Serum Transferrin Receptor Concentrations during Normal Pregnancy, Jong Weon Choi,1,4 Moon Whan Im,2 and Soo Hwan Pai1 (Departments of 1 Clinical Pathology and 2 Obstetrics, College of Medicine, Inha University Hospital, 7-206, 3-ga, Shinheung-dong, Jung-gu, Inchon 400-103, Korea; * author for correspondence: fax 82-32-890-2529, e-mail jwchoi@inha.ac.kr)

Among the physiologic changes during pregnancy, the disproportionate increases in plasma volume and red cell mass produce a decrease in hemoglobin concentration. Pathologic anemia of pregnancy is mostly attributable to iron deficiency associated with increased requirements and inadequate intake (1), but physiologic changes also occur in iron markers. Iron plays an essential role in a spectrum of metabolic processes. Cellular iron uptake is facilitated by transferrin receptor (TfR)-mediated endocytosis (2). Serum TfR (sTfR) is a sensitive indicator of iron deficiency in inflammatory states and in the anemia of chronic diseases because its concentration is not influenced by the acute phase response (3). The sTfR concentration is closely related to erythroid TfR turnover; therefore, sTfR may be a useful marker to monitor erythropoiesis in various clinical situations (4).

Erythropoiesis is a highly dynamic process and can be monitored by quantitative reticulocyte counting. Flow cytometric analysis of reticulocytes provides a quantitative reticulocyte measurement with high sensitivity and precision (5). The fluorescence intensity of reticulocytes is directly proportional to erythrocyte RNA content, and reticulocytes can be divided into three subpopulations by fluorescence intensity: low fluorescence reticulocytes, middle fluorescence reticulocytes, and high fluorescence reticulocytes (5, 6). The reticulocyte maturity index (RMI) is calculated from the proportion of reticulocyte subpopulations and can be used as the earliest and most sensitive predictor of bone marrow erythropoiesis (6).

The sTfR concentration and the RMI during normal pregnancy have not been studied extensively, and the reported values for sTfR show a wide range of discrepancies. Therefore, in the present study, we investigated the sequential changes in sTfR concentrations according to gestational age in healthy pregnant women without anemia and iron deficiency. We also evaluated the relationship between the sTfR concentrations and the RMI during normal pregnancy.

We measured sTfR, hematologic and iron markers, and reticulocyte subpopulations in 355 apparently healthy pregnant, postpartum, or nonpregnant subjects (Table 1). We excluded subjects with evidence of anemia or iron deficiency, or with complications during pregnancy. Gestational age was determined by sonographic examination and the date of the last menstrual period. Second trimester was defined as 12.1–24.0 weeks of gestation. The study was approved by the Committee of Ethics at the Inha University Hospital, and informed consent was obtained from all subjects. Maternal blood (9 mL) was drawn into iron-free evacuated tubes before the introduction of iron supplementation when the subjects visited the outpatient Department of Obstetrics. After blood samples were collected, all pregnant women were supplemented with one 256-mg tablet of ferrous sulfate (80 mg of elemental iron) per day; however, vitamin B₁₂ and folate were not supplemented. The compliance rate for the iron supplementation was checked by personal interviews throughout the pregnancy. Complete blood cell counts and reticulocyte subpopulations were measured with EDTA-anticoagulated blood within 3 h after collection. Sera for the measurement of sTfR were stored in 500-μL aliquots at −70 °C until analysis.

