Day-to-Day Variation in Serum Ferritin Concentration in Healthy Subjects

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We report our observations on day-to-day variation in serum ferritin, serum iron, total iron-binding capacity, and percent saturation of binding proteins with iron in 13 ostensibly healthy subjects during five weeks. The average intrasubject coefficients of variation were 14.5, 28.5, 4.8, and 28.0%, respectively. Precision studies on control samples showed greater within-assay and between-assay analytical variation for serum ferritin than for serum iron or total iron-binding capacity. Evidently, serum ferritin varies less in a given individual from day to day than does serum iron and percent iron saturation. Thus, a single measurement of serum ferritin may be a more reliable index of iron stores than an isolated determination of either serum iron or percent iron saturation.

Additional Keyphrases: iron in serum · total iron-binding capacity · reference intervals · clinical laboratory measurement of iron-related disorders · percentage saturation of serum protein with iron · inter- and intra-individual variation · assessing iron stores

Measurement of ferritin, the main iron-storage protein of the body, has been used widely to evaluate disorders of iron metabolism. Ferritin is found in most tissues, but concentrations are highest in spleen, liver, and bone marrow. Originally it was thought that ferritin was exclusively an intracellular protein, with no measurable quantities in serum, but when a sensitive radiolmmunoassay for it was developed in 1972, ferritin was found to be a normal constituent of serum (1).

Ferritin is made up to 24 subunits. A family of structurally related molecules called "isoforms" is made up of various combinations of ferritin subunits. These ferritin molecules, which are classified as acidic and basic, have a characteristic tissue distribution. Basic isoform is found in the spleen, liver, and serum. Liver ferritin, being similar to serum ferritin in subunit composition, is recommended for use in preparation of antibody and as standards for immunoassay of serum ferritin (2).

The concentration of ferritin in the serum of normal individuals is directly related to body iron stores (3-5). It also reflects iron stores in iron-deficiency and iron-overload states, and can be useful in diagnosis and monitoring of patients with these disorders (3, 6-10). Serum ferritin assay is especially useful in detection of mild iron deficiency without clinically evident anemia, and in differentiating between the anemias of chronic disease and iron deficiency.

The diagnostic value of a measurement depends upon its sensitivity and specificity, and upon the prevalence of the disease in the population. These three factors determine the predictive value of a test (11). In a study by Mazza et al. (12) low ferritin concentrations in serum were found to have a greater specificity—and hence a higher predictive value—than decreased percent iron saturation for detection of iron deficiency. Because the sensitivity of the two assays is equal, a greater number of falsely positive results would be expected for percent iron saturation than for serum ferritin as a diagnostic measurement for iron deficiency.

Intra-individual physiological variation, inter-individual biological variation, subject preparation, and analytical variation of a laboratory measurement determine the reliability of assay results (13). Factors affecting subject preparation and analytical variation can be modified to increase reliability, but intra-individual physiological variation, if excessive, presents an unmodifiable adverse effect on the reliability of assay results. Serum iron concentrations in an individual vary widely day-to-day (14-17); because serum iron is used in calculating percent iron saturation, this latter value is also quite variable. Therefore, because of wide physiological variation, measurement of serum iron or percent iron saturation may be an unreliable index of iron stores. If serum ferritin varies day-to-day as much as serum iron, then a single value also might be an unreliable index of iron stores.

We present a study of day-to-day variation in serum ferritin concentrations, as compared with variations in serum iron, total iron-binding capacity (TIBC), and percent iron saturation.

Material and Methods
In this study we used 13 healthy men and women volunteers (ages, 25 to 39 years) whose hemoglobin values were within our laboratory reference interval. Blood specimens of 8 mL were drawn by an experienced phlebotomist each morning for five weekdays on each of three alternate weeks. Six subjects completed the entire protocol, six subjects missed one venipuncture, and one subject missed two. No restrictions were placed on the subjects other than that specimens were drawn at nearly the same time of the morning for each subject, with the subjects in the sitting position. None of the subjects were taking iron-containing medication and none became ill during the study. On the first day of the study, 3 mL of blood also was obtained for hemoglobin determination by the cyanmethemoglobin method (Model S blood counter; Coulter Electronics, Ltd., Hialeah, FL 33010). Specimens obtained for the other determinations were centrifuged within 1 h of collection, and the serum, separated into two portions, was stored in glass tubes at -20 °C. Before analysis, frozen specimens were thawed slowly and allowed to reach room temperature.

Serum ferritin concentrations were determined by radioimmunoassay (GammaDab; Clinical Assays, Cambridge, MA 02139). Human liver ferritin was used to produce antibody and prepare standards, tracer, and serum controls. Specimens with ferritin values exceeding 200 μg/L were diluted with zero standard and then reassayed. We determined serum iron and TIBC by modification of the bathophenanthroline method (18), using the same lot of aca reagents (DuPont Inc., Wilmington, DE 19898). Serum controls (Hyland Travenel Labs., Inc., Costa Mesa, CA 92626) were used for the iron and TIBC precision studies. Percent iron saturation was calculated from the values for serum iron and TIBC. All specimens from a subject were assayed concurrently and, for ferritin, all specimens were assayed in duplicate. Within-assay and between-
assay precisions for serum iron and serum ferritin were evaluated at two concentrations from assays of control samples run 20 times within one day and once daily for 20 consecutive days. Precision of TIBC was similarly evaluated except that we used only one concentration of control material.

