Plasma Ferritin and Soluble Transferrin Receptor Concentrations and Body Iron Stores Identify Similar Risk Factors for Iron Deficiency but Result in Different Estimates of the National Prevalence of Iron Deficiency and Iron-Deficiency Anemia among Women and Children in Cameroon

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Abstract

Available iron status indicators reflect different aspects of metabolism. We compared the prevalence and distribution of iron deficiency (ID) and iron-deficiency anemia (IDA) among Cameroonian women and children, as measured by plasma ferritin, and soluble transferrin receptor concentrations, body iron stores (BIS), and hemoglobin, and evaluated the impact of adjustments for inflammation on these measures. In a nationally representative survey, we randomly selected 30 clusters in each of 3 zones (north, south, and large cities) and 10 households/cluster, each with a child aged 12–59 mo and a woman 15–49 y. Ferritin and BIS were mathematically adjusted for inflammation, using plasma C-reactive protein and α1-acid glycoprotein both as continuous and categorical variables. Inflammation was present in 48.0% of children and 20.8% of women and anemia was diagnosed in 57.6% of children and 38.8% of women. Depending on the iron status indicator applied, the prevalence of ID ranged from 14.2 to 68.4% among children and 11.5 to 31.8% among women, and the prevalence of IDA ranged from 12.0 to 47.4% among children and 9.0 to 19.4% among women; the proportion of anemia associated with ID ranged from 20.8 to 82.3% among children and 23.2 to 50.0% among women. The different iron indicators generally identified similar groups at greatest risk of deficiency, using both conventional and derived cutoffs: younger children, pregnant women, and women and children in the north and rural areas. Research is needed to clarify the relationships between iron status indicators, particularly in the presence of inflammation, to harmonize global data on prevalence of ID.

Introduction

Iron deficiency (ID) and its more severe form, iron-deficiency anemia (IDA), are public health problems worldwide (1,2) and can lead to impaired cognitive development in children and adverse pregnancy outcomes (3–5). Valid indicators of iron status are required for assessing the prevalence of ID and IDA in populations, identifying high-risk population subgroups, and determining the need for and response to interventions to improve iron status.

Many indicators of iron status are available and each reflects a slightly different aspect of iron metabolism (4). For example, plasma concentrations of ferritin, an iron storage protein, decrease with falling iron stores; however, ferritin concentrations are insensitive to further change during severe ID or negative iron balance (6). In contrast, soluble transferrin receptor (sTfR) concentrations generally begin to change only after iron stores (in the form of ferritin) are depleted (6,7). Body iron stores (BIS) can be estimated using the ratio of sTfR: ferritin (7) and this indicator is thought to reflect iron status over a wider range of iron stores (i.e., from decreased storage iron to functional tissue ID). Because these markers reflect different metabolic processes, they cannot necessarily be directly compared (8), which complicates the comparison of
iron status results across populations and over time when different iron status indicators are used.

Iron status assessment is also affected by the presence of inflammation or infection. The WHO currently recommends plasma ferritin for iron status assessment of populations (2,9). However, plasma ferritin is a positive acute-phase protein that is secreted by the liver during inflammation, independent of iron status (4,10). Thus, measuring iron status by using ferritin in populations with a high prevalence of inflammation provides an underestimation of the “true” prevalence of ID. When calculated using ferritin, BIS is similarly affected by the presence of inflammation.

Several methods have been proposed to help interpret iron status as measured by ferritin (and thus BIS) during inflammation (8). Excluding individuals with inflammation is an unsatisfactory strategy, because it biases the sample and can substantially reduce the sample size in populations with a high prevalence of inflammation. Another method is to increase the ferritin cutoff indicative of deficiency in settings where inflammation or infection is common. The WHO has followed this approach, recommending a ferritin cutoff of <30 μg/L rather than <12 μg/L for children <5 y old in areas where infection is prevalent (2). A third technique is to mathematically adjust individual observations for the presence of inflammation or infection, as measured by acute-phase proteins, such as C-reactive protein (CRP) and/or α1-acid glycoprotein (AGP). One such approach suggested by Thurnham et al. (11) statistically adjusts the ferritin results, depending on whether one or both of the acute-phase proteins are elevated.

Unlike ferritin, sTfR is considered not to be affected by the acute-phase response (10,12). However, in addition to reflecting erythroblast transferrin receptor expression (e.g., increased transferrin receptor expression is indicative of functional tissue ID), sTfR in plasma is also a general marker of erythropoiesis (13,14). Therefore, assessment of iron status using sTfR may be confounded by factors other than iron that affect erythropoiesis, such as age (15), pregnancy (16–18), malaria infection (19–24), and other hemolytic diseases (25).

Given the different roles of each of these indicators as well as the potential confounding effects of inflammation and infection, consensus is needed on the best indicators of iron status and cutoffs to indicate deficiency, particularly in areas where inflammation is prevalent. The WHO has recognized this need and recently initiated a process to update the recommended indicators for the assessment of population iron status (26).

