Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999–2006¹–⁴

Zuguo Mei, Mary E Cogswell, Anne C Looker, Christine M Pfeiffer, Sarah E Cusick, David A Lacher, and Laurence M Grummer-Strawn

ABSTRACT

Background: Total body iron calculated from serum ferritin and soluble transferrin receptor concentrations allows for the evaluation of the full range of iron status.

Objective: We described the distribution of total body iron and the prevalence of iron deficiency (ID) on the basis of total body iron in US pregnant women.

Design: We examined data from the National Health and Nutrition Examination Survey (NHANES) in 1999–2006 for 1171 pregnant women.

Results: ID prevalence (±SE) in US pregnant women, which was defined as total body iron <0 mg/kg, was 18.0 ± 1.4%. Pregnant women in the first trimester had a higher mean total body iron than did pregnant women in the second or third trimesters. ID prevalence in pregnant women increased significantly with each trimester (6.9 ± 2.2%, 14.3 ± 2.1%, and 29.5 ± 2.7% in the first, second, and third trimesters, respectively). Pregnant women with parity ≥2 had the lowest mean total body iron and the highest prevalence of ID compared with values for pregnant women with parity of 0 or 1. The ID prevalence in non-Hispanic white pregnant women was significantly lower than in Mexican American or non-Hispanic black pregnant women. The mean total body iron and the prevalence of ID did not differ by educational level or by family income.

Conclusions: To our knowledge, these are the first data on total body iron distributions for a representative sample of US pregnant women. Low total body iron is more prevalent in pregnant women in the second or third trimesters, in Mexican American pregnant women, in non-Hispanic black pregnant women, and in women with parity ≥2. Am J Clin Nutr doi: 10.3945/ajcn.110.007195.

INTRODUCTION

Iron deficiency (ID) is the most common known form of nutritional deficiency; preschool children and women of childbearing age are at highest risk (1). During pregnancy, the expansion of blood volume by ∼35% and growth of the fetus, placenta, and other maternal tissues increase the demand for iron from 1.0 to 1.5 mg/d to 5.0 mg/d in the second and third trimesters (2, 3). The Centers for Disease Control and Prevention (CDC), the American College of Obstetricians and Gynecologists, and the Institute of Medicine recommended universal consumption of prenatal supplements with iron to meet pregnancy requirements and prevent anemia (4–6). Recent randomized controlled trials indicated that universal supplementation with iron to meet pregnancy requirements can reduce risk of low birth weight and preterm births (7, 8).

In 2003, Cook et al (9, 10) introduced a method for estimating total body iron on the basis of the ratio of soluble transferrin receptor (sTfR) to serum ferritin. This quantitative estimate, which expresses total body iron on the basis of body weight, allows for an evaluation of the full range of iron status from deficiency to excess within a population and provides information on iron status beyond that of the ferritin model, which is traditionally used to assess the ID prevalence in the US population (11). Positive values of total body iron represent storage iron, and negative values of total body iron indicate tissue ID. The suggested cutoff for defining ID is <0 mg/kg (9, 10). Thus, the measure greatly enhanced the evaluation of iron status and the response to iron interventions in populations in which inflammation was uncommon or had been excluded by laboratory screening. Published data on the use of total body iron to assess the iron status of pregnant women supported its use in this important at-risk group. For example, the pattern of total body iron in pregnant Jamaican and Bolivian women (9) or in US African American women (12) followed expected patterns, which provided indirect evidence that the model may be valid for use in pregnancy.

Information on the iron status of pregnant women in the United States has been limited to monitoring anemia in low-income pregnant women or in nonrepresentative samples (7, 8, 13–18). However, an objective to monitor ID in pregnant women was...

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² The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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included, for the first time to our knowledge, in Healthy People 2010 (19). To address these needs, the National Health and Nutrition Examination Survey (NHANES) oversampled pregnant women during 1999–2006, and in 2003, NHANES started measuring sTfR in addition to serum ferritin. A special project that used surplus sera to measure sTfR and ferritin in pregnant women included in NHANES 1999–2002 was undertaken to enhance the sample of pregnant women for whom total body iron can be assessed. In this article, to our knowledge, we present the first estimates of total body iron, sTfR, and serum ferritin for pregnant women included in NHANES 1999–2006. We also examined the distribution of total body iron by survey, age group, race-ethnicity, trimester, parity, education, and family income. Furthermore, we evaluated prevalences and associations of ID on the basis of total body iron with that on the basis of ferritin or sTfR alone or on the basis of anemia in pregnant women. These data addressed important gaps in our knowledge about the full spectrum of iron status in US pregnant women.

