Although iron evaluation on bone marrow aspirates remains the gold standard for assessing iron status, several other methods have been implemented that are less invasive and more practical. Serum iron, percentage of saturation, and total iron-binding capacity, however, lack sensitivity and are too labile to be of value as single determiners (1). Indirect measures of the functional iron compartment, such as mean cell volume and red cell distribution width, have the disadvantage of becoming indicators relatively late in the development of iron deficiency (2). Serum ferritin can be used as a marker of the iron storage compartment because it is the earliest marker to decrease with iron depletion. However, because ferritin is an acute-phase reactant in serum, its concentration may rise disproportionately to the iron status during inflammation, infection, or neoplasia (3), an occurrence that limits utility of ferritin in the differential diagnosis between anemia from iron deficiency and anemia from chronic disease (4). Because the soluble transferrin receptor (sTfR) concentration is not influenced by acute-phase reactions, it remains within reference values in patients with anemia of chronic disease. sTfR, therefore, can be used as a more reliable index of iron deficiency anemia (5, 6). The correlation between sTfR and bone marrow erythropoietic activity allows the use of sTfR for monitoring erythropoietin therapy, with sTfR increasing 4 weeks before the first increase of hemoglobin (7). However, conditions associated with erythropoietic hyperplasia can also lead to an increase of sTfR in the absence of iron deficiency (8). The sTfR/log ferritin ratio (sTfR/ferritin ratio) is reported to be even more sensitive in the presence of borderline normal ferritin and/or sTfR concentrations. This index may also be useful in distinguishing iron deficiency conditions with hyperplastic erythropoiesis (2).

Because of the lack of international standardization, different reference values for sTfR can be found in the literature (9, 10). It also has been shown that the 95% ranges of the sTfR distribution in healthy and iron-deficient individuals may overlap (11), emphasizing the need for well-established reference values. Therefore, we measured iron status characteristics in 456 volunteers and determined reference values for male and female adults. Subsequently, the influence of blood donation among volunteers was evaluated, as well as estrogen therapy and menopausal status in female volunteers.

Blood was collected from 527 healthy volunteers in the morning with the Sarstedt blood collection system in accordance with NCCLS guidelines (C28-A, H3-A3, and H18-A) (12–14) and after a 15-min recumbent rest. All individuals were living in Antwerp at 7.5 m above sea level and were asked to refrain from participation if they were aware of any preexisting illnesses or were taking medication. The institutional committee for medical ethics approved the study protocol and all volunteers gave informed consent. From the total number of 527 Caucasian adults enrolled, 71 subjects were excluded because of a C-reactive protein concentration >7 mg/L, a body mass index >28 kg/m2 [weight/length2 (kg/m2)], and/or a positive drug screening result (abuse of alcohol or drugs). We measured iron-related analytes in 456 apparently healthy individuals (216 men and 240 women) with a mean age of 39 years (range, 19–60 years) within the 6 weeks of the study period. Seventy-four were regular blood donors (38 men and 36 women). In the female subgroup of 193 premenopausal and 47 postmenopausal women, 87 and 26 women received hormone therapy, respectively. According to WHO guidelines (15), 79 subjects in this survey were identified as anemic (hemoglobin limit of 130 g/L for males and 120 g/L for females).

Hemoglobin concentration, hematocrit, mean cellular volume, mean cellular hemoglobin, mean cellular hemoglobin concentration, and erythrocyte counts were measured on a Coulter STKS hematology analyzer (Coulter Electronics) within 4 h after sampling. Iron measurement was performed on the Ektachem Vitros 950IRC analyzer (Ortho-Clinical Diagnostics). Transferrin measurement was done on the BM/Hitachi 911 (Boehringer-Manheim Diagnostics). Soluble transferrin receptor and ferritin concentrations were nephelometrically determined by N Latex reagent sets (Dade Behring) according to the manufacturer’s instructions after storage of the centrifuged (2600 g; 10 min) serum at –80 °C. These latex tests are based on microagglutination of latex particles coated with a monoclonal antibody. The intraassay CVs for the ferritin and sTfR assays were 1.0–4.6% and 1.4–2.1%, respectively. Interassay CVs for ferritin and sTfR were 1.2–3.1% and 0.8–1.2%, respectively, as determined according to NCCLS guidelines (16).

For sTfR and ferritin, means and SDs were calculated. Frequency distribution histograms were plotted, and 2.5 and 97.5 percentiles were determined (Fig. 1). To assess the effects of gender, pre- or postmenopausal status in women, blood donation status, and hormone therapy, the Wilcoxon 2-sample test was applied. For the correlation analysis, a nonparametric approach was chosen using Spearman rank correlation coefficients. Statistical parameters for estimating the regression line were calculated according to the procedure described by Passing and Bablok (17). All statistical evaluations were performed with the statistical analysis software package SAS (SAS Institute Inc.).

Median and 2.5–97.5 percentiles of all iron-related analytes that we obtained from our study population are presented in Table 1. Statistical analysis of our data showed significantly higher ferritin concentrations in men...
than in premenopausal women ($\chi^2 = 157.35; P = 0.0001$). Premenopausal women had ~38% lower values for ferritin than males, whereas no significant difference was found between males and postmenopausal women. Moreover, 14.65% of the 193 premenopausal women included in the study had hemoglobin concentrations <120 g/L, a finding that was far different from the 2.5 percentile usually excluded at each end of the reference distribution for calculating reference intervals. These findings demonstrate that the prevalence of iron deficiency among premenopausal women may be higher than generally assumed. All reference values were calculated without the exclusion of these anemic subjects because no significantly different reference values were found when these individuals were excluded (data not shown). Moreover, exclusion of these subjects would lead to “ideal” reference values, instead of reference values that would be obtained from the general population. The mean cell volume of 88 fL (SD, 4.15) in the anemic population lies within the reference interval, which confirms the low diagnostic value of the mean cell volume in iron deficiency, as was found in earlier studies (18). From our data, ferritin reference intervals of 20–291 mg/L for males and 5–182 mg/L for females could be determined. Statistical analysis showed a significant correlation between ferritin and age ($r = 0.2449; P < 0.0001$), hemoglobin concentration ($r = 0.5522; P < 0.0001$), and transferrin saturation ($r = 0.3653; P < 0.0001$). These correlations, however, are too weak to justify the need for age-specific ferritin reference intervals in a clinical setting.