In the 2009 Cameroon national survey, we found that the prevalence of ID in children 12–59 mo of age ranged from 14 to 68%, depending on the indicator and adjustment for inflammation applied (27). This broad range of estimated prevalence complicates policy decisions, which are generally related to specific levels of disease prevalence. Therefore, to better understand this issue, we conducted a more in-depth analysis of the iron indicators used in this survey. In particular, we compared the prevalence of ID and IDA, as determined by the available indicators, with and without adjustment for inflammation both for the entire nationally representative sample and separately for selected risk groups for ID and IDA. We also assessed the effect of redefining deficiency cutoffs on the risk factors that were identified for ID.

Materials and Methods

Study design. This was a nationally representative, cluster survey of women of reproductive age (15–49 y) and young children (12–59 mo) in the Republic of Cameroon. The study design and data collection methods were reported in greater detail elsewhere (27,28). Dietary intake data, anthropometric measurements, and biochemical indicators of nutritional status were collected from individual women and children, and socio-economic and demographic data were collected at the household (HH) level.

HH selection and eligibility. The study employed a multi-stage, cluster sampling design with 3 strata based on ecological zone: the north, consisting of the 3 northernmost provinces representing the arid Sahel (North, Extreme North, and Adamawa); the south, consisting of the 7 remaining provinces with the exception of the 2 largest cities, representing the humid tropics; and Douala and Yaoundé, the 2 largest urban areas. Thirty clusters (villages, or neighborhoods within cities) were selected from each zone according to the probability-proportional-to-size method using 2005 census data from the Cameroon Central Office of Census and Population Studies. Approximately 10 HHs per cluster were sampled using a random start point and systematic selection of adjacent HHs. HHs were considered eligible if at least one child 12–59 mo and a woman 15–49 y who was the child’s primary caregiver resided in the HH. In most cases, the caregiver was the child’s mother. HHs were not eligible if either the woman or child had lived in the HH for <1 mo or if the woman or child reported fever, diarrhea with dehydration, or other severe illness in the 72 h prior to data collection.

Informed consent was obtained from the index woman, with permission from the head of HH. The study was approved by the Cameroon National Ethics Committee and the Institutional Review Board of the University of California, Davis.

Socioeconomic, demographic, and dietary data collection. Demographic and socio-economic status (SES) data were collected using interviewer-administered questionnaires that included the number of HH members in various age groups, primary HH language, occupation, employment status and educational level of the head of HH and the index woman, and HH possessions, including livestock, sources of energy for cooking and lighting, waste disposal facilities, and source of water. Child age was determined by maternal report or, when available, from vaccination cards or other documents (<5% of children). Pregnancy status was determined by self-report.

Anthropometry. The weight of each index child and caregiver was measured to the nearest 0.1 kg by a battery-powered electronic scale (Seca 899; Seca Weighing and Measuring Systems), which was tested daily with certified calibration weights (4). For children <2 y, length was measured in duplicate to the nearest 0.1 cm using a portable length board (Seca 416; Seca Weighing and Measuring Systems). The standing height of caregivers and of children ≥2 y was measured in duplicate to the nearest 0.1 cm using a portable stadiometer (Seca Leicester Portable Height Measure; Seca Weighing and Measuring Systems). If the 2 measurements were >0.5 cm apart, a third measurement was taken and the 2 closest measurements were recorded. Results represent the mean of the 2 measurements. Anthropometric Z-scores were calculated according to the 2006 WHO standard (29).

Blood collection and processing. Trained phlebotomists collected 5–7 mL of blood by antecubital or metacarpal venipuncture into tubes containing lithium heparin as an anticoagulant (Sarstedt). Hemoglobin was immediately measured in whole blood using a portable photometer (Hb201+, Hemocue). Blood samples were then centrifuged for 10 min at 2500 × g to separate plasma in the field (Hermle Z206A, Hermle LaborTechnik). Plasma was frozen on the day of collection and stored at ≤−20°C until analysis.

Laboratory analysis. Five plasma proteins [ferritin, sTfR, retinol-binding protein (RBP), CRP, and AGP] were analyzed by a combined sandwich ELISA method (30). Ferritin and sTfR were measured as indicators of iron status and to calculate BIS (7). RBP was measured as an indicator of vitamin A status (31). CRP and AGP were measured to serve as indicators of inflammation to assist in the interpretation of the other biomarkers of nutrient status. The inter-assay CVs were 3.1% for ferritin, 2.8% for sTfR, 6.5% for CRP, 3.5% for AGP, and 2.7% for RBP.
Data analysis. Data were analyzed with SAS 9.3 using the SAS survey procedures to account for the sampling design. Descriptive statistics were calculated for all variables. Continuous variables were examined for adherence to a normal distribution using the Shapiro Wilkes ‘W’ and visual examination of histograms. Variables that did not adhere to a normal distribution were arithmetically transformed prior to further analysis.

BIS were calculated using the ratio of sTfR and ferritin concentrations according to a formula derived by Cook et al. (7). Plasma concentrations of ferritin were adjusted for the presence of inflammation using 2 methods. The first method was previously published by Thurnham et al. (11) and involves first stratifying individuals into categories based on elevated CRP and/or AGP: apparently healthy reference group (CRP $\leq 5$ mg/L and AGP $\leq 1$ g/L), incubation (CRP $> 5$ mg/L and AGP $= 1$ g/L), early convalescence (CRP $> 5$ mg/L and AGP $> 1$ g/L), and late convalescence (CRP $\leq 5$ mg/L and AGP $> 1$ g/L). Individual values were then adjusted by multiplying by the ratio of the median of the apparently healthy group to the median of that individual’s inflammation group.

