Transferrin Saturation, Plasma Iron Turnover, and Transferrin Uptake in Normal Humans

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The relationship between plasma iron, transferrin saturation, and plasma iron turnover was studied in 53 normal subjects whose transferrin saturation varied between 17% and 57%, in 25 normal subjects whose transferrin saturation was increased by iron infusion to between 67% and 70%, and in five subjects with early untreated idiopathic hemochromatosis whose transferrin saturation was continually elevated to between 81% and 85%. The plasma iron turnover of all of these subjects ranged from 0.45 to 1.22 mg/dL whole blood/d. The mean values for the above-mentioned three groups were 0.71 ± 0.17, 1.01 ± 0.11, and 1.01 ± 0.13 mg/dL whole blood/d, respectively. Most of this variation, estimated at 72% by regression analysis, was due to a direct relationship between transferrin saturation and plasma iron turnover. This effect was attributed to a competitive advantage of diferric over monoferric transferrin in delivering iron to tissues. This was confirmed by the demonstration of a more rapid clearance of diferric as compared to monoferric transferrin in an additional group of eight normal subjects. Calculations were made of the amount of transferrin reacting with membrane receptors per unit time. Allowance was made for the noncellular (extravascular) exchange and for the 4.2:1 preference of diferric over monoferric transferrin demonstrated in vitro. The amount of iron-bearing transferrin leaving the plasma to bind to tissue receptors for 53 subjects with a transferrin saturation between 17% and 57% was 71 ± 13; for 25 subjects with a saturation from 67% to 100%, 72 ± 12; and for five subjects with early idiopathic hemochromatosis, 82 ± 11 µmol/L whole blood/d. There were no significant differences among these groups. These studies indicate that while the number of iron atoms delivered to the tissues increases with increasing plasma iron and transferrin saturation, the number of iron-bearing transferrin molecules that leave the plasma per unit time to bind to tissue receptors is relatively constant and within the limits studied, independent of transferrin saturation.

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Ferrokinetic studies have been used to evaluate internal iron exchange, and more particularly, to characterize erythropoiesis.1-6 The assumption on which the validity of such calculations rests has been that plasma iron behaves as a single pool. Recent studies have shown this assumption to be invalid.7-9 The plasma transferrin iron pool is composed of three different molecular species, two monoferric and one diferric transferrin. The two monoferric species donate iron similarly in vivo, but are inferior to the diferric moiety in their iron-donating capacity. In this work, in vivo relationships were determined by performing ferrokinetic studies in normal and hyperferremic humans. On the basis of previous in vitro studies, a formula was developed for deriving from the plasma iron turnover the number of iron-bearing transferrin molecules that leave the plasma per unit time to bind to tissue receptors.

MATERIALS AND METHODS

Experimental subjects. In vivo ferrokinetic studies were performed on 53 normal volunteers between the ages of 21 and 40. Experimental protocols for these studies were approved by the University of Washington Human Subjects Committee and informed consent was obtained from all subjects. All subjects were in good health, had a hematocrit (Hct) ≥40 (males) or ≥35 (females), and a transferrin saturation between 20% and 30% in a few instances, the transferrin saturation, when repeated at the time of the study, was found to be outside of this range. In addition, the plasma iron turnover was determined in 25 subjects after the plasma iron had been artificially raised by an infusion of ferrous ammonium sulfate. In 16 of these subjects, plasma iron turnover had been first determined under basal conditions a few hours before. For the second turnover, a cold ferrous iron infusion was given over a period of ten minutes immediately following an injection of a tracer amount of a second isotope of iron. The amount of cold iron injected was designed to increase the in vivo saturation to about 90%, that calculation being based on the unsaturated iron-binding capacity and estimated plasma volume. In six subjects, the single dose was followed by a continuous infusion in an amount calculated to maintain the plasma iron at a near-saturated level. Measurements of plasma iron saturation were made at different times during the study.

