Introduction

Bioavailability has been defined as the fraction of the ingested nutrient that is absorbed and subsequently used for normal physiologic functions (1). Iron bioavailability is of interest because poor absorption is considered to be a major contributing factor to the worldwide prevalence of iron deficiency. Globally, ~1.6 billion people suffer from anemia, which is primarily caused by iron deficiency (2). In the United States, the prevalence of iron deficiency was estimated to be 9% in 20- to 49-y-old nonpregnant women, when the currently popular body iron model was used (3). When the ferritin model was used, however, the estimated prevalence of iron deficiency jumped to 16% (3). Total iron absorption from the diet can be estimated by combining the fractional absorption from nonheme and heme iron sources. It is widely accepted that iron bioavailability is the fraction of ingested iron that is absorbed and subsequently used for normal physiologic functions (1). Iron bioavailability is of interest because iron status and dietary factors (5). Thus, estimates of nonheme iron bioavailability have differed greatly in the literature as a result of different combinations of dietary factors (6–12).

Hallberg et al. (13) estimated that total iron bioavailability for a typical Western-type diet may range from 14–17% in subjects who are borderline iron deficient (serum ferritin concentration ≤15 μg/L). The WHO has proposed a bioavailability of 15% for Western-type diets that are high in fruit, vegetables, meat, and fish (14). The US Institute of Medicine and Health Canada have established that total iron bioavailability is 18%, based on data from a study in which absorption was measured in 15 subjects who consumed their typical diet for 2 wk (15, 16). This value was used in estimating the current DRI for iron except for in pregnant women and children <1 y of age (17).

Iron absorption in humans is usually measured with the use of stable or radioactive isotopes of iron (8, 18–21). However, these methods are not feasible in population studies because they are expensive and cumbersome. Thus, several algorithms have been developed for estimating iron bioavailability in a population (22–31), but the limitation is that they are based on single-meal
studies which exaggerate the effect of dietary factors. Hunt (32) argued that the inability of single-meal algorithms to predict human absorption raises the need to develop algorithms that are based on complete diets.

Our objective in this study was to estimate total iron absorption from the US diet with the use of a complete-diet algorithm that we recently proposed for estimating nonheme iron absorption and a conservative value for heme iron absorption (33). The NHANES (2001–2002) (34), MyPyramid Equivalents Database (MPED)5 version 1.0 (35), and Food and Nutrient Database for Dietary Studies (FNDDS) version 1.0 (36) provided nationally representative data with the information needed at the individual level to estimate iron absorption from the US diet with the use of the algorithm we have proposed.

Methods

Data sources and general procedures. In this analysis we used the NHANES 2001–2002, MPED version 1, and FNDDS version 1.0 data. These data versions were used because they had complete information on serum ferritin concentrations in all age groups (≥ 1 y of age) and in both genders. More recent data has ferritin information for only children 1–5 y and female subjects 12–49 y of age. We used day 1 dietary intake and serum ferritin values for the NHANES 2001–2002 for estimating absorption. Only those individuals with complete information for the variables needed to estimate nonheme iron absorption were included in the analysis (n = 6631). Pregnant and lactating women, as well as individuals with C-reactive protein (CRP) ≥ 6 mg/L, were excluded from the analyses.

Food and nutrient intake estimation. We obtained individual-level daily nutrient intake of vitamin C, total iron, and calcium from the NHANES, and 90% of the total iron intake was estimated as nonheme iron (15). Because the NHANES does not report phytate intake, we estimated intake with the use of information reported by the International Zinc Nutrition Consultative Group (37) that lists the phytate content of different foods and food groups. Through these data, we estimated the phytate content of the various food groups reported in the MPED equivalence data. These values were scaled appropriately in order to obtain the phytate content in milligrams per 100 grams of each food group. We merged the resulting data with the Individual Foods data from the NHANES to estimate the phytate content of each food consumed by each study participant. The total daily phytate intake was the sum of the phytate amounts contributed by the different foods consumed by the individual during the day.

We estimated polyphenol intake as black tea equivalents (cups), because the polyphenol content in foods and beverages is not reported in the NHANES. Tea, coffee, and related beverages reported in the FNDDS were assigned black tea equivalents with the use of the following conversion factors: 2 cups (480 mL) iced tea and 1.5 cups (360 mL) herbal tea or coffee were coded as 1 cup (240 mL) of black tea (38). These values were used to estimate the total number of cups consumed by each subject.