We also examined whether adjusting the results using CRP and AGP as continuous variables, rather than categorical variables, would improve the adjustment for inflammation. In this method, individuals were divided into the same categories as for the Thurnham method based on elevated CRP, AGP, neither, or both. We then developed 3 separate linear regression equations with ferritin as the dependent variable (after transformation to achieve a normal distribution); the independent variables in the 3 equations were CRP only, AGP only, or both CRP and AGP. We confirmed the linearity of the relationships between CRP and AGP and each iron indicator using visual examination of scatter plots and evaluation of higher order terms and interactions in the linear regression models.

Using the regression coefficients from these models, we adjusted individual ferritin values based on whether that person’s CRP and/or AGP values were elevated. Thus, for individuals with only CRP elevated, we used coefficients from the model containing CRP only. If only AGP was elevated, we used coefficients for the model containing AGP only. If both CRP and AGP were elevated, the coefficients from the equation containing both proteins were used. Ferritin values for individuals without elevated CRP and/or AGP were not adjusted.

The reference CRP and AGP concentrations used for the adjustment were CRP $= 3.75$ mg/L and AGP $= 0.75$ g/L. As an example, the formula for adjustment of results for an individual with both CRP $\geq 5$ mg/L and AGP $\geq 1$ g/L would be: adjusted ferritin = unadjusted ferritin $- (\text{regression coefficient for CRP}) \times (\text{CRP} - 3.75) - (\text{regression coefficient for AGP}) \times (\text{AGP} - 0.75)$.

Although sTfR concentrations were significantly correlated with CRP and AGP among both women and children, we chose not to adjust sTfR concentrations for the presence of elevated CRP and/or AGP, because acute-phase proteins are nonspecific indicators of conditions, such as malaria, that could cause elevated sTfR concentrations in the absence of tissue ID. Moreover, despite the significance of the relationship between the acute-phase proteins and sTfR, the magnitude of the effect of inflammation on sTfR was small and the presence of inflammation had minimal impact on the estimated prevalence of ID using this indicator.

The cutoffs used to define deficiency for each indicator were as follows: unadjusted and adjusted ferritin, $< 12$ µg/L for children and $< 15$ µg/L for women; unadjusted ferritin, $< 30$ µg/L for children [WHO recommendation (1,9)]; unadjusted and adjusted BIS, $< 0$ mg/kg for children and women; sTfR, $> 8.3$ mg/L for children and women; and hemoglobin, $< 110$ g/L for children and pregnant women and $< 120$ g/L for nonpregnant women.

To estimate results at the national level, weighting factors were applied to adjust for the different population sizes of each study region (zone). Weighting factors were also applied at the cluster level to adjust for slightly different sample sizes in each cluster (e.g., 9 or 11 instead of 10). Weights were calculated as the inverse probability of selection.

Continuous variables (i.e., concentrations of each iron status indicator) were compared using Spearman correlations and, after appropriate transformations, survey regression procedures (SAS PROC SURVEYREG). Comparisons of continuous variables among groups were also conducted using PROC SURVEYREG. Comparisons of the prevalence of ID and IDA among groups were conducted using survey logistic regression procedures (SAS PROC SURVEYLOGISTIC).

Independent risk factors related to iron status were identified using multivariate linear regression analysis (SAS PROC SURVEYREG). Separate models were developed with adjusted and unadjusted ferritin, BIS, and sTfR as outcome (dependent) variables. Potential predictors (independent variables) included study region; urban or rural location; HH SES (a composite score derived from SES-related variables using factor analysis); caregiver education; presence of inflammation (elevated CRP and/or AGP); age; anthropometric Z-scores and sex (among children); physiological status (i.e., pregnant, lactating, or nonpregnant, nonlactating, among women); other micronutrient deficiencies; and relevant interactions. A backward stepwise procedure was used to eliminate variables that were not significantly related to the iron status indicator ($P \geq 0.05$).

Derivation of new ID cutoffs. We conducted an exercise to assess whether the different indicators show the same patterns/distribution in the population but result in a different estimate of the prevalence of ID and IDA. We chose inflammation-adjusted BIS (using the continuous adjustment for CRP and AGP) as the reference indicator for this analysis. Although we recognize that arguments could be made for or against the use of each of the iron indicators based on physiology, response to intervention (32), etc., we chose this indicator because it takes into account the maximum amount of information available (ferritin, sTfR, CRP, and AGP concentrations) and seems to be a reasonable “tipping point” for justifying intervention.

We then developed cutoffs for unadjusted ferritin (i.e., similar to the WHO approach), inflammation-adjusted ferritin, and sTfR that would achieve the same national-level prevalence of ID as the reference indicator, BIS $< 0$ mg/kg (i.e., $32.0–32.3\%$ for children and $12.6–12.7\%$ for women; separate cutoffs for women and children). If iron indicator concentrations are related to specific subject characteristics, then it is possible that different individuals would be targeted if the cutoff changes. Thus, we also reassessed whether the indicators identified the same risk groups for deficiency after adjustments of the cutoffs to yield the same national prevalence of ID.

