Day-to-day variation of transferrin receptor and ferritin in healthy men and women\textsuperscript{1,3}

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ABSTRACT To determine the total day-to-day intrindividual variability of ferritin and transferrin receptor (TfR), we collected venous serum and plasma and capillary serum and plasma samples during 10 nonconsecutive days over a 4-wk period from a group of healthy men (n = 10) and women (n = 11) aged 19–46 y. On the basis of a method previously established in younger and older adults, biological ($\sigma^2_{\text{day}}$) and analytical ($\sigma^2_{\text{rep}}$) variance components were computed and summed to obtain the total day-to-day intrindividual variability ($\sigma^2_{\text{tot}}$). The total day-to-day intrindividual CV ($\text{CV}_{\text{tot}}$) was lower for TfR than for ferritin measurements. One to two discrete blood samples are required to accurately determine capillary and venous TfR, whereas capillary and venous ferritin, which have a higher CV$_{\text{tot}}$, require three to six samples. Results from the current study showing the low biological and analytical variability of TfR support the use of this new test for assessing a person's iron status. Am J Clin Nutr 1996;64:738–42.

KEY WORDS Day-to-day variation, transferrin receptor, ferritin, venous blood, capillary blood, serum, plasma

INTRODUCTION Decisions regarding which indexes to use in the assessment of a patient’s iron status are affected by the sensitivity, specificity, and variability of the measurements (1). Laboratory measures currently used to diagnose iron-deficiency anemia, including measures of serum iron, total iron-binding capacity, transferrin saturation, and erythrocyte protoporphyrin cannot distinguish between iron-deficiency anemia and the hypoproliferative anemia that accompanies infection, inflammation, or malignancy (anemia of chronic disease) (2, 3). The shortcomings of serum ferritin as a sole indicator of iron depletion have been well documented (4, 5). Many clinical conditions, including liver disease, infection, and alcohol abuse, result in an elevation of ferritin independent of a person’s iron stores (2).

In recent years, serum transferrin receptor (TfR), a measure of tissue iron availability, has been established as a quantitative measure of functional iron deficiency (2). Changes in serum TfR concentration occur earlier in the development of functional iron deficiency than do changes in erythrocyte protoporphyrin or mean corpuscular volume (6). The combination of measurements of serum ferritin and serum TfR has been proposed as a reliable method for assessing iron status (2). By measuring TfR along with hemoglobin in blood, it is possible to distinguish iron-deficiency anemia from the anemia of chronic disease (3, 7).

The reliability of any laboratory result is influenced by intrindividual physiologic variation, interindividul biological variation, subject preparation, and analytical variation (8). Additional factors known to affect the variation of measures of iron status are the site (venous compared with capillary) and type (serum compared with plasma) of sampling (5, 9, 10). Although the day-to-day variation of plasma TfR has been reported for a group of elderly women (1), there is currently a lack of data on the variation of TfR in younger adults.

The objective of the current study was to measure the total day-to-day intrindividual variation of TfR compared with ferritin in a group of healthy adults. Venous serum and plasma and capillary serum and plasma measurements were examined to determine whether the site and type of sample had an effect on variability estimates.

SUBJECTS AND METHODS

Subjects Subjects were recruited from the staff and graduate students of the Hospital for Sick Children in Toronto and from the Nutritional Sciences Department at the University of Toronto. The sample consisted of 10 men and 11 women aged 19–46 y. The subjects were required to be in good health on the basis of self-report and initial hematologic assessment (a hemoglobin value $\geq$ 120 g/L). Subjects were not excluded from participation in the study because of smoking or medication use and none of the subjects was taking supplemental iron. All subjects received a detailed description of the study protocol and signed informed consent forms. This study was approved by the Research Ethics Board at The Hospital for Sick Children.