Anemia was defined as a hemoglobin <110 g/L in pregnant women by WHO criteria (7). Nonanemic women who had a serum ferritin concentration <12 μg/L were classified as having iron deficiency. Routine complete blood cell counts and red cell indices were determined with the electronic counter SE 9000 (Sysmex). Reticulocytes and their subpopulations were analyzed automatically by the R-3000 flow cytometry (Sysmex). RMI was calculated from the equation, RMI = [(middle fluorescence reticulocytes + high fluorescence reticulocytes) × 100]/low fluorescence reticulocytes, and was expressed as the percentage (8). The corrected reticulocyte count was calculated, based on a normal hematocrit of 45%, from the following formula: corrected reticulocyte count (%) = (subject’s hematocrit/45) × reticulocyte count (%). Serum iron and total iron-binding capacity were assayed with the Hitachi 747 automatic chemical analyzer (Hitachi), and ferritin was measured by the ACS-180 chemiluminescence assay (Chiron). The sTfR was measured immunoenzymetrically using IDEATM sTfR kits (Orion Diagnostica). The intraassay CVs (n = 20) for three samples (mean sTfR, 1.3–6.5 mg/L) were 3.2–5.4%; the interassay CVs calculated from duplicate results in 10 subsequent assays were 3.5–6.1%. Data analysis was conducted using SAS 6.12 for Windows (SAS Institute). The Mann–Whitney test was used to compare the difference of values. Correlation coefficients were calculated by the Spearman method for ranked values. P \leq 0.01 was considered statistically significant.

The changes in sTfR concentrations and iron markers during normal pregnancy are summarized in Table 1. The sTfR concentration of pregnant women in the first trimester did not differ significantly from that of nonpregnant women (P = 0.215). However, the sTfR concentration in the second trimester was significantly higher than that in the first trimester (P <0.01). The mean sTfR value increased gradually from the second trimester of pregnancy and reached maximal concentration in the third trimester. The sTfR concentration decreased abruptly within 1–4 weeks after delivery. No significant difference in sTfR concentration was observed between the women 12–16 weeks after delivery and nonpregnant women. Therefore, on the basis of our results, we believe that the sTfR concentrations increase with gestational age during pregnancy and return to nonpregnancy values 12 weeks after delivery.

Our data for the sTfR concentration during pregnancy are in accord with another study showing that the value of sTfR increases from early to late pregnancy (9). How-
ever, contradictory results for the sTfR concentration during pregnancy also have been reported. Carriaga et al. (10) reported that the mean sTfR concentration of pregnant women in the third trimester did not differ from the concentration in nonpregnant individuals and that sTfR concentrations were not influenced by pregnancy per se. They reported that the sTfR concentration for healthy male and female volunteers was 5.63 mg/L, which differs substantially from our result for nonpregnant women. In our study, we selected nonpregnant women in the same age group as the pregnant women, who had no iron deficiency, iron deficiency anemia, or history of pregnancy. In our previous study (11), the mean sTfR concentration in healthy adults was 2.13 mg/L, which was similar to the value for the nonpregnant women in the present study. The differences between our studies and the study by Carriaga et al. (10) seem to be derived from the use of different methods for the sTfR assay and the different ages in the control group.

It has been suggested that the low sTfR concentration in early pregnancy is caused by reduced erythropoiesis, whereas the increase sTfR concentration in late pregnancy reflects increased erythropoiesis (9). However, there are few reports on the direct relationship between the sTfR concentration and RMI in healthy pregnant women. In this study, we evaluated the changes in reticulocyte subpopulations and RMI during pregnancy. In our results, the corrected reticulocyte counts were twofold higher in the third trimester than in the first trimester. The RMI was threefold higher in the third trimester than in the first trimester. On the other hand, there were no significant differences in corrected reticulocyte counts and RMI between the pregnant women in the first trimester and the nonpregnant women. As shown in Fig. 1, the sTfR concentrations increased similarly to the changes in corrected reticulocyte counts and RMI during pregnancy. The sTfR concentrations correlated significantly with the corrected reticulocyte counts ($r = 0.61; P < 0.01$) and the corrected reticulocyte count ($r = 0.61; P < 0.01$).