Results

Sample population characteristics. The sample population has been described in detail elsewhere (27,28). A total of 1002 HHs participated in the study; SES data were collected for 901 HHs, and 879 women and 847 children provided blood samples. In general, the prevalence of anemia and micronutrient deficiencies was high in this population, particularly among children and among HHs in the northern region (Tables 1 and 2) (27,28).

Correlations between iron status indicators. Among children, all of the iron status indicators were highly correlated ($P < 0.001$). Additionally, all iron indicators were correlated with hemoglobin (except unadjusted ferritin, $P = 0.89$) and with CRP (except adjusted BIS, $P = 0.12$ for Thurnham adjustment and $P = 0.57$ for continuous adjustment) and AGP (except adjusted ferritin, $P = 0.07$ for Thurnham adjustment and $P = 0.14$ for continuous adjustment).

Among children, the relationship between unadjusted ferritin and sTfR varied based on the presence of elevated acute-phase proteins (Spearman’s $r = -0.47$, $P < 0.001$ among children without inflammation and $r = -0.05$, $P = 0.32$ among children with inflammation). However, adjusted ferritin values were correlated with sTfR regardless of children’s inflammation status ($r = -0.12$, $P = 0.015$ for Thurnham adjustment and $r = -0.13$, $P = 0.05$ for adjusted BIS).
P = 0.013 for continuous adjustment, among children with infection). Both adjusted and unadjusted BIS were correlated with both sTfR and ferritin regardless of whether inflammation was present.

Among women, all of the iron status indicators were correlated with each other and with hemoglobin (P < 0.001). Each iron status indicator was also correlated with CRP (except adjusted ferritin, Thurnham method only, P = 0.12, and adjusted

### TABLE 1 Characteristics of participating Cameroonian children and women, nationally and by study region

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>National</th>
<th>n</th>
<th>South</th>
<th>n</th>
<th>North</th>
<th>n</th>
<th>Large cities</th>
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<tbody>
<tr>
<td>Children</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age, mo</td>
<td>928</td>
<td>30.2 (29.3, 31.1)</td>
<td>328</td>
<td>31.0 (26.5, 32.4)</td>
<td>304</td>
<td>29.3 (27.8, 30.7)</td>
<td>296</td>
<td>30.0 (28.1, 31.8)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>898</td>
<td>452 (50.2)</td>
<td>308</td>
<td>150 (48.5)</td>
<td>302</td>
<td>158 (51.6)</td>
<td>288</td>
<td>143 (49.4)</td>
</tr>
<tr>
<td>Height-for-age Z-score</td>
<td>826</td>
<td>−1.35 (−1.52, −1.18)</td>
<td>280</td>
<td>−1.35 (−1.67, −1.02)</td>
<td>291</td>
<td>−1.79 (−2.03, −1.56)</td>
<td>255</td>
<td>−0.68 (−0.83, −0.53)</td>
</tr>
<tr>
<td>Weight-for-height Z-score</td>
<td>826</td>
<td>0.13 (0.04, 0.22)</td>
<td>280</td>
<td>0.40 (0.25, 0.55)</td>
<td>291</td>
<td>−0.41 (−0.60, −0.21)</td>
<td>255</td>
<td>0.36 (0.26, 0.46)</td>
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<tr>
<td>CRP, mg/L</td>
<td>838</td>
<td>5.33 (4.76, 5.91)</td>
<td>293</td>
<td>5.50 (4.50, 6.49)</td>
<td>291</td>
<td>5.84 (4.78, 6.91)</td>
<td>254</td>
<td>4.20 (3.55, 4.85)</td>
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<tr>
<td>AGP, mg/L</td>
<td>838</td>
<td>0.96 (0.94, 0.98)</td>
<td>293</td>
<td>0.98 (0.94, 1.02)</td>
<td>291</td>
<td>0.97 (0.93, 1.02)</td>
<td>254</td>
<td>0.90 (0.87, 0.93)</td>
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<tr>
<td>Hemoglobin, g/L</td>
<td>859</td>
<td>106 (104, 107)</td>
<td>294</td>
<td>109 (106, 112)</td>
<td>288</td>
<td>98 (95, 101)</td>
<td>276</td>
<td>110 (108, 112)</td>
</tr>
<tr>
<td>Anemic, %</td>
<td>859</td>
<td>57.6 (53.1, 62.0)</td>
<td>294</td>
<td>49.2 (40.9, 57.5)</td>
<td>288</td>
<td>76.5 (70.1, 82.9)</td>
<td>276</td>
<td>46.8 (40.1, 53.5)</td>
</tr>
</tbody>
</table>

### TABLE 2 Concentrations of iron status indicators among Cameroonian children 12–59 mo of age and women of reproductive age, nationally and by region