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Experimental protocol

Subjects reported to the laboratory at designated times between 0900 and 1400 on 10 nonconsecutive days over a 4-wk period. The selection of 10 nonconsecutive days was based on the research of Borel et al (11), which suggested that between 3 and 10 independent samples from an individual are necessary to accurately assess ferritin. Blood collection was conducted between April 20 and July 7, 1994, with random start and end dates for most subjects. Subjects typically provided samples on the same days (Mondays, Wednesdays, and Fridays). No restrictions were placed on subjects except that samples were drawn at approximately the same time of day throughout the study period so that any effect of diurnal variation would be limited.

When subjects first arrived at the laboratory, a finger-prick blood sample was collected with an automatic lancet (SoftClix; Boehringer Mannheim, Indianapolis) after the finger was cleansed with a disposable alcohol prep (9). To facilitate blood flow, a mini heat pad or warm water was used to heat the finger for ~5 min before blood sampling. A rapid, firm puncture was made at a depth setting of six to maximize blood flow. Finger-prick blood samples were decanted into both microtainers containing EDTA and those with no additive (Becton Dickinson, Lincoln Park, NJ). At each collection, blood was also drawn from an antecubital vein (made visible by temporary use of a tourniquet) by using a 10-mL syringe and size 21 butterfly needle. Plasma and serum were frozen for later analysis of TfR and ferritin.

Hemoglobin was measured in triplicate on the first study day by using a cyanmethemoglobin method (kit 525; Sigma Chemical Co, St Louis). Plasma and serum ferritin were assayed in duplicate with a commercial immunoradiometric assay based on the method developed by Miles et al (12) with the FERRON II kit (Ramco Laboratories, Houston). All ferritin samples from a subject were assayed on the same day (in a single batch) in one 96-well microtiter plate to minimize interassay variation. An external reference standard (Lyphochek Anemia Control; Bio-Rad, Anaheim, CA) was assayed in duplicate on each microtiter plate for the ferritin assay. On the basis of these external controls, the within- and between-assay variations were 7.2% and 15.7%, respectively, for ferritin.

Plasma and serum TfR were measured in triplicate with an indirect enzyme-linked immunosorbent assay method developed in our laboratory (13). The standard used in the assay was TfR isolated from human placental tissue through use of the technique of Turkewitz et al (14). All TfR samples from an individual were assayed on the same day in two 96-well microtiter plates. A reference plasma sample was run in triplicate on each microtiter plate for the TfR assay. On the basis of a reference plasma sample, the average within-assay variation for TfR for all samples was 3.5% and the between-plate variation was 11.6%. Primary monoclonal antibody against human TfR was supplied by Cetus/Chiron (clone 454A12; Emeryville, CA); secondary goat antibody to the mouse enzyme conjugate against the primary antibody was supplied by Calbiochem (San Diego).

Statistical analysis

All statistical analyses were performed with SAS (SAS Institute Inc, Cary, NC). Variance component analysis (PROC VARCOMP) was used to estimate the components of variance (biological and analytical) for each of the iron status variables according to previously established methods (1, 11, 15). A single model statement included subject and day-nested-within-subject effects (1, 11). The day-nested-within-subject variance estimate represented the biological variance component (\(\sigma_{\text{day}}^2\)), whereas the error variance estimate (\(\sigma_{\text{rep}}^2\)) represented the analytical variation within days. The estimates of biological and analytical variation were determined for each subject, for males and females, and for all subjects. It is not uncommon in studies that involve repeated blood sampling that a certain proportion of the samples will be outliers (16, 17). In the current study, outlying days and replicates for each subject were identified and eliminated from the data set before variance estimates were calculated.

The biological and analytical CVs were calculated as the SD (\(\sigma_{\text{day}}^2\) and \(\sigma_{\text{rep}}^2\)) divided by the mean and expressed as a percentage. The total intradiurnal variation (\(\sigma_{\text{tot}}^2\)) was calculated from the two variance components as follows: \(\sigma_{\text{tot}}^2 = \sigma_{\text{day}}^2 + \sigma_{\text{rep}}^2\). The \(\sigma_{\text{tot}}^2\) was used to calculate the total CV (CV\(_{\text{tot}}\)) as well as the predicted number of blood samples that would have to be obtained to approximate the “true” value (the 10-sample average for an individual). The predicted sample number estimate (S) was defined as the number of discrete blood samples necessary to achieve a mean value that was within 20% of the true value 95% of the time. The \(S\) value was calculated with the equation for individuals adapted from Basiotis et al (18) as follows:

\[
S = \frac{(Z_a)^2 \times (\sigma_{\text{tot}}^2/\mu)^2 \times (\sum v_{1-10}/n)^2}{(1)}
\]

where \(Z_a\) is 1.96 for \(a = 0.05\) (19), \(A\) is 20% or 0.2, \(\nu\) represents the individual ferritin and TfR values from a subject, and \(n\) is the total number of samples per subject. The \(\sigma_{\text{tot}}^2\) and \(S\) were determined for each subject and the average \(S\) was calculated for males, females, and the entire group.

There is no known statistical method for determining whether significant differences exist between CV calculations. Therefore, comparisons between the CV calculations for ferritin and TfR measurements are not statistically based.

RESULTS

The means, variance components, analytical and biological CVs, and total intradiurnal CVs for each iron index for all samples from all subjects are shown in Table 1. Overall, the total intradiurnal CVs were higher for ferritin measurements than for TfR. For both ferritin and TfR, the biological CVs (CV\(_{\text{day}}\)) were at least twofold greater than the analytical CVs (CV\(_{\text{rep}}\)). Venous serum and plasma ferritin, with a CV\(_{\text{tot}}\) of 19.1% and 18.9%, respectively, appeared to be the least variable ferritin measurements. Additionally, venous ferritin values were consistently less variable than capillary ferritin values. Capillary serum and plasma TfR had the lowest variability at 10.8% and 10.6%, respectively; additionally, unlike ferritin, capillary TfR samples were less variable than venous TfR samples.

The means, analytical and biological CVs, and total intradiurnal CVs for each iron index segregated by sex are also shown in Table 1. The biological and analytical CVs were similar between males and females for all TfR measurements,
whereas the variability of ferritin measurements was consistently higher in females. Generally, total intraindividual variability for TfR and ferritin (CV$_{tot}$) was higher for females than males.

The number of discrete blood samples that would have to be taken from an individual to derive a measurement within 20% of the true value (the mean of the 10 samples) 95% of the time are shown in Table 2. A single capillary serum or plasma sample would provide an accurate estimate of the true value of TfR regardless of sex. In contrast, three discrete venous (plasma and serum) and capillary serum samples would be required from an individual to provide an accurate estimate of the true ferritin value regardless of sex, whereas six capillary plasma samples would be required in women to obtain the same degree of accuracy and confidence.

**DISCUSSION**

The high variability and lack of specificity of serum ferritin as an index of iron status and the potential clinical utility of TfR as a reliable measure of tissue iron status prompted this investigation. This is the first study to report the variability of repeated values of venous, capillary, serum, and plasma TfR measurements. This study shows that capillary serum and plasma TfR have the lowest analytical and biological variation compared with all other ferritin and TfR measurements and that a single capillary serum or plasma TfR sample is sufficient to obtain an accurate, indirect measure of a person’s tissue iron availability.

In general, reliability refers to both measurement error (reproducibility of repeated measurements of the same sample) and within-person variation (reproducibility of the results from repeated samples collected at different times from the same subject) (20). An iron index with small biological and analytical variability presumably should be a more reliable measurement. In the current study, both measurement error (analytical variation) and within-person variation (biological variation) were measured. The analytical CV (CV$_{rep}$) of venous serum ferritin and of capillary plasma ferritin for all subjects in our study was similar to values published previously (1, 11). In contrast, the CV$_{rep}$ for venous plasma TfR (3.8%) was relatively smaller than that reported previously (6.5%) (1). Differences in the analytical variabilities between studies may be explained by several factors, including differences in the specific ferritin and TfR assays used (1, 11) and random measurement error.