### Table 1. Change in sTfR concentrations and iron markers according to gestational age during normal pregnancy.

<table>
<thead>
<tr>
<th>Population</th>
<th>Age, years</th>
<th>No. of cases</th>
<th>sTfR, mg/L</th>
<th>sTfR reference interval, mg/L</th>
<th>s-Iron, µg/L</th>
<th>TIBC, µg/L</th>
<th>TS, %</th>
<th>Ferritin, µg/L</th>
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</thead>
<tbody>
<tr>
<td>Pregnant women</td>
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<tr>
<td>1st trimester</td>
<td>22–38</td>
<td>62</td>
<td>2.31 ± 0.41</td>
<td>1.49–3.61</td>
<td>1029.8 ± 215.6</td>
<td>3332.5 ± 404.3</td>
<td>29.9 ± 11.3</td>
<td>54.3 ± 14.4</td>
</tr>
<tr>
<td>2nd trimester</td>
<td>23–37</td>
<td>65</td>
<td>3.92 ± 0.43</td>
<td>2.93–4.98</td>
<td>908.4 ± 229.3</td>
<td>3491.6 ± 416.7</td>
<td>26.1 ± 8.7</td>
<td>49.1 ± 13.9</td>
</tr>
<tr>
<td>3rd trimester</td>
<td>21–39</td>
<td>56</td>
<td>4.74 ± 0.49</td>
<td>3.52–5.94</td>
<td>894.2 ± 264.8</td>
<td>3658.2 ± 475.4</td>
<td>24.4 ± 7.2</td>
<td>47.5 ± 10.8</td>
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<tr>
<td>Postpartum women</td>
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<tr>
<td>1–4 weeks</td>
<td>25–41</td>
<td>51</td>
<td>3.43 ± 0.51</td>
<td>2.42–4.49</td>
<td>912.5 ± 238.8</td>
<td>3514.8 ± 440.7</td>
<td>25.9 ± 9.4</td>
<td>49.6 ± 12.6</td>
</tr>
<tr>
<td>12–16 weeks</td>
<td>24–39</td>
<td>57</td>
<td>2.21 ± 0.45</td>
<td>1.41–3.52</td>
<td>983.4 ± 216.7</td>
<td>3249.5 ± 401.3</td>
<td>30.3 ± 10.3</td>
<td>51.1 ± 14.3</td>
</tr>
<tr>
<td>Nonpregnant women</td>
<td>21–38</td>
<td>64</td>
<td>2.17 ± 0.36</td>
<td>1.34–3.27</td>
<td>1059.6 ± 201.1</td>
<td>3310.7 ± 398.6</td>
<td>32.0 ± 9.8</td>
<td>57.4 ± 13.1</td>
</tr>
</tbody>
</table>

a sTfR concentrations (mean ± SD) in the different stages of pregnancy were compared by Mann–Whitney test: 1st trimester vs nonpregnant women, $P = 0.215$; 1st trimester vs 2nd trimester, $P < 0.01$; 2nd trimester vs 3rd trimester, $P < 0.01$; women 1–4 weeks post partum vs women 12–16 weeks post partum, $P < 0.01$; women 12–16 weeks post partum vs nonpregnant women, $P = 0.317$.

b Nonparametric method was used to calculate reference intervals (95% confidence intervals).

c s, serum; TIBC, total iron-binding capacity; TS, transferrin saturation.
d There were no significant differences in iron markers according to gestational age.

![Fig. 1. Comparison of the changes in RMI, corrected reticulocyte count (c-retic), and sTfR concentrations during normal pregnancy.](image-url)

sTfR concentrations ($\Delta$) increased with the RMI (●) and corrected reticulocyte count (♦), and peaked in the third trimester. The sTfR concentrations correlated significantly with the RMI ($r = 0.61; P < 0.01$) and the corrected reticulocyte count ($r = 0.61; P < 0.01$).
believe the increased sTfR concentration during pregnancy reflects increased erythropoietic activity in this period.

In conclusion, we found that sTfR concentrations do exhibit gestational age-related changes during pregnancy: the value of sTfR increased with gestational age during pregnancy and returned to nonpregnancy values 12 weeks after delivery. Increases in sTfR concentrations during pregnancy seem to be influenced more by increased erythropoietic activity than by iron depletion in this period.