**SUBJECTS AND METHODS**

**Study population and sample selection**

Data from NHANES 1999–2006 represented the total civilian, noninstitutionalized population in the United States for those years. Although NHANES 1999–2006 was conducted in 2-y cycles, for the purpose of most analyses in the current study, we pooled data for all 8 y to obtain an adequate sample size of pregnant women. NHANES used a stratified multistage probability sample that was based on the selection of counties, blocks, households, and finally persons within households. The surveys were conducted by the National Center for Health Statistics, CDC, via household interviews followed by standardized physical examinations in mobile examination centers. Ethical approval was obtained, and written consent was received from all participants. Procedures for data collection and analyses were published elsewhere (20–23).

The reproductive status of subjects was based on a positive urine pregnancy test or self-reported pregnancy. Urine pregnancy tests were administered to all female subjects aged 12–59 y. Pregnant women in the NHANES sample fell into 2 groups (24). The first group consisted of women aged 15–39 y who were eligible to be selected for a supplemental sample of pregnant women on the basis of specific sample domain criteria related to race-ethnicity, age, and a self-report of pregnancy at the time of the household screening interview. The pregnancy status of the first group was confirmed at the physical examination via lab examinations in mobile examination centers. Ethical approval was obtained, and written consent was received from all participants. Procedures for data collection and analyses were published elsewhere (20–23).

The second group of pregnant women consisted of women ages 15–39 y who may have been unaware of their pregnancy status at the screening interview or who may have become pregnant in the time between the household interview and physical examination in the mobile exam center and pregnant women aged <15 or >39 y (eg, women who were not eligible to be selected for the supplemental sample).

We restricted our study sample to pregnant women who attended mobile examination centers to undergo blood collection for biochemical analyses (n = 1224). We excluded pregnant women who were missing serum sTfR or ferritin measurements (n = 53). Our final sample included 1171 pregnant women. Of the 1171 pregnant women in our analytic sample, 919 women were part of the supplemental pregnancy sample identified at the screening interview. Because the supplemental pregnancy sample was selected to be included in the survey by using predesigned algorithms, valid response rates could be provided for them. The 919 women from the supplemental sample in the current study represented 65% of the selected supplemental pregnancy sample, 76% of the interviewed supplemental sample, and 81% of the examined supplemental sample.

**Laboratory analysis and variable definition**

Serum sTfR and ferritin assays for 2003–2006 specimens and the surplus specimens from 1999 to 2002 were conducted at the National Center of Environmental Health, CDC. Methodologic details were described earlier (11). In brief, the Tina-quant sTfR assay (Roche Diagnostics, Mannheim, Germany), which is an automated homogeneous immunoturbidimetric assay, was performed on a Hitachi 912 clinical analyzer (Roche Diagnostics, Indianapolis, IN) (25). Because no reference range was provided for sTfR concentrations in pregnant women, we used the manufacturer-specified range for women of 1.9–4.4 mg/L (25) and defined sTfR concentrations >4.4 mg/L as a threshold for ID. The reference range was determined by calculating the 2.5th and 97.5th percentiles for sTfR in 261 women who fulfilled all criteria for the absence of anemia, an acute-phase response, and ID and were consequently included into the reference population (25).

Serum ferritin was measured by using 2 methods. In 2003, a single-incubation 2-site immunoradiometric assay (BioRad Laboratories, Hercules, CA) was used. This assay was discontinued by the manufacturer in early 2004, so ferritin was measured in 2004–2006 by the Roche Tina-quant ferritin immunoturbidimetric assay on the Hitachi 912 clinical analyzer (Roche Diagnostics) (27). The same Roche method as used in 2004–2006 was used to analyze the surplus specimens from pregnant women in NHANES 1999–2002 (20, 21). Because of method differences between the BioRad and Roche ferritin assays, concentrations obtained for 2003 samples with the BioRad assay had to be statistically adjusted to be comparable with those obtained for 2004 samples with the Roche assay for NHANES 2003–2004. This was accomplished before the data release by the National Center for Health Statistics NCHS by applying 3 piecewise linear-regression equations described in detail elsewhere (26). An abnormal value for ferritin concentrations was defined as <12.0 μg/L (4).

