Relationship Among Plasma Iron, Plasma Iron Turnover, and Reticuloendothelial Iron Release

By Tatsumi Uchida, Tsuyoshi Akitsuki, Hideo Kimura, Tetsugoro Tanaka, Shin Matsuda, and Shigeo Kariyone

A circadian rhythm was demonstrated in 10 males and 10 females with respective mean decreases in plasma iron concentration at 18 hr of 62% and 47% of morning values. Ferrokinetic studies performed on 5 normal males and 5 normal females showed a more rapid disappearance rate and lower plasma iron turnover in the evening. Parallel studies were done on 6 normal males in the morning and 4 normal males in the evening of the release of reticuloendothelial iron at 8 and 18 hr after intravenous injection of 59Fe chondroitin ferrous sulfate. The 6-hr release in the morning was 54.1% and in the evening 25.9%. Composite data from morning and evening showed a correlation between plasma iron level and plasma iron turnover \( r = 0.76, p < 0.001 \). A similar correlation existed between the plasma iron level and the percent of radioiron released from the reticuloendothelial system \( r = 0.67, 0.02 < p < 0.05 \). These data are consistent with a fluctuating iron release from the reticuloendothelial cell in normal subjects, which would account for the diurnal variation in plasma iron.

It is well known that the plasma iron concentration shows a diurnal variation, showing a morning peak and an evening nadir. These changes might suggest a variation in the release of iron to plasma by the reticuloendothelial (RE) system. The present study is an attempt to evaluate physiologic relationship between plasma iron and internal and RE iron kinetic studies by using the parameters of plasma iron concentration, plasma iron turnover (PIT), and RE iron release in daily variation.

MATERIALS AND METHODS

The diurnal variation in plasma iron was studied in 20 normal subjects (10 males and 10 females) who worked by day and slept by night. Fifteen milliliters of venous blood was taken at 0:00, 8:00, 12:00, 16:00, and 20:00. Ferrokinetic studies were performed twice at 8:00 and at 18:00 in 5 normal males and 5 females. RE iron kinetics were performed on 6 normal men at 8:00 and on another at 18:00. In accordance with the Helsinki Declaration, all the subjects were informed about the procedure, and their consent was obtained.

The plasma iron was determined according to the recommendation of the Standardization Committee of the International Society of Hematology (SCIH). Ferrokinetic studies were performed twice in the morning and evening on each patient. The first group (cases 1, 2, 3, 6, 7) was done at 8:00 and then 18:00, and the second group (cases 4, 5, 8, 9, 10) was done at 18:00 and 8:00 on the following day. Five milliliters of heparinized plasma obtained in the morning or evening was labeled with 5 μCi of 59Fe-citrate for 30 min before injection (59Fe was supplied by the Radichemical Centre, England). Five milliliters of plasma that had more than 100 μg/dl of UIBC was used in order to ensure that adequate binding of 59Fe to transferrin occurs. Five milliliters of blood was withdrawn at 5, 15, 30, 60, and 120 min after the infusion in the morning or evening. The plasma iron disappearance rate (PIT T½) was determined for all points from the plasma radioactivity by the least-squares method. In the first or second group, the plasma 59Fe radioactivity in the evening or morning was corrected for remaining radioactivity from the preceding study. The radioactivity before the injection was subtracted from that after the second injection, although the correction was small. The plasma iron turnover rate (PIT) was calculated according to the following formula:

\[
PIT \ (mg/dl \ whole \ blood/day) = \frac{PI (mg/dl)}{PIT T^{1/2}} \times 100 - (Hct \times 0.92) \]

\[\frac{100}{100}\]

