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References


Ferritin Is Not an Indicator of Available Hepatic Iron Stores in Anemia of Copper Deficiency in Rats, Meira Fields,*1 Isabelle Bureau,2 and Charles G. Lewis†(1USDA, BHNRC, NRFL, Bldg. 307, Rd. 330, BARC-East, Beltsville, MD 20705-2350;2visiting scientist, Université Joseph Fourier, La Tronche, France; †author for correspondence: fax 301-504-9062, e-mail fields@307.bhnrc.usda.gov)

Serum ferritin is a sensitive indicator of available iron stores [1], but in certain instances it cannot be used in diagnosis, e.g., in anemias of chronic disease, infections, inflammation, liver disease, and malignancies [2–7]. Iron stores may be normal or increased, though accompanied by increased serum ferritin, in anemias of chronic disorders, aplastic anemia, sideroblastic anemia, and chronic hemolytic anemia. Because ferritin is also a positive acute-phase reactant protein that is increased in inflammation [2], serum ferritin concentration is not a reliable index of available iron stores in individuals with chronic diseases. There is no information, however, on whether ferritin can be used as a marker of available iron stores in the anemia of copper deficiency. Unlike iron-deficiency anemia, in which body iron stores are usually depleted as evidenced by diminished serum ferritin concentrations, anemia of copper deficiency [8–10] results from increased hepatic iron stores and impaired mobilization and delivery of iron from storage to bone marrow for heme synthesis, leading to iron-deficient erythropoiesis [11]. Can serum ferritin be utilized as a reliable tool to measure available iron stores in anemia of copper deficiency? We evaluated in experimental copper deficiency the potential usefulness of three different concentrations of dietary iron and their effects on iron availability and degrees of anemia. To measure accurately body iron stores, hepatic iron concentration was determined. The reliability of ferritin as an iron index was tested by comparison with hepatic iron concentration.

We fed weanling male Sprague-Dawley rats one of six diets [12] for 6 weeks. All rats were fed either a copper-deficient diet containing 0.6 µg Cu/g diet or a copper-adequate diet containing 6.0 µg Cu/g as analyzed by atomic absorption spectrophotometry. Cupric carbonate and ferric citrate were added to the copper- and iron-deficient diets. Analysis of the diets revealed that the dietary iron was either 19 µg Fe/g (low), 48 µg Fe/g (adequate), or 88 µg Fe/g (high). Added dietary iron was within the concentrations recommended for optimal growth of rodents. Rats were killed after an overnight fast. Livers were removed, rinsed in saline, and portions used for the quantitative analysis of copper and iron concentrations [13]. Blood was collected into heparinized test tubes. Ferritin was measured in plasma with rat ferritin test kit (cat. no. RF69; Ramco Labs., Houston, TX), a sandwich solid-phase enzyme immunoassay. Rat liver ferritin was used as a calibrator. Hematocrit and hemoglobin were measured by conventional procedures.

All data were expressed as mean ± SE and analyzed by ANOVA with two concentrations of copper and three concentrations of iron. The independent effects of copper and iron and the interaction between them were examined. Differences at P <0.05 were considered statistically significant.

Forty percent of rats fed the copper-deficient diet containing 88 µg Fe/g and 28% of copper-deficient rats fed 48 µg Fe/g died prematurely because of ruptured hearts in the apex. No mortality occurred in either copper-deficient rats fed the low, 19 µg Fe/g diet or any of the copper-adequate controls.

Liver copper and iron, hemoglobin, hematocrit, and ferritin are presented in Table 1. All copper-deficient rats exhibited reduced liver copper compared with copper-adequate rats. The lowest copper concentration was found in copper-deficient rats fed the added concentration of dietary iron. The highest liver iron stores were found in copper-deficient rats fed the fortified concentrations of dietary iron. The combination of copper defi-
ciency with added iron resulted in the most severe anemia, reflected in the lowest hematocrit and hemoglobin. The highest ferritin concentrations were found with the adequate-copper, added-iron diet and the lowest ferritin values with the adequate-copper, low-iron diet.

Correlations between concentrations of ferritin and hepatic iron and ferritin and hematocrit of copper-deficient and copper-adequate rats are shown in Fig. 1A. Plasma ferritin was significantly correlated with hepatic iron concentration \( (r^2 = 0.860) \) and \( (r^2 = 0.738) \) in copper-adequate and copper-deficient rats, respectively (Fig. 1). In copper-adequate rats plasma ferritin was significantly correlated with hematocrit \( (r^2 = 0.861) \) but not correlated with hematocrit \( (r^2 = 0.044) \) in copper-deficient rats (Fig. 1B).

The results of the present study clearly show that only in copper-adequate rats was there a direct relation between hematocrit and ferritin. The highest hepatic iron concentrations were correlated with the highest concentrations of plasma ferritin and hematocrit, and the lowest concentrations of plasma ferritin predicted the presence of the lowest hemoglobin, hematocrit, and hepatic iron. On the basis of data from copper-adequate rats it is clear that under normal conditions the liver is capable of mobilizing iron and making it available for utilization by hemopoietic tissues for heme synthesis, and therefore ferritin is a sensitive measure to assess available body iron stores. In contrast, there was no relation between degree of anemia, concentrations of hepatic iron, and ferritin in copper-deficient animals. Ferritin should not be used to assess functional liver iron stores in copper deficiency and as such may not provide a clue to potentially serious underlying disorders.