Hemoglobin was measured as part of a complete blood count in the mobile examination centers with the Beckman Coulter MAXIM hematology flow cytometer (Beckman Coulter Inc, Fullerton, CA) (20–23). We defined anemia for pregnant women according to CDC thresholds (4) of a hemoglobin concentration <110 g/L for pregnant women in the first trimester; <105 g/L for pregnant women in the second trimester; <110 g/L for pregnant women in the third trimester; and <110 g/L for pregnant women in an unknown trimester. C-reactive protein (CRP) was measured at the University of Washington by latex-enhanced nephelometry (Dade Behring Inc, Deerfield, IL) (20–23). We defined signs of inflammation as abnormal CRP concentrations >5.0 mg/L (27).

Parity was based on self-report of the number of pregnancies that resulted in a live birth in women aged ≥12 y who reported...
that they had ever been pregnant. Parity was categorized into 0, 1, or \( \geq 2 \) births.

The trimester was based on the number of months pregnant reported by the mother. Only female subjects who reported that they were pregnant at the time of the medical examination were asked about their trimester. The first trimester was defined as \( \leq 3 \) mo pregnant, the second trimester was defined as 4–6 mo pregnant, and the third trimester was defined as \( \geq 7 \) mo pregnant. The trimester value for women who did not know, were not asked, or did not report how long they had been pregnant was categorized as unknown.

Race-ethnicity was based on self-reported data. Participants who reported they were of a Latino ethnicity other than Mexican American and participants who reported they were from more than one race-ethnic group were excluded from comparisons by race-ethnic group because of the small sample size of these groups.

Pregnant women’s educational levels were based on the highest grade or level of education completed (less than high school, high school diploma, and more than high school). Family income was based on the poverty-income ratio, which is the ratio of household income to the family’s appropriate poverty threshold from the US Census Bureau.

**Statistical analysis**

We plotted the serum sTfR and ferritin distributions for pregnant women. We log transformed ferritin \([\ln(\text{ferritin})]\) and sTfR \([\ln(s\text{TfR})]\) to normalize the distributions because ferritin and sTfR concentrations were positively skewed (28, 29). We described the serum ferritin and sTfR distributions [geometric means and 50th (median), 25th, and 75th percentiles]. For the distributions of total body iron and hemoglobin, arithmetic means and percentiles were used because both total body iron and hemoglobin were not skewed distributed, unlike sTfR or ferritin (9, 10). Percentages of abnormal values for total body iron, ferritin, stTfR, and hemoglobin were also calculated.

Total body iron was calculated as previously described in detail (11) from sTfR and ferritin concentrations by using a formula from Cook et al (9, 10) after converting Roche sTfR concentrations to those equivalent to the Flowers assay (30) used in the development of the total body iron model (9, 10).

\[
\text{Total body iron (mg/kg)} = - \left[ \log_{10}(s\text{TfR} \times 1000 \div \text{ferritin}) - 2.8229 \right] \div 0.1207 \quad (1)
\]

To convert the Roche sTfR concentrations to those equivalent to the Flowers assay (30), we applied a conversion equation derived from a previous comparison (24) of the 2 assays (\( n = 40 \)):

\[
\text{Flowers sTfR} = 1.5 \times \text{Roche sTfR} + 0.35 \text{ mg/L} \quad (2)
\]

We used the original Roche ferritin concentrations for the total-body iron calculation because a previous comparison of the Roche assay with the enzyme-linked immunosorbent assay method used to develop the total-body iron model (9, 10) indicated that these 2 methods generated similar values (11).

For total body iron, we presented the mean and ID prevalence (total body iron \(< 0 \text{ mg/kg}\)) in pregnant women by each 2-y survey cycle, age groups (12–19, 20–34, and 35–49 y), trimester (first, second, third, and unknown), parity (0, 1, and \( \geq 2 \)), race-ethnic group (non-Hispanic white, non-Hispanic black, and Mexican American), education (less than high school, high school diploma, and more than high school), and family income poverty income ratio (\(< 130\%\) compared with \( \geq 130\%\)).

We also conducted secondary analyses to explore the possible effect of inflammation on our results because pregnancy can be considered an inflammatory state. In specific, we deleted women with elevated CRP concentrations (\( > 5 \text{ mg/L} \)) and compared the prevalence of ID defined by the total body iron with those obtained in the main analyses. In addition, we evaluated the associations of ID on the basis of total body iron and with that on the basis of ferritin or sTfR alone or on the basis of anemia overall and by trimesters and race-ethnic groups.