Consumption of meat–fish–poultry (MFP) was estimated with the use of the appropriate MPED data, which provide daily intake of MFP for each subject in MyPyramid equivalents. The MFP equivalents were converted to grams with the use of a conversion factor of 28.35 g per ounce.

Nonheme iron absorption was estimated with the use of the following equation (33):

\[
\begin{align*}
\text{Ln Absorption} & = 6.294 - 0.709 \ln(SF) + 0.119 \ln(C) \\
& + 0.006 \ln(MFP) + 0.1 - 0.055 \ln(T + 0.1) \\
& - 0.247 \ln(P) - 0.137 \ln(Ca) - 0.083 \ln(NH)
\end{align*}
\]

where SF is Serum ferritin concentration (micrograms per liter), C is vitamin C intake (milligrams), MFP is meat–fish–poultry (grams), T is black tea equivalents (number of cups), P is estimated phytate (milligrams), Ca is calcium (milligrams) and NH is nonheme iron (milligrams).

Nonheme iron absorption was estimated at the individual level and geometric means were estimated for different groups and subpopulations. In estimating total iron absorption for the US population, we used 2 different approaches. In the first approach, we adjusted the geometric mean of nonheme iron absorption to a serum ferritin concentration of 15 μg/L. In the second approach, we estimated iron absorption, choosing only the subjects who were iron deficient (nonanemic but with a ferritin concentration ≤ 15 μg/L). In the first approach, the geometric mean of nonheme iron absorption was adjusted for serum ferritin with the use of the following equation, which was developed based on the inverse relation between ferritin and nonheme iron absorption (16), as follows:

\[
\text{Log(Adj Abs(%)}) = \log(\text{Obs Abs(%)}) + \log(\text{Obs Ferritin(μg/L)})
\]

where \( \text{Adj Abs} \) is adjusted nonheme absorption, \( \text{Obs Abs} \) is observed nonheme absorption (geometric mean), and \( \text{Obs Ferritin} \) is observed serum ferritin concentration (geometric mean). To estimate total absorption, we used the following equation (15):

\[
\text{Tot Abs(%)} = [\text{Adj Nonheme Abs(%) \times 0.9}] + [\text{Heme Abs(%) \times 0.1}]
\]

where \( \text{Tot Abs} \) is total absorption, \( \text{Adj Nonheme Abs} \) is the adjusted nonheme iron absorption, and \( \text{Heme Abs} \) is heme iron absorption.

We used a conservative value of 25% for heme iron absorption (4), and assumed that 90% of dietary iron in the typical US diet is nonheme, with 10% being heme iron as used in developing the DRI for iron (15). In the second approach, instead of adjusting the geometric mean of nonheme iron absorption to 15 μg/L, we estimated absorption for only a subsample (n = 678) of the subjects who had no iron stores but were not anemic (13, 15). The presence or absence of anemia was established with the use of WHO cutoff values for hemoglobin concentration (39). To determine whether an individual had no iron stores, we used a threshold of serum ferritin concentration ≤ 15 μg/L. Thus, we excluded anemic individuals, as well as individuals with ferritin >15 μg/L.

We used R software version 3.1.2 (40) for statistical analysis. We estimated means and CIs with the use of the “Survey” package, taking into account the strata, primary sampling unit, and appropriate sampling weight. Nutrient intake, serum ferritin concentration, and nonheme iron absorption values were log-transformed with the use of an ln before any statistical tests were performed, and the geometric means (95% CIs) were reported for these variables. Means (95% CIs) were reported for CRP and hemoglobin concentrations. Student’s t test was used to compare means and geometric means between subpopulations. P values were adjusted for multiple comparisons with the use of the Benjamini–Hochberg procedure for controlling false discovery rate. Statistical significance was set at \( P \leq 0.05 \).