The results of the current study concur with previous findings that biological variation is the main contributor to total intraindividual variation for various iron measurements (1, 11). Low biological variability is desirable if the intent is to take either serial measurements or a single sample from an individual (21). The biological CVs for venous and capillary ferritin measurements in the present study were higher than those reported in other studies (1, 11, 22). Specifically, the biological and total intraindividual variation for venous serum ferritin (26.7% and 27.4%) for younger women in this study was higher than the variability reported in older women (9.7% and 11.2%) (1). The biological variation for capillary plasma ferritin for all subjects in the current study (21.1%) was comparable with that reported for a similar cohort (17.6%) (11). In addition, the values for CV$_{tot}$ for capillary plasma ferritin in the younger men and women in the current study (19.1% and 27.8%) were similar to values published previously for younger adults (15.2% and 26.8%) (11). The results of the current study (Table 1) are consistent with the observation that

**TABLE 1**

Means, variance components, and CVs for samples from all subjects combined and from males and females separately

<table>
<thead>
<tr>
<th>Index</th>
<th>$\bar{x}$</th>
<th>$\sigma^2_{rep}$</th>
<th>$\sigma^2_{day}$</th>
<th>CV$_{rep}$</th>
<th>CV$_{day}$</th>
<th>CV$_{tot}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
<td>All</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS (n = 402)</td>
<td>44.1</td>
<td>7.2</td>
<td>63.7</td>
<td>5.8</td>
<td>6.1</td>
<td>6.1</td>
</tr>
<tr>
<td>VP (n = 396)</td>
<td>43.2</td>
<td>5.9</td>
<td>61.0</td>
<td>5.0</td>
<td>6.6</td>
<td>5.6</td>
</tr>
<tr>
<td>CS (n = 380)</td>
<td>47.0</td>
<td>8.0</td>
<td>118.9</td>
<td>4.7</td>
<td>6.2</td>
<td>6.0</td>
</tr>
<tr>
<td>CP (n = 379)</td>
<td>45.3</td>
<td>13.6</td>
<td>91.0</td>
<td>6.0</td>
<td>11.4</td>
<td>8.1</td>
</tr>
<tr>
<td>TfR (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS (n = 579)</td>
<td>3.87</td>
<td>0.023</td>
<td>0.21</td>
<td>4.2</td>
<td>4.0</td>
<td>3.9</td>
</tr>
<tr>
<td>VP (n = 608)</td>
<td>3.63</td>
<td>0.019</td>
<td>0.23</td>
<td>3.4</td>
<td>4.0</td>
<td>3.8</td>
</tr>
<tr>
<td>CS (n = 579)</td>
<td>3.72</td>
<td>0.021</td>
<td>0.14</td>
<td>3.2</td>
<td>4.0</td>
<td>3.9</td>
</tr>
<tr>
<td>CP (n = 596)</td>
<td>4.09</td>
<td>0.018</td>
<td>0.17</td>
<td>3.0</td>
<td>4.0</td>
<td>3.3</td>
</tr>
</tbody>
</table>

1 $\sigma^2_{rep}$ is the analytical variance component estimate for each iron index calculated for all subjects, $\sigma^2_{day}$ is the biological variance component estimate for each iron index calculated for all subjects, CV$_{rep}$ and CV$_{day}$ are the CV for the analytical and biological variance component estimates for each iron index, and CV$_{tot}$ is the total CV and is calculated from $\sigma^2_{rep}$ and $\sigma^2_{day}$ for males, females, and all subjects. VS, venous serum; VP, venous plasma; CS, capillary serum; CP, capillary plasma; Tfr, transferrin receptor.

**TABLE 2**

Number of samples required to estimate ferritin and transferrin receptor (TfR) with 95% confidence and 20% accuracy

<table>
<thead>
<tr>
<th>Index</th>
<th>Males</th>
<th>Females</th>
<th>All subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS Ferritin</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>VP Ferritin</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>CS Ferritin</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CP Ferritin</td>
<td>3</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>VS TfR</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>VP TfR</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CS TfR</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CP TfR</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Number of samples are rounded to the nearest whole number. VS, venous serum; VP, venous plasma; CS, capillary serum; CP, capillary plasma.
mean plasma ferritin values are lower in younger persons than in healthy, active elderly women (1, 11, 23). A plausible explanation for the difference in the absolute ferritin values as well as the variability in women of different ages is the signif-
ificant loss of iron associated with menstruation in younger populations (an average loss of 1.3 mg/d) (24). Kim et al (25) showed lower means and significantly different and variable ferritin values across phases of the menstrual cycle in women aged 18–44 y. Hence, age appears to play a role in the variability of ferritin concentrations in women.

