 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. An international consensus on nonheme iron bioavailability has not been reached, indicating that for setting iron DRIs, 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). 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; 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 in-
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 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 $^{57}$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 $^{58}$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$_2$SO$_4$ 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 $^{57}$FeSO$_4$ and $^{58}$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 $^{58}$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 Survey$^{21}$ 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.

Steam buns containing $^{57}$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.

**Figure 1.** Conceptual diagram of this study.
participant. All the $^{57}$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 $^{57}$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 $^{57}$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.\(^ \text{19} \) 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 $-20^\circ$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.\(^ \text{15,20,22,23} \)

**Blood analysis**

Whole blood was collected and centrifuged, after which serum was separated and frozen at $-20^\circ$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 μg/L on the basis of the reference range of the kit.\(^ \text{5} \) Iron deficiency anemia was defined when SF $<$15 μg/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 HNO$_3$/H$_2$O 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 $^{54}$Ni on $^{56}$Fe. An argon and hydrogen mixture was used as a collision gas to eliminate interference.\(^ \text{24} \) In optimized conditions, external precisions for $^{57}$Fe/$^{56}$Fe and $^{58}$Fe/$^{56}$Fe were in the order of 0.01%–0.03% relative standard deviation (RSD) and 0.1%–0.3% RSD, respectively. Enriched proportions of $^{57}$Fe/$^{56}$Fe% and $^{58}$Fe/$^{56}$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 $^{57}$FeSO$_4$ dose and intravenously administered $^{58}$FeSO$_4$ dose into the erythrocytes of the participants.\(^ \text{17,19} \) The formulas for total oral $^{57}$FeSO$_4$ absorption, iron mass, and circulating $^{58}$FeSO$_4$ mass were described previously.\(^ \text{19,25,26} \)

**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); there-

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m$^2$)</th>
<th>CRP (mg/L)</th>
<th>UIBC (μmol/L)</th>
<th>SI (μmol/L)</th>
<th>sTfR (mg/L)</th>
<th>SF (μg/L)</th>
<th>Hb (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
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<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>21.6±1.50</td>
<td>56.0±7.0</td>
<td>163±3.30</td>
<td>21.2±2.40</td>
<td>0.17±0.13</td>
<td>29.7±15.2</td>
<td>9.20±5.60</td>
<td>3.03±1.00</td>
<td>41.3±27.2</td>
<td>130±13.4</td>
</tr>
</tbody>
</table>

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.
fore, 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, 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 $^{57}$Fe formulation comprised 96.73% $^{57}$Fe, 2.11% $^{58}$Fe, 1.16% $^{56}$Fe, and 0.01% $^{54}$Fe, whereas the $^{58}$Fe formulation comprised 90.97% $^{58}$Fe, 8.83% $^{57}$Fe, 0.19% $^{56}$Fe, and 0.01% $^{54}$Fe. 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. The percentages of energy supplies from protein, fat, and carbohydrates were 18.7% (63.7 × 400/1361), 30.4% (46 × 900/1361), and 49% (67.5 × 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). 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 $^{57}$Fe (oral) and 3.60 mg of $^{58}$Fe (intravenous) was administered to each participant (Table 4). No significant difference in the actual oral or intravenous doses of the meal-administered $^{57}$Fe or $^{58}$Fe, basal and 2-week post-experimental blood $^{57}$Fe ($^{57}$Fe:$^{58}$Fe) and $^{58}$Fe ($^{58}$Fe:$^{56}$Fe) levels, and $^{58}$Fe bioavailability and infused $^{58}$Fe incorporation after 2 weeks was observed between the two groups (p>0.05).

After 2 weeks, the mean $^{57}$Fe 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