Results

The geometric means for daily intake of vitamin C, calcium, phytate, and MFP for all subjects were 52 mg/d, 0.75 g/d, 0.56 g/d, and 59 g/d, respectively (Table 1). Girls 9–18 y of age had lower vitamin C intake than men >50 y (\( P = 0.03 \)) and women >50 y (\( P = 0.04 \)). Similarly, women aged 19–50 y had lower vitamin C intake than men >50 y (\( P = 0.03 \)) and women >50 y (\( P = 0.04 \)). All other pairwise comparisons for vitamin C intake did not show any statistically significant difference (\( P > 0.05 \)). Boys 9–18 y of age had higher calcium intake than all other groups (\( P < 0.05 \)). Men aged 19–50 y had higher MFP (\( P < 0.05 \)) and phytate intake (\( P < 0.01 \)) than all other groups. Tea intake was not significantly different between men >50 y of age

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5 Abbreviations used: CRP, C-reactive protein, FNDDS, Food and Nutrient Database for Dietary Studies; MPED, MyPyramid Equivalents Database; MFP, meat–fish–poultry.
and women aged ≥50 years (P = 0.77). However, both groups had significantly higher tea intake than all other groups (P < 0.001). Non-Hispanic white iron intake was higher in men and ≥50 years than all other groups (P < 0.05) except boys aged 9–18 years (P = 0.10). All pairwise comparisons for ferritin concentration showed a significant difference except between children 3–8 years of age and girls 9–18 years of age (P = 0.94), and between men aged 19–50 years and men aged ≥50 years (P = 0.94). Similarly, all pairwise comparisons for non-Hispanic iron absorption showed a significant difference except between children 3–8 years of age and girls 9–18 years of age (P = 0.79), and between men aged 19–50 years and men ≥50 years (P = 0.30). Mean CRP concentration was higher in women aged ≥50 years than all other groups (P < 0.001). All pairwise comparisons for hemoglobin concentration showed a significant difference (P < 0.01) except between girls 9–18 years of age and women 19–50 years of age (P = 0.36). Further demographic differences in intake of the dietary components, ferritin concentration, and non-Hispanic iron absorption are shown in Table 2. Non-Hispanic iron absorption was significantly lower for non-Hispanic whites than for both non-Hispanic blacks (P < 0.0001) and Mexican Americans (P < 0.0001). Non-Hispanic whites had lower MFP intake and higher ferritin concentrations and calcium, phytate, nonheme iron, and black tea-equivalent intake than non-Hispanic blacks (P < 0.05). Similarly, non-Hispanic whites had higher ferritin concentrations and tea intake, and lower vitamin C intake than Mexican Americans (P < 0.05). These differences may explain the variation in estimated nonheme iron absorption. Non-Hispanic iron absorption was significantly higher in female subjects (5.6%) than in male subjects (2.6%) (P < 0.0001). Male subjects had significantly higher intake of calcium, phytate, MFP, nonheme iron, and vitamin C than female subjects (P < 0.01), whereas black tea-equivalent intake did not differ between male and female subjects (P = 0.46). Female subjects also had a lower serum ferritin concentration than male subjects (36 μg/L and 92 μg/L for female and male subjects, respectively; P < 0.0001).

After correcting nonheme iron absorption to a serum ferritin concentration of 15 μg/L and adding fractional absorption from heme iron, the percentage total absorption was 15.5% (n = 6631). In iron-deficient individuals (nonanemic, n = 678), total iron absorption was 15.1%.

### Discussion

Despite global efforts to improve iron nutrition in at-risk populations, iron deficiency anemia remains a challenge (41). In

### Table 2: Nutrient intake, nonheme iron absorption, and serum ferritin concentrations in NHANES 2001–2002 participants by gender and ethnicity