**Results**

Table 1 shows the values at the beginning of the study. All hemoglobin values were within the reference interval. For the other variables eight subjects had at least one value outside the reference interval. Comparison of serum ferritin values for each subject at the beginning and end of the study by paired *t*-test indicated no significant difference (*p* < 0.05). In Table 2 we compare within-assay and between-assay precision for serum ferritin, TIBC, and serum iron.

Table 3 lists results of day-to-day variation. In all subjects except subject 8, the coefficient of variation (CV) for serum ferritin was less than for serum iron or percent iron saturation. TIBC values varied the least for each subject. The average intrasubject CV was 14.5% for serum ferritin, 28.5% for serum iron, 28.0% for percent iron saturation, and 4.8% for TIBC. Figure 1 shows the variation in values for serum ferritin and serum iron of three representative subjects.

Thirteen specimens from eight of the 13 subjects showed low values for both serum iron and percent iron saturation. Four of these were associated with an above-normal TIBC; however, none was associated with serum ferritin of less than 12 μg/L (Table 4). One of the subjects (subject 4), however, did have a borderline low serum ferritin. Two subjects had above-normal mean TIBC and low mean percent iron saturation. One subject (subject 1) had 11 specimens with above-normal values for serum iron, percent iron saturation, and serum iron. The means for these also were above normal. Mean values for all assays in 10 of the 13 subjects were within the reference interval.

**Discussion**

We initially determined hemoglobin values for all subjects, to exclude those with anemia. In all subjects this value was within the reference interval, even though eight subjects had at least one other parameter (serum iron, TIBC, percent iron saturation, or serum ferritin) outside the reference interval. During the study each subject had from 107 to 123 mL of blood drawn over a five-week period; because serum ferritin decreases with phlebotomy (4, 19), the loss of blood through phlebotomy might be responsible for significant assay variation of results. However, comparison of initial and final serum ferritin values in the 13 subjects showed no significant difference (*p* < 0.05), as might be expected if an inconsequential volume of blood had been withdrawn. Therefore, because serum ferritin (known to be a more sensitive index of iron stores than serum iron, TIBC, or percent iron saturation) did not decrease over the five-week study, no significant change from phlebotomy would be expected in the other three factors determined.

Our method for measuring serum iron compares favorably with other methods of serum iron measurement. In the College of American Pathologists Comprehensive Chemistry Survey, the CV for modification of our bithaphenanthroline method was much less than for all other methods (20). Our within-assay and between-assay variations for serum ferritin were similar to reported values for other ferritin assays (21). Comparison of within-assay and between-assay precision revealed considerably more analytical variation for ferritin than for iron or TIBC. Thus, we would expect greater day-to-day variation in serum ferritin than in serum iron or TIBC if physiological variation is equivalent.

In general, intrasubject CV for serum ferritin was much less than for serum iron and percent iron saturation (Table 3). The percent deviation of serum iron and serum ferritin in relation to the initial value as plotted in Figure 1 demonstrates the variability for these parameters. In Figure 1a, subject 7, whose values were typical of the group, demonstrated greater day-to-day variation of serum iron than of serum ferritin. Figure 1b illustrates the values from subject 2, who showed the widest day-to-day variation for serum iron, with values above and

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Table 1. Values for the 13 Subjects at the Beginning of the Study

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Hemoglobin (F 115–155, M 135–175) mg/L</th>
<th>Iron (500–1500) μg/L</th>
<th>TIBC (2500–4500)</th>
<th>Iron saturation (20–55), %</th>
<th>Ferritin (10–300), μg/L</th>
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<td>1</td>
<td>M</td>
<td>159</td>
<td>2060</td>
<td>2600</td>
<td>79</td>
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<td>2</td>
<td>F</td>
<td>132</td>
<td>2080</td>
<td>3000</td>
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<td>3</td>
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<td>155</td>
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<td>530</td>
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<tr>
<td>7</td>
<td>F</td>
<td>134</td>
<td>870</td>
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<tr>
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<tr>
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*Values in parentheses are reference intervals. M, male; F, female.*
Table 3. Summary of Day-to-Day Study

<table>
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<tr>
<th>No. specimen assayed</th>
<th>Serum iron, µg/L</th>
<th>TIBC, µg/L</th>
<th>Iron saturation, %</th>
<th>Serum ferritin, µg/L</th>
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<td>3920-4220</td>
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</table>

Subjects are in the same order as in Table 1.

below our reference interval during the three-week phlebotomy period. Thus both iron overload and deficiency were indicated from isolated serum iron values. In Figure 1c the data from subject 8 show the widest day-to-day variation in serum ferritin. This was the only subject in whom the day-to-day variation in serum ferritin exceeded the variation in serum iron.