data missing

Table 1. Results of laboratory tests reflecting the iron status of the reference population.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Males (n = 216)</th>
<th>Females (n = 240)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>146 (131–165)</td>
<td>128 (111–146)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.424 (0.384–0.477)</td>
<td>0.376 (0.332–0.423)</td>
</tr>
<tr>
<td>Erythrocyte count, $\times 10^{12}$/L</td>
<td>4.74 (4.14–5.51)</td>
<td>4.18 (3.70–4.74)</td>
</tr>
<tr>
<td>Serum iron, $\mu$mol/L</td>
<td>21.5 (10.7–35.6)</td>
<td>20.6 (8.8–36.2)</td>
</tr>
<tr>
<td>Serum ferritin, $\mu$g/L</td>
<td>91.5 (20.0–291.0)</td>
<td>36.5 (4.5–182.0)</td>
</tr>
<tr>
<td>Serum transferrin, mg/L</td>
<td>251 (186–342)</td>
<td>267 (196–393)</td>
</tr>
<tr>
<td>Serum transferrin saturation</td>
<td>0.33 (0.15–0.62)</td>
<td>0.29 (0.11–0.64)</td>
</tr>
<tr>
<td>sTfR, mg/L</td>
<td>1.14 (0.76–1.74)</td>
<td>1.09 (0.76–1.82)</td>
</tr>
<tr>
<td>sTfR-ferritin index</td>
<td>0.58 (0.38–1.07)</td>
<td>0.70 (0.39–2.27)</td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of sTfR and ferritin in the study population.

Table 1. Results of laboratory tests reflecting the iron status of the reference population.

Postmenopausal women had statistically higher ferritin concentrations than premenopausal women ($\chi^2 = 6.5076; P = 0.0107$), indicating the influence of menstrual bleeding on the iron depletion in premenopausal women. However, estrogen intake in the form of birth control pills increased the ferritin concentrations significantly in pre-
menopausal women ($\chi^2 = 10.988; P = 0.0009$), whereas hormone replacement therapy in postmenopausal women did not significantly alter ferritin concentrations. Whether this is because of different dosage schedules of the estrogens used or because of less blood loss in postmenopausal women has to be further evaluated.

When subjects donated blood on a regular basis, a significantly lower ferritin concentration could be observed for men ($\chi^2 = 23.242; P = 0.0001$) as well as for premenopausal women (not shown). This correlates well with the results of Punnonen and Rajamäki (23), who showed that 17% of Finnish women who frequently donated blood had completely lost their iron stores. Unlike the findings of Vernet and Doyen (18), who found increased sTfR concentrations in males who regularly donated blood, we found that blood donation did not affect sTfR concentrations. This may be caused by compensation of the chronic blood loss by mobilizing iron from storage pools. These findings suggest that serum sTfR concentrations will only be increased when erythropoiesis becomes deprived of iron, whereas a decrease in serum ferritin will reflect changes over a broad range of body iron stores. In premenopausal women, we found significantly higher sTfR concentrations compared with postmenopausal women ($\chi^2 = 6.5076; P = 0.0107$), which is in contrast with the earlier findings of Allen et al. (11).

In our opinion, this difference may be a result of the more rigid exclusion criteria or the low number of postmenopausal women involved in their study.

We would like to acknowledge Annick Wauters and Ermine Van Boeckel for their valuable advice in performing this study.

References

13. National Committee for Clinical Laboratory Standards. Procedures for col-

Determination of Blood Total, Reduced, and Oxidized Glutathione in Pediatric Subjects, Anna Pastore,1 Fiorella Piemonte, Mattia Locatelli,2 Anna Lo Russo,1 Laura Maria Gaeta,2 Giulia Tozzi,2 and Giorgio Federici1 1Laboratory of Biochemistry, 2Molecular Medicine Unit, and 3Scientific Directorate, Children’s Hospital and Research Institute “Bambino Gesù”, Piazza S. Onofrio, 4, 00165 Rome, Italy; *author for correspondence: fax 39-0620902270, e-mail apastore@opbg.net

Glutathione ([1–γ-glutamyl-L-cysteinylglycine], which is present in virtually all mammalian tissues, provides reducing capacity for several reactions and plays an important role in detoxification of hydrogen peroxide, other peroxides, and free radicals (1). The synthesis and degradation of glutathione are controlled by reactions of the γ-glutamyl cycle; a decrease in blood reduced glutathione (GSH) has been reported in patients affected by deficiencies of the enzymes involved in the synthesis of glutathione (1).

In cells, total glutathione can be free or bound to proteins; measurement of free glutathione in blood samples is essential for evaluation of the redox and detoxification status of cells in relation to its protective role against oxidative and free radical-mediated cell injury; moreover, GSH measurement is important for the diagnosis of γ-glutamyl cycle disorders.

Recently, several methods to measure glutathione in blood have been described, but little is known about the concentrations of various forms of blood glutathione in pediatric subjects (2–6). We report a rapid and fully automated HPLC method for determining total (GSH), reduced (GSH), and oxidized glutathione (GSG) in