<table>
<thead>
<tr>
<th>Gender</th>
<th>n</th>
<th>Phytate, g/d</th>
<th>MFP, g/d</th>
<th>Tea, cups/d</th>
<th>Calcium, g/d</th>
<th>Vitamin C, mg/d</th>
<th>Nonheme iron, mg/d</th>
<th>Ferritin, μg/L</th>
<th>Nonheme iron absorption, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>M</td>
<td>3456</td>
<td>0.62 (0.59, 0.66)</td>
<td>80 [74, 86]</td>
<td>0.14 (0.12, 0.16)</td>
<td>0.84 [0.81, 0.88]</td>
<td>56 [51, 61]</td>
<td>14.0 (13.5, 14.6)</td>
<td>92 [89, 96]</td>
<td>2.6 (2.5, 2.7)</td>
</tr>
<tr>
<td>F</td>
<td>3175</td>
<td>0.50 (0.48, 0.52)</td>
<td>42 [36, 49]</td>
<td>0.13 (0.11, 0.15)</td>
<td>0.65 [0.63, 0.68]</td>
<td>49 [46, 52]</td>
<td>10.5 (10.3, 10.8)</td>
<td>36 [34, 37]</td>
<td>5.6 (5.4, 5.7)</td>
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<tr>
<td><strong>Ethnicity</strong></td>
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<tr>
<td>Non-Hispanic white</td>
<td>2844</td>
<td>0.58 (0.54, 0.61)</td>
<td>56 [50, 63]</td>
<td>0.15 (0.13, 0.17)</td>
<td>0.79 [0.76, 0.83]</td>
<td>50 [46, 55]</td>
<td>12.4 (12.0, 12.9)</td>
<td>61 [58, 64]</td>
<td>3.5 (3.4, 3.6)</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>1571</td>
<td>0.47 (0.45, 0.50)</td>
<td>86 [76, 99]</td>
<td>0.07 (0.06, 0.09)</td>
<td>0.57 [0.55, 0.59]</td>
<td>56 [50, 62]</td>
<td>11.4 (11.1, 11.8)</td>
<td>54 [49, 58]</td>
<td>4.4 (4.1, 4.7)</td>
</tr>
<tr>
<td>Mexican American</td>
<td>1676</td>
<td>0.56 (0.52, 0.59)</td>
<td>60 [55, 67]</td>
<td>0.08 (0.06, 0.09)</td>
<td>0.76 [0.72, 0.79]</td>
<td>61 [56, 68]</td>
<td>12.1 (11.5, 12.7)</td>
<td>47 [43, 51]</td>
<td>4.5 (4.2, 4.8)</td>
</tr>
</tbody>
</table>

1 Values are geometric means [95% CIs]. Estimates are only for subjects with complete data for estimating nonheme iron absorption (n = 6631). Comparisons between gender groups were conducted separately from comparisons between ethnicity groups. Within gender and ethnicity categories, labeled values in a column without a common letter differ, P < 0.05. P values for pairwise comparisons between ethnicity groups were adjusted for multiple comparisons with the use of the Benjamini-Hochberg procedure. Estimates for "other" race were not reported, although data were included in the overall analyses. MFP, meat–fish–poultry.

2 One cup (240 mL) black tea = 2 cups iced tea or 1.5 cups herbal tea or coffee.
the United States, the prevalence of iron deficiency is high even at the present in certain populations (42, 43), even when the diet is high in heme iron and most foods are fortified with iron. One possible explanation for this persistent deficiency is that the bioavailability of iron in the diet of Americans has been overestimated. As a consequence, the prevalence of inadequate intake may have been underestimated. In this study, we revisited the problem of estimating iron absorption from dietary intake and iron status information with the use of data from a nationwide food consumption and health survey.

Various studies have estimated iron absorption from the complete diet, particularly in the case of nonheme iron absorption. In 4 studies that estimated nonheme iron absorption from typical US diets, the values ranged from 4.6% to 7.4% (7, 11, 16, 44). However, where meal compositions were varied to increase or decrease intake of selected factors, reported nonheme iron absorption values varied from 0.7% to >10% (6, 9, 10, 12). In this study, geometric means for nonheme iron absorption ranged from 1.9% in men (19–50 y of age) to 7.3% in children (3–8 y of age) and girls (9–18 y of age). It was also clear that groups that had high ferritin concentrations had low nonheme iron absorption, and vice versa (Table 1), which is due to the fact that high iron stores increase hepcidin expression, which reduces iron absorption (45). Our study also found that nonheme iron absorption was higher in female subjects from all age groups combined (5.6%) than in male subjects (2.6%). Again, this difference largely may be due to the lower serum ferritin concentration in female subjects compared with that in male subjects. In addition, a lower intake of inhibitors (calcium and phytate) in female subjects might contribute to this difference. Black tea-equivalent intake did not differ significantly by gender and thus may not have contributed to the discrepancy in nonheme iron absorption between the gender groups. With respect to ethnicity, nonheme iron absorption was lower in non-Hispanic whites than in both non-Hispanic blacks and Mexican Americans mainly because of higher ferritin concentrations, as well as differences in the intake of dietary factors that affect nonheme iron absorption.