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>National</th>
<th>n</th>
<th>South</th>
<th>n</th>
<th>North</th>
<th>n</th>
<th>Large cities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>945</td>
<td>27.1 (26.5, 27.7)</td>
<td>334</td>
<td>27.8 (26.8, 28.9)</td>
<td>307</td>
<td>26.1 (25.0, 27.1)</td>
<td>304</td>
<td>27.0 (26.3, 27.7)</td>
</tr>
<tr>
<td>Physiological status, n (%)</td>
<td>948</td>
<td>353</td>
<td>302</td>
<td>293</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>96</td>
<td>10.0</td>
<td>29</td>
<td>8.2</td>
<td>41</td>
<td>13.6</td>
<td>26</td>
<td>8.9</td>
</tr>
<tr>
<td>Lactating</td>
<td>226</td>
<td>25.0</td>
<td>100</td>
<td>28.3</td>
<td>70</td>
<td>23.2</td>
<td>56</td>
<td>19.2</td>
</tr>
<tr>
<td>Nonpregnant, nonlactating</td>
<td>626</td>
<td>65.0</td>
<td>224</td>
<td>63.5</td>
<td>191</td>
<td>63.2</td>
<td>211</td>
<td>71.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>836</td>
<td>23.8 (23.4, 24.2)</td>
<td>313</td>
<td>24.6 (24.0, 25.3)</td>
<td>278</td>
<td>24.0 (23.0, 25.1)</td>
<td>246</td>
<td>24.5 (23.8, 25.3)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>872</td>
<td>2.95 (2.61, 3.28)</td>
<td>302</td>
<td>2.69 (2.12, 3.27)</td>
<td>297</td>
<td>3.44 (2.95, 4.04)</td>
<td>273</td>
<td>2.71 (2.26, 3.16)</td>
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<tr>
<td>AGP, mg/L</td>
<td>872</td>
<td>0.75 (0.74, 0.77)</td>
<td>302</td>
<td>0.76 (0.73, 0.78)</td>
<td>297</td>
<td>0.76 (0.73, 0.79)</td>
<td>273</td>
<td>0.73 (0.71, 0.75)</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>888</td>
<td>122 (120, 124)</td>
<td>304</td>
<td>126 (123, 129)</td>
<td>299</td>
<td>119 (116, 122)</td>
<td>285</td>
<td>119 (117, 121)</td>
</tr>
<tr>
<td>Anemic, %</td>
<td>888</td>
<td>38.8 (34.4, 42.3)</td>
<td>304</td>
<td>32.4 (25.0, 39.5)</td>
<td>298</td>
<td>42.7 (34.6, 50.6)</td>
<td>285</td>
<td>46.7 (40.7, 52.7)</td>
</tr>
</tbody>
</table>

### Notes

1. Values are mean (95% CI), n (percent), or percent (95% CI). Values in a row with superscripts without a common letter differ, P < 0.05, using linear or logistic regression, as appropriate (SAS PROC SURVEYPEOUG or SURVEYLOGISTIC).

2. Hemoglobin <110 g/L for children and pregnant women; hemoglobin <120 g/L for nonpregnant women.

3. BMI results exclude pregnant women.

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BIS, $P = 0.96$ for Thurnham adjustment and $P = 0.14$ for continuous adjustment) and AGP (except adjusted BIS, $P = 0.94$ for Thurnham adjustment and $P = 0.27$ for continuous adjustment). Iron status indicators were correlated ($P < 0.001$) with or without the presence of elevated CRP and/or AGP.

Vitamin A status, as measured by inflammation-adjusted plasma RBP, did not alter the relationships between iron status indicators and hemoglobin among children or women.

**Prevalence of ID and IDA nationally.** The prevalence of ID and IDA and the proportion of anemia associated with ID varied widely by indicator and adjustment for inflammation (Table 3). The prevalence of ID was lowest according to unadjusted ferritin among children (14.2%) and unadjusted BIS among women (11.5%), whereas the prevalence of ID was highest when defined by elevated sTfR (68.4% among children and 31.9% among women). Adjustment of ferritin and BIS for inflammation increased the measured prevalence of ID, regardless of the method of adjustment. The proportion of anemia associated with ID ranged from 21 to 82% among children and from 23 to 50% among women, depending on the indicator applied. The prevalence of ID was positively related to the proportion of anemia that was associated with ID.

**Multivariate analysis of predictors of iron status.** Among women, factors that were independently related to one or more of the iron indicators included study region (all indicators), inflammation-adjusted RBP (all indicators), caregiver education (all indicators), urban or rural area, reproductive status (i.e., pregnant, lactating, or nonpregnant, nonlactating), and SES. Among children, factors that independently predicted one or more of the iron status indicators were study region (all indicators); child age (all indicators); inflammation-adjusted RBP; height-for-age Z-score, weight-for-height Z-score; urban or rural location; SES; and caregiver education. The terms that were significant predictors of iron status in the above models were used to assess whether each indicator identified similar risk groups for ID in subsequent analyses.