We used SUDAAN (version 9.2; Research Triangle Institute, Research Triangle Park, NC) with 8-y sample weights to account for the complex survey design. An 8-y weight variable was created by assigning one-half of the 4-y weight (WTMEC4YR) for 1999–2002 if the person was sampled in 1999–2002, one-quarter of the 2-y weight (WTMEC2YR) for 2003–2004 if the person was sampled in 2003–2004, and one-quarter of the 2-y weight (WTMEC2YR) for 2005–2006 if the person was sampled in 2005–2006. We calculated crude and adjusted point estimates for total body iron. We used multiple linear regression models to calculate the adjusted mean total body iron and predicted marginal from multiple logistic regression models to calculate adjusted ID prevalence. In our analyses, multiple comparisons were made across sociodemographic characteristics in total-body iron indicator. The point of these analyses was not to test joint hypotheses. Instead, we tested a priori expectations about total body iron in relation to each characteristic. Thus, we set statistical significance at \( P < 0.05 \), and no adjustments were made with the recognition that some of the differences in these comparisons may have been due to chance (31).

**RESULTS**

In the total sample of US pregnant women from 1999 to 2006, the mean age was 27.5 y, and the mean parity was 1.95. The proportions of US pregnant women who were Mexican American, non-Hispanic white, and non-Hispanic black, were 15.2%, 56.3%, and 15.3%, respectively. The proportions of US pregnant women who were in their first, second, third, and unknown trimesters at the time of the medical examination were 19.1%, 32.4%, 28.7%, and 19.8%, respectively. Approximately 23.3% of US pregnant women had less than a high school education, 19.6% of US pregnant women had high school diploma, and 57.3% of US pregnant women had more than high school education. For 27.3% of US pregnant women, the family income was below the 130% poverty income ratio.

Means, medians, and selected percentiles and percentages of abnormal values (prevalences of anemia or ID) of total body iron, ferritin, serum sTfR, and hemoglobin for US pregnant women are shown in Table 1. The prevalence of ID from ferritin (25.0%) was significant higher (paired \( t \) test on the difference of probability of ID, \( P < 0.01 \)) than that from sTfR (17.4%) or total body iron (18.0%). However the prevalence of ID from sTfR and from total body iron was not statistical significant (\( P > 0.05 \)). The prevalence of anemia was 5.4% (Table 1).
Unadjusted and adjusted estimates for total body iron (means and prevalence of ID) stratified by selected characteristics are presented in Table 2. Differences in the unadjusted mean total body iron across the 2-y survey periods were not significant. However, after adjusting for age, race-ethnic group, trimester, parity, education and family income, mean total body iron was significantly higher in 2003–2004 and 2005–2006 than in 1999–2000 and 2001–2002. The unadjusted prevalence of ID of pregnant women from 1999 to 2000 was significantly higher than that from 2001 to 2002 and 2003–2004 but did not differ significantly from that from 2005 to 2006. The adjusted prevalence of ID by survey period showed a similar pattern to the unadjusted rates by survey years except that the prevalence for 1999–2000 was significantly different from that for 2005–2006.

Pregnant women aged 35–49 y had a higher mean total body iron than did pregnant women <35 y old. The prevalence of ID in pregnant women aged 12–19 and 20–34 y was >3 times higher (unadjusted prevalence) or >5 times higher (adjusted prevalence) than the rate for pregnant women aged 35–49 y (Table 2). The distribution of total body iron by 3 age groups presented in Figure 1A showed a clear shift in the distribution between 35–49 y pregnant women and the other age groups (12–19 and 20–34 y). However, the distribution of total body iron in pregnant women 35–49 y was much flatter than in the other 2 age groups (Figure 1A).

Pregnant women in the first trimester had the highest mean total body iron compared with that of pregnant women in the second or third trimesters. The prevalence of ID in pregnant women increased with trimester. For example, the prevalence of ID for pregnant women in the second trimester was >2 times higher (both unadjusted and adjusted rates) than the rate for pregnant women in the first trimester; and the prevalence of ID for pregnant women in the third trimester was >2 times higher (both unadjusted and adjusted rates) than the rate for pregnant women in the second trimester (Table 2). Not surprising, the distribution of total body iron among 3 trimesters in pregnant women were well separated (Figure 1B) and indicated a complete shift in the distribution in each trimester rather than only a shift below the cutoff (<0 mg/kg). Pregnant women with parity ≥2 had the lowest mean total body iron and the highest prevalence of ID compared with pregnant women with parity of 0 or 1.