The circadian rhythm of RE iron kinetics was investigated. The labeling of RE cells was carried out by modification of the method of Bentley and coworkers. Briefly, 59Fe chondroitin ferrous sulfate (5 μCi in 3.3 μg of iron, Dainabot Radioisotope Laboratory, Tokyo, Japan) was infused intravenously together with 40 μCi of 59Fe-labeled transferrin. Radiation doses in a 70-kg subject from a 10-μCi intravenous injection of 59Fe are 230 in the whole body, 700 in the blood, and 470 mrad in the bone marrow. Radiation dosage from 40 μCi of 59Fe are 128 mrad in the whole body, 1920 in the blood, and 640 in the bone marrow. Venous blood samples were taken at 15, 30, 60, 120, 240, and 360 min after the infusion. Radioactivity of 59Fe and 59Fe in 2 ml of plasma was measured simultaneously by a modification of the Eakins and Brown liquid scintillation counting method. The 59Fe-transferrin, which reappeared in the plasma, was expressed as the percentage of plasma radioactivity to the initial 59Fe dose. This study could not be performed twice in one day because the released 59Fe radioactivity still remained after 9–12 hr, so two groups of people having definite diurnal variations of plasma iron were used. The cases studied in the morning or evening were selected at random.

Calculation of RE release of iron was based on the mean clearance rate of 59Fe-transferrin and the reappearance curve of transferrin 59Fe derived from chondroitin ferrous sulfate. The values of the reappearance curve of 59Fe were determined at 0.5-hr intervals, and the amount of 59Fe released from RE system over the first 6 hr was calculated at 8:00 and 18:00 in normal individuals.

RESULTS

Plasma Iron

The plasma iron level reached its peak at 8:00 and nadir at 0:00 in males and 2:00 in females. Table 1 shows the diurnal iron concentration in 10 males and 10 females. There are significant differences in plasma iron in the morning and in the evening in males (123.9 ± 10.6 μg/dl, 76.5 ± 6.3, mean ± SEM,
Diurnal Variation in Plasma Iron in Normal Males and Females

Curves were fitted by least squares, and a statistical estimate was made for each individual studied. PIT T/2 at 18:00 is more significantly rapid than at 8:00 except for 2, 3, and 5 in females. PIT (mg/dl whole blood/day) is $0.84 \pm 0.09$ versus $0.57 \pm 0.01$ (NS) in males and $1.05 \pm 0.21$ versus $0.43 \pm 0.04$ ($0.02 < p < 0.05$) in females.

Diurnal RE Iron Kinetics

When 5 μCi of $^{59}$Fe chondroitin sulfate iron was injected, the radioactivity in plasma decreased rapidly, showing an exponential line with a half-life of 4–5 min. Then, when the fall in plasma radioactivity reached a minimum, there was a rapid rise in $^{59}$Fe transferrin activity within the first 15 min after the injection of the colloid. This finding suggested that the colloidal iron was taken up rapidly by RE cells and reappeared in the plasma as transferrin iron. RE iron kinetics in normal individuals in which the plasma iron concentration fluctuated were performed twice at 8:00 and 18:00. The results of the radioactivity of $^{59}$Fe-

<table>
<thead>
<tr>
<th>Case</th>
<th>Plasma Iron (μg/dl)</th>
<th>PID T/2 (min)</th>
<th>PIT (mg/dl Whole Blood/Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td>8:00</td>
<td>18:00</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>160</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>180</td>
<td>65</td>
<td>107</td>
</tr>
<tr>
<td>4</td>
<td>130</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>140.0 ± 17.0</td>
<td>71.4 ± 9.8*</td>
<td>99.0 ± 2.7</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>8:00</td>
<td>18:00</td>
</tr>
<tr>
<td>1</td>
<td>145</td>
<td>25</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>50</td>
<td>105</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>50</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>75</td>
<td>105</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>128.0 ± 9.8</td>
<td>46.0 ± 8.9*</td>
<td>85.4 ± 10.9</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.

*Significant difference between 8:00 and 18:00 (0.001 < p < 0.01).
†(0.02 < p < 0.05).
‡No significant difference.