**Patterns of ID by risk group.** We compared the prevalence of ID and IDA among various potential risk groups using different indicators of iron status and adjustments for inflammation (see examples in Fig. 1; other data available as Supplemental Figs. 1–14). The patterns of ID and IDA by risk group were similar (i.e., groups with higher prevalence of ID also tended to have higher prevalence of IDA); thus, we limited the comparison to prevalence of ID. We generated graphs of the prevalence of ID according to each potential risk group (e.g., zone, SES category, etc.) as shown in Figure 1 and Supplemental Figures 1–14, and interpreted the graphs as follows: if the lines did not cross over (or crossed over minimally, i.e., the difference was not significant), the different indicators were considered to rank the groups similarly in terms of risk of ID (thus implying that any indicator could be measured for the goal of identifying high-risk subgroups). However, substantial crossing over of the lines indicated that the ranking of risk groups by the different indicators was inconsistent (implying that different indicators will not only estimate a different national prevalence of ID but will also identify different subgroups at highest risk).

The rankings of risk groups for ID were generally similar among the different iron indicators examined (Fig. 1A; Supplemental Figs. 1–14A), although the differences in prevalence of ID among risk groups were not significant for all indicators. The groups that were identified as having the highest prevalence of ID were pregnant women, younger children, and women and children in the northern region, in rural areas, in the poorest SES group, and HHs in which the caregiver had no formal education.

Although the different iron indicators generally identified the same groups at highest risk of ID, there were several differences

### Table 3: National prevalence of ID, IDA, and proportion of anemia associated with ID among Cameroonian children and women, by iron indicator and adjustment for inflammation

<table>
<thead>
<tr>
<th>Indicator and adjustment for inflammation</th>
<th>ID</th>
<th>IDA</th>
<th>Anemia associated with ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted ferritin $&lt;12 \mu g/L$</td>
<td>14.2 (11.6–16.9)</td>
<td>12.0 (9.5–14.4)</td>
<td>20.8</td>
</tr>
<tr>
<td>Unadjusted ferritin $&lt;30 \mu g/L$ (WHO)</td>
<td>43.3 (38.5–48.1)</td>
<td>25.9 (22.4–29.4)</td>
<td>45.0</td>
</tr>
<tr>
<td>Inflammation-adjusted ferritin $&lt;12 \mu g/L$</td>
<td>20.6 (17.5–23.6)</td>
<td>16.5 (13.6–19.3)</td>
<td>28.6</td>
</tr>
<tr>
<td>Unadjusted body Fe stores $&lt;0 mg/kg$</td>
<td>21.9 (18.6–25.3)</td>
<td>17.5 (14.4–20.6)</td>
<td>30.4</td>
</tr>
<tr>
<td>Inflammation-adjusted body Fe stores $&lt;0 mg/kg$</td>
<td>23.4 (20.0–26.9)</td>
<td>18.8 (15.7–22.0)</td>
<td>32.3</td>
</tr>
<tr>
<td>Inflammation-adjusted body Fe stores $&lt;0 mg/kg$</td>
<td>29.8 (26.3–33.2)</td>
<td>23.7 (20.6–26.8)</td>
<td>41.1</td>
</tr>
<tr>
<td>Inflammation-adjusted body Fe stores $&lt;0 mg/kg$</td>
<td>32.2 (28.4–36.0)</td>
<td>25.2 (21.7–28.8)</td>
<td>43.8</td>
</tr>
<tr>
<td>sTfR $&gt;8.3 mg/L$</td>
<td>68.4 (64.0–72.8)</td>
<td>47.4 (43.0–51.9)</td>
<td>82.3</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted ferritin $&lt;15 \mu g/L$</td>
<td>13.5 (11.0–16.0)</td>
<td>9.9 (7.7–12.1)</td>
<td>25.5</td>
</tr>
<tr>
<td>Inflammation-adjusted ferritin $&lt;15 \mu g/L$</td>
<td>15.3 (12.7–17.9)</td>
<td>11.1 (8.7–13.5)</td>
<td>28.6</td>
</tr>
<tr>
<td>Unadjusted body Fe stores $&lt;0 mg/kg$</td>
<td>14.5 (11.9–17.1)</td>
<td>10.4 (8.0–12.8)</td>
<td>26.8</td>
</tr>
<tr>
<td>Inflammation-adjusted body Fe stores $&lt;0 mg/kg$</td>
<td>11.5 (9.3–13.7)</td>
<td>9.0 (6.9–11.1)</td>
<td>23.2</td>
</tr>
<tr>
<td>Inflammation-adjusted body Fe stores $&lt;0 mg/kg$</td>
<td>13.3 (13.9–18.7)</td>
<td>10.1 (7.8–12.3)</td>
<td>31.7</td>
</tr>
<tr>
<td>sTfR $&gt;8.3 mg/L$</td>
<td>12.6 (10.3–14.9)</td>
<td>9.9 (7.6–12.2)</td>
<td>25.5</td>
</tr>
</tbody>
</table>

1 Values are percent (95% CI) or percent, $n = 838$ children (798 for IDA) and 872 women (857 for IDA). IDA, iron-deficiency anemia. sTfR, soluble transferrin receptor.