Unlike most iron-deficiency anemias, the anemia of copper deficiency reported herein was not due to depleted iron stores but to hepatic iron overload and an impaired release of iron from body iron stores. Unlike the anemia of iron

![Fig. 1. Correlation between (A) concentrations of ferritin and hepatic iron and (B) concentrations of ferritin and hematocrit in copper-adequate ● and copper-deficient ● rats.](https://academic.oup.com/clinchem/article-abstract/43/8/1457/5640862)

**Table 1. Liver copper and iron, hemoglobin, hematocrit, and ferritin (mean ± SEM).**

<table>
<thead>
<tr>
<th></th>
<th>Copper adequate</th>
<th></th>
<th>Copper deficient</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(High)Fe</td>
<td>(Adequate)Fe</td>
<td>(Low)Fe</td>
<td>(High)Fe</td>
</tr>
<tr>
<td>Liver Cu, µg/g wet wt.</td>
<td>3.9 ± 0.2</td>
<td>4.5 ± 0.1</td>
<td>6.0 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Liver Fe, µg/g wet wt.</td>
<td>158 ± 17</td>
<td>117 ± 7</td>
<td>38 ± 1</td>
<td>352 ± 33</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>183 ± 9.0</td>
<td>181 ± 7.0</td>
<td>129 ± 3.0</td>
<td>59 ± 5.0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42.0 ± 0.5</td>
<td>38.9 ± 0.7</td>
<td>30.3 ± 0.6</td>
<td>15.9 ± 0.8</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>309 ± 24</td>
<td>264 ± 18</td>
<td>67 ± 12</td>
<td>185 ± 27</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>Fe</th>
<th>Cu × Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Cu</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Liver Fe</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ferritin</td>
<td>S</td>
<td>NS</td>
<td>S</td>
</tr>
</tbody>
</table>

S = significant \( (P < 0.05) \); NS = not significant.

High Fe = 88 µg/g diet; adequate Fe = 48 µg/g; low Fe = 19 µg/g.
deficiency that responds to iron supplementation [14], the anemia of copper deficiency should not be treated by iron supplementation but should be treated by either lowering the intake of dietary iron or by chelation therapy [15–17]. As can be seen in the present study, the less severe anemia of copper deficiency was caused by the consumption of a low-iron diet. In contrast, the most severe anemia in copper-deficient rats was induced by consumption of additional concentrations of dietary iron and was associated with the highest concentrations of liver copper. This hepatic copper retention, however, could be toxic [18, 19]. Plasma ferritin did not reflect the magnitude of these abnormalities. This is the first report, however, that demonstrates that serum ferritin, a key conventional laboratory test, is inadequate in identifying anemia and assessing functional iron stores in copper deficiency. This finding may have practical significance to clinicians dealing with cases presenting as anemias of iron deficiency.

References

Electrochemical Enzyme Immunoassay for Serum Prostate-Specific Antigen at Low Concentrations, Sung-Fang Chen, Yan Xu,* and Michael Po-Chee Ip† (Dept. of Chem., Cleveland State Univ., Cleveland, OH 44115 and 1 Dept. of Pathol., MetroHealth Med. Center, Cleveland, OH 44109; *author for correspondence: fax 216-687-9298, e-mail y.xu@popmail.csuohio.edu)

Serum prostate-specific antigen (PSA) has been recognized as a sensitive indicator of recurrent prostate cancer after radical prostatectomy [1–5]. In the past 5 years, numerous PSA assays with improved limits of detection [6–15] have been developed by both clinical researchers and diagnostic assay manufacturers. The rationale behind the development of more sensitive PSA assays (e.g., lower limits of detection) is that the relapse of prostate cancer or the tumor-doubling time after radical prostatectomy can be detected much earlier if patients are monitored with more sensitive assays [16, 17].

Here we report a rapid enzyme immunoassay (EIA) for serum PSA at low concentrations by flow-injection electrochemical detection, which is based on the modification of the Tandem®-E PSA assay (Hybritech, San Diego, CA). EIA coupled with electrochemical detection offers low limits of detection with high specificity [18–21]. Electrochemical EIA is usually based on the conversion of an electroinactive substrate to an electroactive product by the enzyme label. In this work, the enzyme was alkaline phosphatase (EC 3.1.3.1), which converted p-aminophenyl phosphate to p-aminophenol [22]. The concentration of p-aminophenol was then determined amperometrically in the flow-injection system.

We had illustrated the flow-injection electrochemical detection system used for this work elsewhere [21]. Basically, it was a BAS chromatograph (Bioanalytical Systems, West Lafayette, IN) without the separation column. The thin-layer electrochemical cell had dual glassy carbon working electrodes (in the parallel mode), a Ag/AgCl (3 mol/L NaCl) reference electrode, and a stainless-steel auxiliary electrode. The detection voltage between the working and reference electrode was set at +500 mV. The system had a custom-built injector with 1-μL sample loop. The carrier fluid (0.1 mol/L Tris, 1 mmol/L MgCl₂, 65 mmol/L oxalic acid, and 0.2 g/L NaN₃ at pH 3.2) was pumped at 0.2 mL/min.

In our assay procedure, Tandem-E PSA assay kit was used. A set of low-concentration PSA calibrators (0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 μg/L) was prepared through serial dilutions of the PSA calibrators (2, 10, and 50 μg/L) with the zero diluent. The assay was carried out in duplicate as follows: (a) Pipette 100 μL of the zero diluent, calibrators, controls, and serum specimens, as well as 100 μL of anti-PSA antibody–alkaline phosphatase conjugate, into each appropriately labeled test tube; (b) add one anti-PSA antibody-coated bead to each tube and vortex-mix the tubes; (c) shake the test tube rack gently in the water bath of the shaking incubator at room temperature (~23 °C) for 2 h; (d) aspirate the liquid from the tubes and wash the beads with the wash solution (4 × 1 mL); (e)