Using the 2 different approaches to estimate total iron absorption yielded similar values (15.5% and 15.1%). Our results, therefore, suggest that the bioavailability of iron in the US diet is ~15% rather than the currently assumed 18%. To validate our results, we considered data arising from 3 studies in which nonheme iron absorption was measured from the typical US diet consumed over a 5 d period (7, 11, 44). From these studies we selected only those subjects with ferritin concentrations of ≤15 μg/L. Total iron absorption for these subjects based on measured nonheme iron absorption plus assumed 25% heme iron absorption was 15.5%. Although the number of subjects meeting this criteria was small (n = 5), the absorption value was similar to our national estimate. In their estimation of iron bioavailability from Western-type diets, Hallberg and Rosander-Hultén (13) obtained a value of 16.6% for the US diet. However, this estimate was only based on data for women. The researchers suggested that an average value for long-term iron bioavailability in Western-type diets may be ~15% in borderline iron-deficient subjects, with a likely range of 14–17%. In a systematic review of iron absorption from whole diets, Collings et al. (46) have suggested that even among individuals with low iron stores, bioavailability may be <15%, particularly if these individuals are consuming a low-bioavailability diet. Their model suggests that individuals with ferritin concentrations of 12 μg/L and 15 μg/L consuming high-bioavailability diets will absorb 13.9% and 11.8% of nonheme iron, respectively. This translates to 15.0% and 13.1% total iron absorption, assuming that 90% of the dietary iron is nonheme and 25% of heme iron is absorbed.

An important issue that deserves revisiting is the whole idea of estimating absorption with the use of information from individuals with no iron stores (ferritin concentration ≤15 μg/L) or adjusting absorption to a ferritin concentration of 15 μg/L. This practice is questionable when we consider that the geometric mean of ferritin for the entire US population is well above this value. By basing estimation of absorption on individuals with no iron stores, we tend to obtain higher estimates for bioavailability than we would when considering the entire population. Furthermore, this approach raises questions about iron absorption in individuals with high iron stores (5). However, the current methodology results in maximum absorption from the US diet and thus is useful in setting lowest ceilings for the different DRIs for iron (13). When we estimated total iron absorption for all subjects without adjusting for serum ferritin by either of the 2 different approaches described earlier, the estimated absorption for the entire population was 5.8%. This value is less useful because it hugely underestimates iron absorption for individuals with low iron stores and at the same time overestimates absorption for individuals with very high iron stores. From a public health point of view, using the maximum absorption value as the national estimate is the better option because it leads to overestimation of absorption only in individuals with adequate iron stores. For example, if we estimate the prevalence of inadequate iron intake in men with the use of the maximum iron absorption threshold, we are likely to underestimate the prevalence of inadequacy. However, it is also the case that we rarely find iron deficiencies in men.

In order to demonstrate that nonheme iron absorption is higher in populations that are vulnerable to iron deficiency, we estimated nonheme iron absorption for subjects who were <19 y of age and women 19–50 y of age. We obtained a value of 6.3% (compared with 2.3% for men ≥19 y of age and women ≥50 y of age) without adjusting ferritin concentration to 15 μg/L.

This study had some limitations. Because phytate intake was not part of the NHANES survey variables, we had to estimate with the use of data from another reliable source (37). Polyphenols constitute one of the main inhibitors of iron absorption, yet consumption of polyphenols is not included in the NHANES. To overcome this problem, we used tea, coffee, and other polyphenol-containing beverage intake expressed in tea, coffee, and other polyphenol-containing beverage intake expressed in black tea equivalents to approximate intake of polyphenols at the individual level. Also, because it is not feasible from a practical point of view to measure iron absorption in a nationally representative sample of individuals, we estimated nonheme iron absorption with the use of an algorithm proposed by Armah et al. (33). The algorithm we implemented is based on a complete diet and includes iron status as part of the model. The prediction model for nonheme iron absorption has been validated with the use of published data with reliable predictive power. The algorithm, however, does not capture the effect of genetic and interpersonal variability in iron absorption. Also, the algorithm was developed with data for adults aged 19–38 y, and thus absorption prediction with this model may be most appropriate for only this age group. In conclusion, the results of this study suggest that the bioavailability of iron in the US diet is ~15%, lower than the 18% assumed at present. This implies that the current DRI for iron may need to be revisited to be adjusted for lower bioavailability.

Acknowledgments

SMA, ALC, and MBR designed the study and wrote the paper; SMA performed the statistical analysis; and MBR had primary responsibility for the final content of the paper. All authors read and approved the final manuscript.
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