2 Calculated as the prevalence of IDA divided by the prevalence of all anemia (anemia prevalence was 57.6% among 859 children and 38.8% among 888 women).
in the patterns of ID by group when different iron indicators were used. Among children, the prevalence of ID by indicator varied among groups with and without inflammation (Fig. 1B). In particular, unadjusted ferritin and BIS identified children with inflammation as having the lowest prevalence of ID, whereas there was no difference in ID by inflammation group after applying the Thurnham correction. The WHO ferritin cutoff ranked children without inflammation at highest risk of ID and children with both CRP and AGP elevated at lowest risk of ID; in contrast, sTfR ranked children without inflammation as having the lowest risk of ID. Additionally, there were inconsistencies among indicators in the patterns of ID by SES group and in children with and without stunting, mainly regarding the WHO ferritin cutoff (<30 μg/L) compared with the other indicators (Supplemental Figs. 4A and 6A).

Women with and without inflammation and women in different SES groups were also ranked differently by the different indicators (Supplemental Figs. 9A and 12A); in particular, women in the richest SES group were ranked at higher risk of ID according to ferritin (adjusted or unadjusted) but at lower risk according to BIS and sTfR (Supplemental Fig. 12A). In addition, sTfR did not identify pregnant women as being at higher risk of ID (Fig. 1A), but the indicators differentially ranked children with and without inflammation (Fig. 1B).

Patterns of ID by risk group: derived cutoffs. We also conducted an exercise to evaluate whether the differences in ID prevalence among indicators were due solely to the different cutoffs used to indicate deficiency or if the underlying distribution of the indicator in these populations was different. Using inflammation-adjusted BIS <0 mg/kg as a reference indicator for ID, we created new cutoffs for unadjusted ferritin (i.e., as for the WHO cutoff), inflammation-adjusted ferritin, and sTfR concentrations that gave the same national prevalence of ID as the reference indicator (32.0–32.3% of children and 12.6–12.7% of women). For children, the derived cutoffs were 22.9 μg/L unadjusted ferritin, 17.4 μg/L inflammation-adjusted ferritin (continuous adjustment), and 11.5 mg/L sTfR. For women, the derived cutoffs were 13.9 μg/L unadjusted ferritin, 13.5 μg/L inflammation-adjusted ferritin (continuous adjustment), and 10.9 mg/L sTfR.

We compared the prevalence of ID among potential risk groups using the derived cutoffs for unadjusted and adjusted ferritin and sTfR (Fig. 2; Supplemental Figs. 1B–14B). The patterns of ID by risk group were similar using the conventional™ cutoffs and the derived cutoffs. That is, adjusting the cutoffs from the conventional cutoffs to the derived cutoffs changed the overall prevalence of ID but did not affect the relative rankings of population subgroups at risk of ID according to the different indicators. For example, all indicators identified children in the north as having a higher risk of ID (Fig. 2A), but the indicators differentially ranked children with and without inflammation (Fig. 2B).
Discussion

The iron indicators used in this population (with conventional cutoffs) produced very different estimates of the prevalence of both ID and IDA. However, with some exceptions, the indicators identified similar groups at highest risk of ID: children and women in the north, in rural areas, and in the poorest SES group, and younger children and pregnant women. Moreover, when equivalent cutoffs were derived to match the prevalence of ID as measured by inflammation-adjusted BIS <0 mg/kg, the indicators still generally identified similar risk groups. However, there were some exceptions: the rankings of prevalence of ID differed among women and children with and without inflammation and among women of different SES groups when different indicators were applied. Additionally, sTfR ranked women differently by physiological status, and the WHO cutoff ranked children differently according to SES and presence of stunting. We also found that mathematically adjusting ferritin concentrations using CRP and AGP as continuous (rather than categorical) variables did not appreciably change the estimates of ID and IDA. However, it is important to note that the extent to which this adjustment changes ferritin concentrations depends on the reference CRP and AGP values used for the adjustment (e.g., if the ferritin values are adjusted to concentrations that represent CRP = 0.1 mg/L and AGP = 0.1 g/L, the magnitude of change in ferritin concentrations will be greater than for reference values of CRP = 3.75 mg/L and AGP = 0.75 g/L).

The absence of data on specific causes of inflammation, such as intestinal parasites, malaria, HIV, or respiratory infections, is a limitation of this study. In particular, malaria infection may alter concentrations of iron indicators, independent of iron status. However, by mathematically adjusting for CRP and AGP concentrations, we can correct for the effect of malaria and other infections to the extent that their effect on iron status indicators is mediated through the acute-phase response. We chose not to adjust sTfR concentrations for elevated CRP and AGP, because sTfR is not considered to be an acute-phase protein (10). Moreover, among children, adjusting sTfR concentrations using the Thurnham method would reduce the prevalence of ID only from 68 to 60%. Thus, lack of adjustment of sTfR for elevated CRP and/or AGP does not explain our observation that the estimated prevalence of ID was much greater as measured by sTfR compared with ferritin or BIS. However, as discussed below, sTfR concentrations may need to be interpreted differently in populations with endemic malaria.

A second limitation is that we did not measure other indicators of iron status, such as transferrin saturation, erythrocyte zinc protoporphyrin, hepcidin, etc. Nevertheless, this study represents an attempt to compare several of the most commonly applied indicators of iron status and harmonize the results obtained following the application of each indicator. Other studies with additional indicators and in different populations are needed to reach a consensus on the best method to assess iron status of populations and to identify high risk subgroups, particularly where inflammation and infection are prevalent.