This is the first study to report the variability of TfR measurements in younger adults. The biological variation for venous plasma TfR for younger women in the current study (13.8%) is comparable with that reported for older women (10.9%) (1). The intraindividual variability for venous and capillary TfR measurements for the men and women (Table 1) in this study were also similar to the variability reported for venous plasma TfR for elderly women (12.7%) (1). On the basis of previous research that determined TfR concentrations to be no different between subjects older and younger than 35 y of age (3, 26), it has been hypothesized that intraindividual variability would be unaffected by age in healthy adults (1). Our results confirm that TfR variability is affected neither by age nor sex.

Results of the current study, which show that two venous plasma TfR samples will provide a reliable estimate of the true TfR value for women, confirm previous research (1). In con-
trast, a single measurement of ferritin may provide an unreliable estimation of iron status, independent of whether the sample is capillary, venous, serum, or plasma. In the current study, all samples from an individual were analyzed in a single batch on the same day. Thus, an estimation of batch-to-batch analytical variance could not be determined. Previous studies have shown, however, that biological variation, total day-to-
day variation, and predicted number of samples to achieve a true value with ferritin or TfR are not affected by the addition of batch-to-batch variance (27). We conclude, therefore, that TfR is a less variable measurement than ferritin.

Clinically significant differences in reported values of iron indexes from similar subjects may result from alternate sites of sampling (venous compared with capillary) and type of blood used for analysis (serum compared with plasma). Several studies have reported small differences in mean values of iron indexes (excluding TfR) between capillary and venous measurements (5, 10). In the current study, mean capillary plasma and serum ferritin values were higher than comparable venous samples, although the differences were small and not significant. There was, however, greater total intraindividual variability of capillary than venous ferritin values, confirming previous observations of a small difference in the variability due to the site of sampling (10). The current research confirms previous work that found no differences in the values of venous serum and plasma ferritin samples (12).

No previous reports have commented on differences in TfR values based on site of sampling or type of blood used for analysis. Our results show no differences between capillary plasma and serum TfR values compared with venous TfR values. However, unexpectedly, total intraindividual variability of capillary values was lower than that of venous values. [Capillary samples, which are often diluted by tissue fluid, would normally be expected to be more variable (5).]

Specificity and sensitivity ratings, along with measures of reliability, influence decisions regarding the suitability of a marker for population studies. Serum TfR concentrations reflect cell surface TfR numbers. Serum TfR has been shown to rise significantly with tissue iron deficiency and with increased erythropoiesis (3, 7, 24, 28). TfR is not an acute-phase reactant (like ferritin), and concentrations of TfR appear not to be elevated in persons with clinical conditions such as acute infection, chronic liver disease, or anemia of chronic disease (3, 7, 29). Thus, TfR has been touted as a more specific and sensitive measure of iron deficiency than the more traditional indexes (3, 7, 28). We found capillary TfR to be less variable than either venous or capillary ferritin measurements in healthy adults. Other investigators have shown wide intraindividual variability in most conventional measures of iron status, including serum iron (1, 11, 22, 30, 31) and transferrin saturation (1); the exception is total iron-binding capacity, which has a small intraindividual variability (1, 30, 31). In agreement with Cook et al (2), we propose that the optimal approach to fully assess the iron status of a population may be to use venous plasma or serum ferritin samples, which are known to reflect the magnitude of iron depletion, and capillary or venous plasma or serum TfR samples, which are sensitive, specific, and potentially reliable measures of tissue iron availability. Results from the current study showing the low biological and analytical variability of TfR support the use of this new tool for assessing iron status.

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REFERENCES

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