Compared with the isolated initial value, the mean values of the components measured appeared to give a more accurate picture of the subjects’ iron status. Only three subjects had mean values that were outside the reference interval, whereas eight subjects had an initial value outside the interval. Two of these three subjects (8 and 11) had above-normal mean TIBC values and low mean percent iron saturation values. Because mean serum iron and ferritin values were within our reference interval for subject 8, it is unlikely that this subject was iron depleted. The above-normal TIBC may have been related to birth-control pills the subject was taking during this study [birth-control pills are known to increase TIBC and serum iron (22, 23)]. The exceptionally high CV for serum ferritin in this subject may also be related to the birth control pills; however, the effect of contraceptive pills on serum ferritin is unknown.

The mean serum ferritin value for subject 11 was 19 µg/L, which might be considered a borderline low value for a male; hence, this subject may indeed have been iron depleted. The same also may be true for female subjects 4 and 9, whose mean ferritin concentration was 15 µg/L. Reported means and ranges for serum ferritin in normal subjects vary considerably (1, 5, 7, 9). Jacobs et al. reported a mean of 69.2 µg/L (range 6-186 µg/L) in 75 healthy males, and a mean of 34.8 µg/L (range 9-125 µg/L) for healthy females (9). Cook et al. reported a mean serum ferritin of 94 µg/L (range 27-329 µg/L) for healthy males (n = 174) and a mean of 34 µg/L (range 9-125 µg/L) for healthy females (n = 153) (5). Although there are differences between reported means and ranges for healthy subjects, there is general agreement that the reference interval for males is significantly higher than for females and that a serum ferritin value less than 10 µg/L is associated with iron deficiency (21). It is of interest that subject 11 was a regular blood donor. Subject 1, whose mean serum iron, mean percent iron saturation, and mean serum ferritin values were above normal, was not taking iron medication, had never been transfused, and had no family history of hemochromatosis. These values do not likely indicate idiopathic hemochromatosis because serum ferritin in this condition almost always exceeds 1000 µg/L, as reported by many investigators (1, 7, 9, 24).

The results of our study show an average intrasubject day-to-day CV for serum ferritin of only 14.5%. This variation is substantially less than that of 28.5% for serum iron, even though the analytical variation for our ferritin assay was greater than for our iron assay. Thus, because of the intrasubject physiologic variation, we conclude that an isolated serum ferritin value is more reliable than an isolated serum iron value. The lower physiologic variation of serum ferritin may be related to the direct relationship of serum ferritin to iron stores and to its rapid turnover in plasma (3).

In a recent review of serum ferritin, Worwood (21) reported a mean CV of 15% (range 6-28%) from a study of ferritin
variation over a seven-week period in 18 normal subjects; no differences were observed in specimens obtained between 1000 and 1600 hours. The details of this study have recently been published (25), and it is apparent that their values are quite similar to ours. Leyland et al. (26), however, have demonstrated a biphasic circadian rhythm for serum ferritin, ranging by about 10% above and below the mean at about noon and 1600 hours in seven normal subjects (26). We have investigated the within-day variation of serum ferritin in healthy subjects, and our data (27) support the observations of Leyland et al.

Other investigators have demonstrated the substantial day-to-day variation of serum iron (14–17). In studies of day-to-day variation of serum iron by Statland and Winkel, ingestion of food before the phlebotomy, time of phlebotomy, tourniquet application, and posture were all controlled, to decrease the effect of these variables on iron variation (14, 15). They reported an average intrasubject day-to-day CV for serum iron of 26.6 and 29.3 for 11 men and nine women, respectively, in two separate reports. The conditions for our subjects were not so restrictive, yet our CV (28.5%) is remarkably similar to theirs; hence, these variables do not appear to affect normal day-to-day variation of serum iron significantly. Although studies have shown that the concentration of serum iron is higher in the morning and lower in the evening, this diurnal variation apparently had little effect on day-to-day variation in a study by Bowie et al. (16). The average intrasubject CV for serum iron obtained at 0800 and 1630 hours were 21.3% and 19.8%, respectively.

If we considered only isolated values of serum iron and percent iron saturation, 13 specimens from eight of the subjects in our study could be classified as iron deficient (percent iron saturation <16%) according to the criteria of Bainton and Finch (28). However, only one of these specimens was associated with a serum ferritin consistent with iron deficiency (12 μg/L). The range of serum ferritin values in these 13 specimens was 12.0 to 312 μg/L.

In summary, our findings that intrasubject day-to-day physiologic variation for serum ferritin are substantially lower than for serum iron and percent iron saturation demonstrate the danger of interpreting isolated values for serum iron and percent iron saturation. Cook et al. have shown that the combination of serum ferritin, percent iron saturation, and free erythrocyte protoporphyrin offers better accuracy in detection of iron deficiency than the use of a single assay (29).

Our findings indicate that serum ferritin values, when combined with other indicators of iron metabolism, will increase the reliability of detecting iron deficiency.

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References


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