Influence of Blood Donation and Iron Supplementation on Indicators of Iron Status

To the Editor:

Under the new requirements of the Clinical Laboratory Improvement Amendments of 1988, laboratories are required to verify or establish reference ranges for new analytical methods before reporting patients' test results (1). Historically, ferritin reference ranges have been based on "normal, apparently healthy adults," with "normal" being based on test results for blood counts, liver enzymes, and, in some cases, erythrocyte sedimentation rates (2, 3). Our data and evidence from other studies (3-8) suggest that the impacts of blood donation and iron supplementation on reference ranges for ferritin have not received adequate attention.

We collected 186 serum samples (54 from men, 132 from women) for the purpose of refining reference intervals for serum iron, transferrin, percentage saturation of transferrin (%TS), and ferritin and for testing the effect of blood donation on these values. Five milliliters of blood was collected from nonfasting volunteers participating in an employer-sponsored blood drive. Each donor gave written informed consent and completed a brief questionnaire regarding past medical history, blood donation activity, and intake of iron supplements.

After phlebotomy, the blood was separated and the serum was stored at 4-8°C and tested within 24 h of collection. Iron was measured colorimetrically with a Cobas Fara (Roche Diagnostics, Nutley, NJ), with reagents and protocol from Diagnostic Chemicals (Monroe, CT). Transferrin was determined immunoturbidimetrically as previously described (9). Ferritin was measured with the Tandem-E ELISA from Hybritech (San Diego, CA). We calculated %TS as the ratio of iron to transferrin, using a factor of 25.2 (assuming a molecular mass of 79,570 Da for transferrin) to convert transferrin concentration (g/L) to an equivalent total iron-binding capacity (µmol/L). Because inflammation is known to affect iron status adversely (4), we also measured serum concentrations of C-reactive protein (CRP) on the Behring Nephelometer (Behring Diagnostics, Somerville, NJ) (10). Fourteen subjects had CRP concentrations exceeding the 95th percentile (8.5 mg/L, the upper limit of normal for adults (11)) and were excluded from the study group. Results for an additional 14 volunteers who did not respond to questions regarding their donation history or vitamin/mineral supplement use were also excluded. The final study group comprised 48 men and 110 women (ages 35 ± 9.4 and 34 ± 9.8 years, respectively).

We examined the relationship between the number of past blood donations, grouped by quintiles, on these indices of iron status (Table 1). No significant relationships were observed between the number of donations and the serum concentrations of iron, transferrin, or %TS among men and women. However, ferritin concentrations (log-transformed to yield a gaussian distribution) decreased significantly as the number of past donations increased (P = 0.004 and 0.01 for men and women, respectively, by linear regression analysis).

Next, we examined the effect of iron supplementation on the measured variables in donors and in nondonors (Table 1). For iron, transferrin, and %TS values were higher in donors than in nondonors; however, except for iron concentrations in the unsupplemented group (P = 0.02), these differences were not significant (by independent t-test, two-tailed). In both the unsupplemented and supplemented groups, ferritin concentrations were significantly lower in donors than in nondonors (P = 0.03 and 0.007, respectively). Furthermore, ferritin contents in nondonors taking iron supplements (115 ± 76 µg/L) were twice as high as in nondonors who did not take iron (52 ± 41 µg/L), a significant difference (P = 0.010).

A number of studies have reported widely varying ferritin values (geometric means) for men and women. In the present study group, the geometric means for male (all ages) and female (<50 years) nondonors were 80 and 38 µg/L, respectively. However, in blood donors, the picture is quite different: For both male and female blood donors, the mean ferritin concentrations were 50% less, 37 and 17 µg/L, respectively. Similar observations of the graded effects of blood donation on iron stores as measured by serum ferritin have been reported previously (6, 8). Finch et al. (6), in agreement with our data, noted that the removal of 1 unit of blood from first-time blood donors halved the ferritin concentrations in men (from 127 to 66 µg/L) and reduced those in women by nearly one-third (from 46 to 33 µg/L). Also, Leggett et al. (7) noted that, apart from

Table 1. Effect of blood donation history and iron supplementation on indicators of iron status.

<table>
<thead>
<tr>
<th>No. of donations</th>
<th>No. of subjects</th>
<th>Iron, µmol/L</th>
<th>Transferrin, g/L</th>
<th>%TS</th>
<th>Ferritin, µg/L</th>
<th>Log ferritin conc</th>
</tr>
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<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7</td>
<td>16.3</td>
<td>2.65</td>
<td>32</td>
<td>132</td>
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<td>1-5</td>
<td>10</td>
<td>18.3</td>
<td>2.75</td>
<td>38</td>
<td>69</td>
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<tr>
<td>6-10</td>
<td>9</td>
<td>18.8</td>
<td>2.93</td>
<td>34</td>
<td>52</td>
<td>1.716</td>
</tr>
<tr>
<td>11-18</td>
<td>12</td>
<td>16.5</td>
<td>2.79</td>
<td>33</td>
<td>39</td>
<td>1.591</td>
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<tr>
<td>&gt;18</td>
<td>10</td>
<td>17.6</td>
<td>2.77</td>
<td>33</td>
<td>30</td>
<td>1.477</td>
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<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21</td>
<td>12.4</td>
<td>2.81</td>
<td>22</td>
<td>56</td>
<td>1.748</td>
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<tr>
<td>1-3</td>
<td>18</td>
<td>16.6</td>
<td>2.98</td>
<td>29</td>
<td>38</td>
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</tr>
<tr>
<td>4-7</td>
<td>25</td>
<td>13.2</td>
<td>3.08</td>
<td>23</td>
<td>12</td>
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<td>8-13</td>
<td>25</td>
<td>17.2</td>
<td>3.11</td>
<td>31</td>
<td>15</td>
<td>1.176</td>
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<tr>
<td>&gt;13</td>
<td>21</td>
<td>14.1</td>
<td>3.10</td>
<td>26</td>
<td>14</td>
<td>1.146</td>
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<tr>
<td><strong>Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No iron supplement</td>
<td></td>
<td>12.9 (4.9)</td>
<td>2.86 (0.45)</td>
<td>25 (10)</td>
<td>52 (41)</td>
<td>1.583 (0.550)</td>
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<tr>
<td>Iron supplementation</td>
<td></td>
<td>16.8 (7.4)</td>
<td>3.08 (0.48)</td>
<td>30 (14)</td>
<td>36 (36)</td>
<td>1.341 (0.461)</td>
</tr>
</tbody>
</table>