Non-Hispanic white pregnant women had a higher unadjusted mean total body iron than either Mexican American pregnant women or non-Hispanic black pregnant women. However, after the adjustment, the mean total body iron in non-Hispanic white pregnant women differed significantly from that in non-Hispanic black pregnant women but not from that in Mexican American pregnant women. The prevalence of ID in non-Hispanic white pregnant women was significantly lower than the prevalence in Mexican American pregnant women and in non-Hispanic black pregnant women, even after the adjustment (Table 2). The distribution of total body iron by the 3 race-ethnic groups shown in Figure 1C indicated that the distribution shifted to the right for non-Hispanic white pregnant women and to the left for non-Hispanic black pregnant women. The mean total body iron and the prevalence of ID did not differ by educational level or family income (Table 2).

Results of the secondary analyses conducted to assess the effect of inflammation on study results indicated that 43.9% of the sample had elevated CRP concentrations (>5 mg/L). Prevalences of ID after excluding subjects with elevated CRP concentration did not substantially differ from the results on the basis of the full sample shown in Table 2. After excluding subjects with elevated CRP concentration, prevalences of ID by using total body iron were 18.6% overall and 4.7%, 13.5%, and 33.1% for women in the first, second, and third trimesters, respectively.

In pregnant women who were ID as defined by total body iron <0 mg/kg, 93.3% of women were ID as defined by low ferritin concentrations (<12 μg/L) compared with only 10.0% of women among those with total body iron ≥0 mg/kg. In pregnant women who were ID as defined by total body iron <0 mg/kg, 64.0% of women were ID as defined by high sTfR concentrations (>4.4 mg/L), but only 7.2% of women had sTfR concentrations >4.4 mg/L among those with total body iron ≥0 mg/kg. In pregnant women who were ID as defined by total body iron <0 mg/kg, 16.2% of women were anemic as defined by low hemoglobin concentrations compared with 3.1% of women in those with total body iron ≥0 mg/kg.

With the use of low ferritin concentrations (<12 μg/L) to define ID, a similar pattern and prevalence of ID was observed by trimester and by race-ethnic group (Table 3) compared with that on the basis of total body iron (Table 2). A similar pattern and prevalence of ID on the basis of high sTfR concentrations (>4.4 mg/L) by trimester and by race-ethnic group (Table 3) were also observed compared with total body iron (Table 2). The observed higher prevalence of anemia pattern in the third trimester also was similar to that of total body iron (Table 3).
Table 2

Values for total body iron (calculated from serum ferritin and soluble transferrin receptor concentrations) and iron deficiency (total body iron <0 mg/kg) in US pregnant women in the National Health and Nutrition Examination Survey (NHANES), 1999–2006