References

Assay for Free and Total Carnitine in Human Plasma Using Tandem Mass Spectrometry, Robert D. Stevens, Steven L. Hillman, Steven Worthy, Doris Sanders, and David S. Millington (Duke University Medical Center, Research Triangle Park, NC 27709; * address correspondence to this author at: Duke University Medical Center, PO Box 14991, 99 T.W. Alexander Drive, Research Triangle Park, NC 27709; fax 919-549-0709, e-mail rdjestev@acpub.duke.edu)

Measurements of free and total carnitine in plasma are important in the diagnosis and clinical management of patients with carnitine deficiency syndromes and certain inborn errors of metabolism (1). The most popular analytical methods are based on the enzymatic reaction in which an acetyl group is transferred from acetyl-CoA to carnitine with release of free CoA (2). A spectrophotometric assay based on this method, using a Cobas centrifugal analyzer (Roche Diagnostics), has been in use in this laboratory for the past several years (3). Limitations of the Cobas enzymatic method in our view include the initial filtration step, which requires a minimum sample volume of 300 μL, and the number and cost of the reagents required. Assay results are critically dependent on two labile reagents, the enzyme carnitine acetyltransferase and the color reagent 5,5′-dithiobis(2-nitrobenzoic acid). We have also observed that patients on valproic acid therapy sometimes give total carnitine values that are spuriously low for reasons that are not clear. Another limitation of the Cobas assay is that acylcarnitines with chain lengths >10 are lost in the filtration step (3), and the method will underestimate the total carnitine content of patients with defects in the oxidative degradation of long-chain or multiple acyl-CoAs, especially when metabolically decompensated. We have emphasized previously that the most valuable applications of the routine Cobas assay are the identification of patients with carnitine deficiency and the monitoring of patients on carnitine therapy (3).

We have developed a new carnitine assay method based on isotope-dilution tandem mass spectrometry (TMS), which is inherently more straightforward and accurate than previously reported methods. This is true primarily because it has absolute molecular specificity, uses an isotope-labeled internal standard to compensate for any losses or variance resulting from the sample preparation, and has no known chemical interference. The new assay procedure has been developed using a total specimen volume of 150 μL, but it can easily be scaled down to much smaller volumes. A fixed amount of stable-isotope-labeled free carnitine is added, and one aliquot of the mixture is used directly for the free carnitine assay. Another aliquot is subjected to base-catalyzed hydrolysis before measuring total carnitine. The analyte is not subjected to acid-catalyzed esterification, thus avoiding any inaccuracies in free carnitine measurement from the hydrolysis of acylcarnitines or incomplete derivatization. The specimen can be either serum or plasma, and any anticoagulant can be used. In this report, the specimens from patients were predominantly heparinized plasma (green-topped tubes).

To the unfiltered specimen (150 μL), we added 30 μL of a 250 μmol/L solution of the internal standard, 3H-H3-1-carnitine (Cambridge Isotope Laboratories). After vortex mixing, 50 μL of the mixture was applied to a 6-mm disk of cotton fiber filter paper (no. 900; 1.88 mm thick; Schleicher & Schuell) placed in a well of a 96-well microtiter plate. The remaining mixture was hydrolyzed by the addition of 10 μL of 1 mol/L KOH and incubation at 65 °C for 15 min, and then neutralized by the addition of 10 μL of 1 mol/L HCl. The mixture was centrifuged, and an aliquot of the supernate (50 μL) was applied to a disk of cotton fiber placed in a well of a second microtiter plate as described. The specimens were air-dried in an oven at 40 °C for 30 min, cooled to ambient temperature, and then extracted with 200 μL of a mixture of methanol, water, and 6 mol/L HCl (400:100:0.5, by volume). After 20 min on an orbital shaker, ~100 μL of extract was transferred to the adjacent well of the next row and was diluted with an additional 100 μL of the acidified methanol/water mixture. Up to 48 samples were processed for either free or total carnitine on a single microtiter plate. The microtiter plates were sealed with a thin sheet of aluminum foil (Fisher Scientific) to limit solvent evaporation before analysis.

Aliquots of the samples (20 μL) were injected directly into the electrospray ion source of a tandem mass spec-