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Platelet-Poor Plasma Not Suitable for Clinical Endotoxin Testing, Demonstrated in Horses

To the Editor:

Endotoxins are the lipopolysaccharide (LPS) constituents of the outer cell wall of Gram-negative bacteria. They initiate clinical phenomena associated with septicemia by triggering synthesis of mediators such as tumor necrosis factor (TNF) and interleukin 1 (IL-1), IL-6, and IL-8. Therapeutic clinical trials have been performed in septic patients with monoclonal antibodies to LPS or TNF, or soluble IL-1 receptor antagonists (1-4). In one study, patients with detectable LPS in their circulation benefitted most from administration of anti-LPS antibodies (5). LPS was not measured in the other studies.

In our opinion, LPS measurements are essential for selecting which patients will be most likely to benefit from anti-LPS, anti-TNF, and other treatment strategies. However, reliable quantification is imperative. Clinical LPS testing can be hampered by environmental contamination during blood collection and handling procedures, providing false-positive results. Conversely, the possible removal of LPS by cellular binding and cell removal during the blood handling would provide false-negative results. Specifically, LPS binding to platelets is controversial. Experiments with LPS-supplemented human whole blood provided similar LPS concentrations in platelet-rich plasma (PRP) and platelet-poor plasma (PPP) (6, 7), but binding to platelets in human blood in vivo has been reported (8-11). Recently, Redl et al. (12) reported the usefulness of a commercially available LPS-free blood collection tube for LPS and cytokine testing (Endo Tube ET®; Chromogenix, Mölndal, Sweden). The recommended use of the tube provides PPP (centrifugation at 3000g generates an intermediate gel layer for optimal separation of cells and plasma), which may result in substantially less LPS being detected in clinical samples.

From 48 horses with naturally occurring acute intestinal disorders, we collected by aseptic venipuncture of the jugular vein duplicate 4-mL blood samples for the LPS assay from Endo Tube ET containing 120 IU of sodium heparin. Blood was obtained just before anesthetization, after apparent restoration of bowel perfusion (as estimated by color and motility in the segments bordering the affected bowel part and the part itself), and every 8 h thereafter for a 48-h follow-up period. The samples were immediately immersed in melting ice, and within 20 min centrifuged at 4°C either at 170g for 15 min to obtain PRP or at 2200g for 15 min to obtain PPP. We then removed the plasma layers and stored each in two aliquots at -23°C. Platelet counts were performed in PRP of EDTA-anticoagulated blood samples drawn at the same collection times and sites during the study periods, with a Sysmex K-1000 cell counter (Toa Medical Electronics, Kobe, Japan). After 10-fold dilution in LPS-free water and heating for 15 min at 75°C to remove plasma inhibitory activity, LPS was measured with a chromogenic Limulus assay (Comtest®; Endotoxin; Chromogenix) as described elsewhere (7). The assay has a detection limit of 3 ng/L in PRP, as determined by the LPS concentration corresponding to the mean +3 SD absorbance (A_{405}) of the blank measured 10 consecutive times. The clinical decision limit for the presence or absence of endotoxemia, i.e., endotoxin positivity, was arbitrarily set at LPS = 5 ng/L PRP, similar to the decision limit for humans (13), given the absence of studies to establish this limit in horses. For the 12 healthy horses, LPS concentrations in PRP never exceeded 4 ng/L (data not shown).

The blood collection tube proved to be convenient and suitable: Only 8 samples of 307 (145 concurrently prepared PRP and PPP and 17 PPP only) were thought to be contaminated during blood collection or further handling on the basis of marked differences (>100 ng/L) between LPS concentrations in the paired PRP and PPP samples. Of the PRP samples, 38 were LPS-positive (mean 19.6, range 5-197 ng/L). However, the LPS concentrations in PPP were significantly higher (P = 0.008, Wilcoxon signed rank test) than in the concurrently prepared PPP (mean 10.9, range <3-145 ng/L). The lower LPS concentrations in the PPP samples meant that 7 of the 38 LPS-positive PRP samples were graded as negative (LPS <5 ng/L). The percentage difference (expressed as % binding of LPS to platelets) between the LPS concentrations from these samples was correlated with the platelet count in the PRP sample (r = 0.53, P = 0.001, n = 38, range 8-77%, Fig. 1). This weak but highly significant correlation suggests that binding of LPS to platelets may be the cause of LPS loss during preparation of PPP. This hypothesis needs further investigation.

We conclude that the Endo Tube ET

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