<table>
<thead>
<tr>
<th>Survey years</th>
<th>n</th>
<th>Unadjusted mean (95% CI)</th>
<th>Adjusted mean (95% CI)</th>
<th>Unadjusted prevalence (95% CI)</th>
<th>Adjusted prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1999–2000</td>
<td>258</td>
<td>3.27 (2.22, 4.33)</td>
<td>3.00 (2.13, 3.87)</td>
<td>24.5 (19.6, 29.4)</td>
<td>26.4 (21.2, 31.7)</td>
</tr>
<tr>
<td>2001–2002</td>
<td>319</td>
<td>3.83 (3.00, 4.66)</td>
<td>2.98 (2.45, 3.51)</td>
<td>14.4 (8.8, 20.0)</td>
<td>16.8 (10.7, 22.9)</td>
</tr>
<tr>
<td>2003–2004</td>
<td>241</td>
<td>4.69 (3.70, 5.67)</td>
<td>4.81 (4.15, 5.48)</td>
<td>12.4 (7.4, 17.4)</td>
<td>11.3 (6.9, 15.7)</td>
</tr>
<tr>
<td>2005–2006</td>
<td>353</td>
<td>3.93 (3.18, 4.69)</td>
<td>4.11 (3.62, 4.59)</td>
<td>18.5 (12.7, 24.4)</td>
<td>18.1 (13.5, 22.6)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
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</tr>
<tr>
<td>12–19 y</td>
<td>161</td>
<td>3.44 (2.70, 4.18)</td>
<td>2.54 (1.53, 3.54)</td>
<td>18.4 (9.6, 27.2)</td>
<td>29.9 (11.4, 48.4)</td>
</tr>
<tr>
<td>20–34 y</td>
<td>903</td>
<td>3.54 (3.05, 4.03)</td>
<td>3.34 (2.95, 3.72)</td>
<td>20.3 (16.8, 23.7)</td>
<td>20.7 (17.4, 24.1)</td>
</tr>
<tr>
<td>35–49 y</td>
<td>107</td>
<td>5.79 (4.35, 7.23)</td>
<td>6.16 (5.13, 7.19)</td>
<td>5.7 (1.5, 10.0)</td>
<td>4.5 (0.8, 8.2)</td>
</tr>
<tr>
<td>Trimester</td>
<td></td>
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</tr>
<tr>
<td>First</td>
<td>189</td>
<td>6.43 (5.60, 7.26)</td>
<td>6.28 (5.70, 6.86)</td>
<td>5.8 (2.4, 9.2)</td>
<td>6.9 (2.6, 11.2)</td>
</tr>
<tr>
<td>Second</td>
<td>416</td>
<td>4.20 (3.35, 5.04)</td>
<td>4.25 (3.57, 4.93)</td>
<td>14.7 (9.2, 20.2)</td>
<td>14.3 (10.2, 18.5)</td>
</tr>
<tr>
<td>Third</td>
<td>384</td>
<td>1.39 (0.79, 1.99)</td>
<td>1.26 (0.77, 1.75)</td>
<td>29.5 (21.9, 37.0)</td>
<td>29.7 (24.4, 34.9)</td>
</tr>
<tr>
<td>Unknown</td>
<td>182</td>
<td>4.49 (3.00, 5.98)</td>
<td>3.89 (2.39, 5.39)</td>
<td>18.0 (7.6, 28.3)</td>
<td>19.6 (7.8, 31.4)</td>
</tr>
<tr>
<td>Parity</td>
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<tr>
<td>0</td>
<td>369</td>
<td>4.45 (3.44, 5.47)</td>
<td>4.45 (3.68, 5.21)</td>
<td>12.2 (5.8, 18.5)</td>
<td>12.2 (5.6, 18.8)</td>
</tr>
<tr>
<td>1</td>
<td>370</td>
<td>3.63 (2.95, 4.32)</td>
<td>3.66 (3.13, 4.19)</td>
<td>16.2 (9.8, 22.6)</td>
<td>16.6 (10.4, 22.8)</td>
</tr>
<tr>
<td>≥2</td>
<td>348</td>
<td>2.94 (2.32, 3.56)</td>
<td>2.87 (2.33, 3.41)</td>
<td>27.7 (20.3, 35.1)</td>
<td>28.4 (21.0, 35.9)</td>
</tr>
<tr>
<td>Race-ethnic group</td>
<td></td>
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<tr>
<td>Mexican American</td>
<td>347</td>
<td>3.14 (2.59, 3.69)</td>
<td>3.28 (2.65, 3.92)</td>
<td>24.1 (19.7, 28.4)</td>
<td>23.6 (17.8, 29.4)</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>517</td>
<td>4.15 (3.59, 5.09)</td>
<td>3.87 (3.39, 4.36)</td>
<td>12.1 (8.7, 15.6)</td>
<td>13.9 (10.3, 17.5)</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>182</td>
<td>2.51 (1.49, 3.54)</td>
<td>2.61 (1.75, 3.48)</td>
<td>31.0 (21.7, 40.2)</td>
<td>29.6 (21.1, 38.1)</td>
</tr>
<tr>
<td>Other</td>
<td>125</td>
<td>3.70 (2.51, 4.88)</td>
<td>4.50 (3.50, 5.49)</td>
<td>21.1 (8.5, 33.8)</td>
<td>18.8 (9.3, 28.4)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Less than high school</td>
<td>371</td>
<td>4.02 (3.06, 4.98)</td>
<td>4.27 (3.34, 5.20)</td>
<td>17.5 (12.3, 22.8)</td>
<td>13.6 (7.8, 19.4)</td>
</tr>
<tr>
<td>High school diploma</td>
<td>259</td>
<td>3.27 (2.40, 4.14)</td>
<td>3.18 (2.32, 4.05)</td>
<td>23.3 (15.3, 31.2)</td>
<td>21.0 (12.0, 30.1)</td>
</tr>
<tr>
<td>More than high school</td>
<td>540</td>
<td>3.99 (3.27, 4.72)</td>
<td>3.60 (3.01, 4.19)</td>
<td>16.4 (11.5, 21.3)</td>
<td>20.3 (13.6, 27.0)</td>
</tr>
<tr>
<td>Family income</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;130% of poverty-income ratio</td>
<td>401</td>
<td>3.48 (2.74, 4.22)</td>
<td>3.67 (2.88, 4.47)</td>
<td>20.1 (14.1, 26.0)</td>
<td>18.1 (10.8, 25.4)</td>
</tr>
<tr>
<td>≥130% of poverty-income ratio</td>
<td>699</td>
<td>3.94 (3.32, 4.57)</td>
<td>3.67 (2.37, 4.08)</td>
<td>17.4 (12.2, 21.1)</td>
<td>19.0 (15.4, 22.5)</td>
</tr>
</tbody>
</table>