It is also important to note that the equation for estimating BIS using the ratio of log(sTfR:BIS) has been validated only in adults (7) and it is possible that the relationship between body iron content and these 2 plasma markers is different in young children. Nevertheless, this equation has been applied among children in other settings (33,34).

Despite these limitations, we show that the different iron indicators identify similar high-risk groups. Thus, both sTfR and BIS are still useful for identifying the population subgroups at highest risk of ID. However, additional validation is needed to improve their use in children and populations with endemic malaria.

The observation that a greater proportion of this population had elevated sTfR compared with the proportion with low values for inflammation-adjusted plasma ferritin has been observed in other populations with (35,36) and without (37) endemic malaria. This finding is inconsistent with the concept that, as ID develops, ferritin stores are depleted before tissue ID, as measured by sTfR concentrations, develops (6). It is possible that this discrepancy is related to the cutoffs used to indicate low iron status (e.g., that the cutoff for elevated sTfR is too low, and/or, conversely, that the cutoff for low adjusted ferritin is too high). Children may have higher sTfR concentrations than adults under normal conditions (15), so different sTfR cutoffs may be appropriate for young children. However, we saw a similarly elevated prevalence of ID as measured by sTfR in relation to that measured by ferritin among both women and children, so this does not fully explain the discrepancy between the prevalence of ID as measured by the 2 indicators.

Hemoglobin variants such as Hb-S (sickle cell trait) may be associated with increased sTfR (25) and are likely prevalent in Cameroon (38); however, because we did not measure hemoglobin variants, we are unable to examine the extent to which these conditions contribute to elevated sTfR in this population.

The elevated prevalence of high sTfR concentrations could also be related to the endemic malaria infection in Cameroon (39). Although we excluded women and children who reported severe fever in the 3 d prior to blood collection, it is likely that many of the participants had asymptomatic malaria infection, which may still be associated with inflammation and/or changes in sTfR (21–23,35). sTfR is thought to be a more reliable indicator than ferritin when inflammation is present (10), however, malaria may induce changes in sTfR concentration independent of both the acute-phase response (i.e., cytokine-mediated inflammation) and body iron content, although the magnitude and direction of this relationship are not clear (12). On one hand, the hemolysis associated with malaria infection could increase sTfR concentrations by stimulating erythropoiesis (22,23). However, there is also evidence of inhibition of erythropoiesis during acute malaria infection, which would be expected to decrease sTfR concentrations (20). In this study, sTfR was positively correlated with CRP and AGP. It is unclear whether the correlation between sTfR and acute-phase proteins reflects worse iron status or increased erythropoiesis related to malaria infection. Longitudinal studies with assessments of indicators of both iron status and malaria infection are needed to clarify these relationships.

These results also call into question the assumption that 50% of anemia is due to ID (2), which is often applied in areas where data are available for hemoglobin, but not specific iron status indicators. Our study demonstrates that the proportion of anemia associated with ID varies depending on the iron status indicator used. Because data on the likely etiology of anemia have important implications for anemia control programs, improved methods to assess the prevalence of IDA consistently across populations are needed.

It is perhaps not surprising to researchers that the different iron indicators yield different estimates of the prevalence of ID, because they are measuring different aspects of iron metabolism (depleted stores, tissue availability, etc.). However, this is an important issue for large-scale surveys in which the results are reported primarily to policy makers and program managers who are interested in the “bottom line” prevalence of ID or IDA. In this setting, the different values for ID and IDA have very
different implications for national policy and resource allocation. For example, in the present survey, the results as measured by sTfR suggest that ID is the primary cause of anemia, whereas the same results using ferritin concentrations suggest that anemia control programs should focus on other strategies, such as infection control.

Because the indicators identify similar risk groups, most indicators could be used if the goal is simply to identify which groups to target with iron interventions. However, if the goal is to assess the prevalence of ID or IDA, the decision should be based on what is considered the "best" indicator of iron status, taking into account the physiological role of each indicator. This issue needs to be addressed to achieve a consensus on the indicators and cutoffs to use as well as consistent terminology (e.g., ID vs. depleted iron stores vs. tissue iron availability, etc.).

The WHO cutoff for ID among children in areas where inflammation and infection are prevalent (ferritin <30 μg/L) appears to underestimate the prevalence of ID in children with inflammation and overestimate the prevalence of ID in children without inflammation. Thus, the measured prevalence of ID at the population level using this cutoff will depend both on population iron status and the prevalence of inflammation. This approach may be useful for estimating the prevalence of ID at the national level if data have already been collected and inflammation is known to be present in the population but cannot be assessed among individuals in the study. However, this approach should not be included in studies that are still in the planning stages. Indicators of inflammation and infection (such as CRP and AGP) must be measured to assist in the interpretation of ferritin concentrations (and those of other nutritional status indicators) at the individual level (11,36).

We limited our assessment to the prevalence of the ID and IDA, so these results do not necessarily apply to assessment of the response to intervention. The results also may be population specific, depending on the age structure and prevalence of inflammation and other micronutrient deficiencies that affect iron status and/or anemia prevalence. Studies in other populations are needed to explore these relationships further and ultimately reach a consensus on assessment of iron status in populations where inflammation and infection are common.

Acknowledgments
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Literature Cited