### Table 3. Daily average intakes of nutrients

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>Rice group</th>
<th>Steamed buns group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (Kcal)</td>
<td>1361±91.8</td>
<td>1332±69.9</td>
<td>1389±105</td>
<td>0.156</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>7.20±1.60</td>
<td>5.90±0.60</td>
<td>8.40±1.20</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>5.10±0.60</td>
<td>5.40±0.40</td>
<td>4.90±0.60</td>
<td>0.05</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>692±154</td>
<td>599±75.3</td>
<td>784±159</td>
<td>0.002*</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>168±12.2</td>
<td>178±6.10</td>
<td>157±3.80</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>46.0±8.00</td>
<td>40.2±3.50</td>
<td>51.8±8.80</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>63.7±7.80</td>
<td>54.1±5.90</td>
<td>68.0±7.30</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>10.9±1.80</td>
<td>9.60±1.10</td>
<td>12.1±1.60</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ascorbic acid (mg)</td>
<td>3.70±1.10</td>
<td>2.80±0.40</td>
<td>4.70±0.70</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Phytic acid (mg)</td>
<td>4.50±1.40</td>
<td>3.20±0.30</td>
<td>5.70±0.70</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>Rice group</th>
<th>Steamed buns group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral $^{57}$Fe dose (mg)</td>
<td>4.61±0.125</td>
<td>4.64±0.15</td>
<td>4.58±0.09</td>
<td>0.187</td>
</tr>
<tr>
<td>Oral $^{58}$Fe dose (mg)</td>
<td>0.100±0.003</td>
<td>0.101±0.003</td>
<td>0.100±0.002</td>
<td>0.185</td>
</tr>
<tr>
<td>Oral $^{56}$Fe dose (mg)</td>
<td>3.60±0.174</td>
<td>3.64±0.04</td>
<td>3.55±0.239</td>
<td>0.224</td>
</tr>
<tr>
<td>Oral $^{54}$Fe dose (mg)</td>
<td>0.349±0.017</td>
<td>0.354±0.004</td>
<td>0.345±0.023</td>
<td>0.224</td>
</tr>
<tr>
<td>Oral $^{57}$Fe at two weeks</td>
<td>0.231±0.00001</td>
<td>0.223±0.00001</td>
<td>0.223±0.00001</td>
<td>0.678</td>
</tr>
<tr>
<td>Oral $^{58}$Fe at two weeks</td>
<td>0.0239±0.00030</td>
<td>0.0239±0.00032</td>
<td>0.0239±0.00028</td>
<td>0.825</td>
</tr>
<tr>
<td>Oral $^{56}$Fe at two weeks</td>
<td>0.00085±0.00030</td>
<td>0.00080±0.000033</td>
<td>0.00080±0.00028</td>
<td>0.850</td>
</tr>
<tr>
<td>Oral $^{54}$Fe at two weeks</td>
<td>0.00037±0.000001</td>
<td>0.00030±0.000001</td>
<td>0.00030±0.000001</td>
<td>0.902</td>
</tr>
<tr>
<td>Oral $^{57}$Fe absorption (mg)</td>
<td>0.239±0.103</td>
<td>0.239±0.115</td>
<td>0.239±0.110</td>
<td>0.995</td>
</tr>
</tbody>
</table>

Iv: intravenously administered; SD: standard deviation.

*Oral $^{54}$Fe dose indicates the dose of the oral $^{54}$Fe among the mixed oral $^{57}$Fe preparation.

*Dose $^{54}$Fe indicates the dose of $^{54}$Fe from the mixed iv $^{58}$Fe preparation.
erythrocyte iron incorporation rate of the infused \(^{58}\)Fe 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 Survey\(^{21}\) 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.\(^{37}\) We thus strictly measured the participants’ iron nutritional statuses and ruled out both iron deficiency anemia and early iron deficiency.\(^{29}\) 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 \(^{57}\)FeSO\(_4\) 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,\(^{39}\) 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 \(^{57}\)Fe + 3.60 mg \(^{58}\)Fe]/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 absorption.
incorporation for the two groups. Compared with the healthy young men (consuming 2364-kcal energy) in the same experimental period, 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 women and 14.0 mg/day in Chinese childbearing age women has been recommended, whereas in the present study, the average iron intake was 7.2 mg/day—lower than the recommended iron DRIs for both the aforementioned female populations and of healthy young Chinese men. 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 57Fe-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 57Fe 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 57Fe 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 streamed 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 nonheme iron 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. 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 57Fe into erythrocytes. The present study revealed a mean dietary nonheme iron absorption of 22.2±9.6% in total for young Chinese women eating typical comprehensive diets, 22.2±10.6% for the rice group, and 22.2±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 women and 3.2%–11.6% for young Swiss women. Conversely, it is similar to the nonheme iron absorption of 20.3% for a British nonpregnant female population. 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 57Fe into erythrocytes. At 14 days after the 57Fe infusion, the mean overall iron incorporation rate was 91.6±8.2% in total, 93%±7.3% for the rice group, and 90%±9.1% for the steamed buns group. These results are similar to what was reported for a British nonpregnant female population (90.1±6.0%).

Iron storage (i.e., SF level) is negatively correlated with iron absorption, 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. 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 µL for SF. 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 (±27.2) µL, representing normal SF levels (by using a reference range of 15–150 µG/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 streamed 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 stTR), and oral 57Fe and intravenously administered 57Fe 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. By contrast, we found a 57Fe absorption rate of 22.2±9.6% in young Chinese women was much higher (about by one times) than in young Chinese men (11%±7%) as determined in a parallel study conducted during the same experimental period as the present study. 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 afore-
mentioned 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%). 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. 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