Within a group, values with different superscript letters were significantly different (P < 0.05, 2-tailed t test). n values were unweighted; all other analyses were weighted. 95% CIs took into account the design effect. Analyses for adjusted means or prevalences were restricted to subjects with no missing information for all variables listed in the table (n = 1057). Adjusted means or prevalences took into account all sociodemographic characteristic variables listed in the table. For example, the adjusted mean total body iron or prevalence of iron deficiency for each age group was adjusted for the survey, race-ethnic group, trimester, parity, education, and family income.

The relation between the prevalence of anemia and total body iron is shown in Figure 2. The prevalence of anemia was ~32% in pregnant women with less than ~4 mg total body iron/kg, which decreased exponentially (logistic regression model for trend test, P < 0.01) in pregnant women with greater concentrations of total-body iron stores, and flattened to <1% in pregnant women with total body iron >8 mg/kg.

**DISCUSSION**

The public health importance of monitoring ID in pregnant women is reflected by the inclusion of an objective to monitor the iron status of this at-risk group in Healthy People 2010 (19). In the current article, to our knowledge, we present the first nationally representative data on the estimated average total body iron and prevalence of ID on the basis of total body iron for pregnant women in the US, which is to reflect that some of this data had already been reported to HP 2010 for monitoring purposes. The monitoring of the iron status of pregnant women in previous versions of Healthy People was restricted to anemia, which is the last stage of ID. It is important to identify and monitor ID during pregnancy because anemia is caused by factors other than ID; anemia with but not without ID has been associated with low birth weights, preterm deliveries, and perinatal mortality (32), and in women without anemia in early pregnancy, randomized controlled trials suggested iron supplements were needed to prevent third trimester anemia and low birth weights and/or preterm births (7, 8).

We observed that the overall prevalence of ID for US pregnant women defined by total body iron <0 mg/kg was 18.0 ± 1.4%, and ~30% of women in the third trimester were iron deficient. The CDC recommended universal supplementation to meet the iron requirements of pregnancy (4). The iron intake in pregnant women during NHANES III was observed to be less than the estimated average requirement of 22 mg Fe/d, which led the Institute of Medicine committee on Dietary Reference Intakes to note the need for iron supplementation during pregnancy (6).

The prevalence of total body iron <0 mg/kg in pregnant women in the current study was double the prevalence of total body iron <0 mg/kg that we previously reported in nonpregnant women and 1.3–4.9 times higher than the prevalence observed in children age 1–5 y (11). Prevalences of ID in pregnant women observed in
FIGURE 1. Distributions of total body iron (calculated from serum ferritin and soluble transferrin receptor concentrations) in US pregnant women by age group (A), trimester (B), and race-ethnic group (C) from the National Health and Nutrition Examination Survey (NHANES), 1999–2006.
previous smaller studies conducted in the United States were not directly comparable with our results because the previous studies used other approaches to define an impaired iron status, such as low hemoglobin or low ferritin amounts (7, 8, 13–18).

We also showed that low total body iron was more prevalent in women in the second or third trimester, Mexican American pregnant women, non-Hispanic black pregnant women, and women with parity ≥2. However, neither the mean total body iron nor the prevalence of ID differed significantly by educational level or family income. However, some of our results cannot be interpreted by sociodemographic characteristics alone without detailed information on iron intakes from diets or supplements. Data from NHANES 1988–1994 indicated that, in pregnant participants, the use of supplements that contained iron did not include pregnant women (9). However, our secondary analyses showed that the prevalence of anemia decreased exponentially (Figure 2) in pregnant women with greater concentrations of total-body iron stores. This suggested, as in analyses in nonpregnant women (11), that total body iron measured in pregnant women might be used to determine the severity of ID. Although not all anemia is due to ID, the observed higher prevalence of anemia pattern in the third trimester was also similar to that of total body iron. These additional results may provide indirect support for the validity of total body iron in pregnant women. Second, sTfR concentrations from NHANES had to be adjusted to be comparable with values produced by the Flowers sTfR assay used in the development of the total-body iron store model (4, 28), and an adjustment factor had to be

![FIGURE 2. Prevalence (95% CI) of anemia indicated by total body iron for US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999–2006. Anemia was defined as hemoglobin concentrations <110, <105, <110, and <110 g/L in pregnant women in the first, second, third, and unknown trimesters, respectively.](image)

TABLE 3
Unadjusted prevalence of iron deficiency (ID) on the basis of low serum ferritin or high serum soluble transferrin receptor (sTfR) concentrations and the unadjusted prevalence of anemia on the basis of low hemoglobin concentrations in US pregnant women in the National Health and Nutrition Examination Survey (NHANES), 1999–2006.