Table 3. Percentage of $^{59}$Fe Radioactivity at a Given Time in the Plasma to the Injected Dose of $^{59}$Fe Chondroitin Ferrous Sulfate in Normal Individuals

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma Iron (μg/dl)</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00</td>
<td>(n = 6)</td>
<td>108.3 ± 7.6</td>
<td>3.1 ± 1.8</td>
<td>6.7 ± 1.7</td>
<td>12.1 ± 1.7</td>
<td>16.4 ± 2.0</td>
<td>15.9 ± 2.2</td>
</tr>
<tr>
<td>18:00</td>
<td>(n = 4)</td>
<td>67.8 ± 10.5</td>
<td>0.6 ± 0.6</td>
<td>2.3 ± 0.8</td>
<td>6.3 ± 0.4</td>
<td>7.4 ± 0.9</td>
<td>6.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>0.02 &lt; p &lt; 0.05</td>
<td>NS</td>
<td>0.01 &lt; p &lt; 0.02</td>
<td>0.01 &lt; p &lt; 0.02</td>
<td>0.02 &lt; p &lt; 0.05</td>
<td>0.01 &lt; p &lt; 0.02</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
</tbody>
</table>

p, Significance of difference between 8:00 and 18:00.
NS, no significance.
Values are mean ± SEM.
transferrin released from RE system are shown in Table 3. The percentage of $^{59}$Fe-transferrin radioactivity was significantly higher at 8:00 than at 18:00.

**The Amount of Iron Released From RE System**

The amount of iron released from RE cells up to 6 hr as calculated by the method of Fillet et al. was an average of 54.1% of the administered $^{59}$Fe radioactivity at 8:00, 25.9% at 18:00 in normal subjects.

**Correlation Between Plasma Iron and PIT or RE Iron Release**

Figure 1 shows the relationship between the plasma iron level and PIT (Fig. 1A) or percentage of radioactivity released from the RE system at 240 min (Fig. 1B), which correlated well with each other (Fe versus PIT: $r = 0.76$, $p < 0.01$; Fe versus percent radioactivity released: $r = 0.67$, 0.02 $< p < 0.05$).

**DISCUSSION**

The plasma iron concentration shows circadian variations, exhibiting a morning peak and an evening nadir. In these studies, the peak was at 8:00 and the nadir at 20:00 or 0:00. The present studies confirm the observations that the plasma iron concentration in man shows definite diurnal variation; however, it is well established that TIBC and serum ferritin do not vary. Although there are no reports concerning the RE iron kinetics in the morning and evening in man, it might be suggested that the circadian fluctuation is due to variations in the release of iron to the plasma by the RE system.

Ferrokinetic studies were performed in the morning and the evening. There was a 32% mean decrease in PIT in the male and a 59% difference in the female between morning and evening studies. A good correlation was shown between plasma iron level and PIT. Since there has been shown to be random loading of the open iron binding sites on transferrin, the level of the plasma iron correlates with the amount of diferric transferrin. Diferric transferrin has been shown to have a greater capacity than monoferric transferrin in delivering iron. Thus, the increased morning PIT would be explainable on the basis of the increase in plasma iron concentration.

The RE iron kinetics have demonstrated a significantly greater RE iron release in the morning. Since there was a direct correlation between plasma iron concentration and radioactivity released from the RE system ($r = 0.76$, $p < 0.01$) and also a direct correlation between plasma iron and PIT ($r = 0.67$, 0.02 $< p < 0.05$), it follows that the changes in plasma iron concentration and in turnover must have been due to changes in RE input. The greater PIT at a higher plasma iron concentration is not associated with any change in distribution between erythroid and nonerythroid tissues.

RE iron kinetic studies in man are still obscure because of difficulties of methodology. However, the present study demonstrates that there is a variation in RE iron release that is responsible for the circadian fluctuations in plasma iron and that this in turn alters the amount, but not the distribution, of iron delivered to tissue receptors.

**REFERENCES**


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