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Prevalence of ID on the basis of low serum ferritin concentrations</th>
<th>Prevalence of ID on the basis of high sTfR concentrations</th>
<th>Prevalence of anemia on the basis of low hemoglobin concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>189</td>
<td>7.3 (3.7, 10.9)a</td>
<td>5.8 (2.4, 9.2)a</td>
</tr>
<tr>
<td>Second</td>
<td>416</td>
<td>23.7 (15.3, 32.1)b</td>
<td>10.6 (6.3, 14.9)a</td>
</tr>
<tr>
<td>Third</td>
<td>384</td>
<td>39.2 (29.3, 49.2)c</td>
<td>27.9 (20.6, 35.3)b</td>
</tr>
<tr>
<td>Unknown</td>
<td>182</td>
<td>22.8 (11.6, 34.1)c</td>
<td>23.3 (12.4, 34.3)c</td>
</tr>
<tr>
<td>Race-ethnic group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>347</td>
<td>32.2 (26.1, 38.2)a</td>
<td>14.8 (9.3, 20.3)a</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>517</td>
<td>19.6 (15.2, 24.1)b</td>
<td>11.9 (7.4, 16.4)a</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>182</td>
<td>39.3 (27.8, 50.8)c</td>
<td>31.2 (21.8, 40.6)c</td>
</tr>
<tr>
<td>Other</td>
<td>125</td>
<td>23.1 (12.8, 33.3)c</td>
<td>28.0 (14.3, 41.7)c</td>
</tr>
</tbody>
</table>

*1 n values were unweighted; all other analyses were weighted. 95% CIs took into account the design effect. Within a group, values with different superscript letters were significantly different (P < 0.05, 2-tailed t test). Abnormal values for ferritin and sTfR concentrations were <12.0 μg/L and >4.4 mg/L, respectively. Abnormal values for hemoglobin concentrations were <110, <105, <110, and <110 g/L in pregnant women in the first, second, third, and unknown trimesters, respectively.

*2 Percentage; 95% CI in parentheses (all such values).
applied to make ferritin concentrations in 2003 comparable with those in surrounding survey years (19). Third, regression equations used to adjust sTfR and ferritin concentrations were based on the assumption that the assays remain stable over time. Fourth, a nonresponse bias may have been present in the estimates presented in the current study. A nonresponse bias because of a refusal to participate in the public health examinations in NHANES was reduced by a nonresponse adjustment factor included in the calculation of the sample weights for use with examinee data. Approximately 19% of the pregnant women in the analytic sample who were part of the supplemental sample that came to the examination centers lacked usable ferritin and sTfR data, and this nonresponse was not addressed by the sample-weight adjustments. However, previous studies have not shown that reweighting existing sample weights to account for this additional nonresponse had much effect on estimates (36, 37). Fifth, our results did not take inflammation and infection into account, even though pregnancy was considered an inflammatory state. However, results from secondary analyses suggested that the potential effect of inflammation on our results was small. Specifically, 43.9% of our sample had elevated CRP concentrations (>5 mg/L), but the prevalence of ID after their exclusion was not substantially different from the results on the basis of the entire analytic sample. Finally, the lack of a standard sTfR assay method and a standard reference material will limit the use of total body iron in other studies.

In conclusion, to our knowledge, we present the first data on total-body iron distributions from a representative sample of US pregnant women. These data fill an important need for monitoring the iron status of pregnant women, which has been identified as a public health objective in Healthy People 2010. We showed that ID defined by total body iron <0 mg/kg in pregnant women was more prevalent than we previously reported for nonpregnant women and for children aged 1–5 y (11).

The authors’ responsibilities were as follows—ZM: had full access to all data in this study and was responsible for the integrity of data and accuracy of the data analysis; MEC, ACL, CMP, SEC, DAL, and LGMS: study concept and design, analysis and interpretation of data, and critical revision of the manuscript; and ZM and MEC: drafting of the manuscript and statistical analysis. None of the authors had a financial or personal interest in any company or organization connected with the research represented in the article.

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