Five subjects who were incidentally found to have an elevated transferrin saturation after repeated determinations were also studied. These individuals had no symptoms or splenomegaly on physical examination, had a normal Hct (43.6% ± 1.3%), a normal reticuloocyte count (1.1% ± 0.3%), a plasma ferritin concentration >500 µg/L, and a liver biopsy showing parenchymal iron deposition but no hepatic fibrosis. These subjects were considered to represent individuals with precirrhotic idiopathic hemochromatosis.10

Ferrokinetic studies. The amount of radioiron used for an individual measurement of plasma iron turnover was 4 µCi of 59Fe and/or 2 µCi of 59Fe. Each isotope had a specific activity of about 10 µCi/µg of iron. Measurements were carried out in the morning after the subject had fasted. Initially, several different methods of tagging were evaluated. In these studies, two different isotopes were used to label transferrin in different fashions and were injected simultaneously. In vitro tagging was carried out by adding a tracer amount of 59FeSO4 (pH 2) to 10 mL of heparinized plasma with constant...
agitation so as to optimize dispersion of the added iron and permit random loading of binding sites. $^{59}$Fe citrate was similarly added to plasma and its clearance was compared with plasma tagged in vitro with $^{59}$FeSO$_4$ injected intravenously at the same time. In vivo tagging was carried out by injecting trace amounts of $^{59}$FeSO$_4$ (pH 2) in a volume of 2 mL intravenously by pump over a period of five minutes. From these studies, it was concluded that trace labeling by any of the methods used gave similar results in normal subjects* (Table 1).

The effect of altering the proportion of monoferric and diferric transferrin was examined by labeling plasma at low and high saturation, injecting two plasma aliquots intravenously, and determining their simultaneous clearance from the circulating blood in eight normal subjects. In order to provide plasma of low saturation, 6 mL of the subject's plasma was exposed to a phosphate buffer (pH 7.1) containing 30 mg of desferrioxamine and this was passed through a Sephadex G50 column (Pharmacia, Piscataway, NJ).$^7$ The transferrin aliquot obtained had a plasma iron level of <20 µg/dL and to this, $^{59}$FeSO$_4$ in tracer amounts was added and incubated for 15 minutes. Carrier iron as ferrous ammonium sulfate was then added in an amount calculated to saturate the binding capacity of the plasma. The mean iron saturation of the subject's plasma was trace-labeled with $^{59}$FeSO$_4$ and incubated for 15 minutes. 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transferrin turnover in terms of transferrin, milligrams of iron are first converted to micromoles of iron:

\[ IU(\mu\text{mol/L whole blood/d}) = IU(\text{mg/dL wb/d}) \times (10,000/56). \]

The molecular ratio between transferrin receptor turnover and tissue iron uptake is then related to transferrin saturation. The proportions of monoferric and diferric transferrin being \( TIFe_1 = 2S/(100 - S)/100 \) and \( TIFe_2 = S^2/100 \), respectively, and 4.2 the molecular advantage of diferric transferrin, it is possible to express iron uptake from monoferric and diferric transferrin as follows:

\[
\text{iron uptake from } TIFe_1 = \frac{2S(100 - S)/100}{2S(100 - S)/100 + 8.4S^2/100} \times \text{total iron uptake}
\]

\[
\text{iron uptake from } TIFe_2 = \frac{8.4S^2/100}{2S(100 - S)/100 + 8.4S^2/100} \times \text{total iron uptake.}
\]

Considering that monoferric transferrin delivers one iron atom and diferric transferrin delivers two iron atoms at all times, the above-reported relationships permitted to derive the molecular ratio between transferrin and iron uptake:

\[
\text{tissue transferrin uptake} \quad \frac{\text{tissue iron uptake}}{\text{iron uptake from } TIFe_1} + \frac{1}{2}(\text{iron uptake from } TIFe_2) = \frac{\text{iron uptake from } TIFe_2}{\text{iron uptake from } TIFe_1 + \text{iron uptake from } TIFe_2}.
\]

Thus, by substituting iron uptake from monoferric and diferric transferrin with the above-reported relationships, it follows:

\[
\text{TU}(\mu\text{mol/L whole blood/d}) = IU(\mu\text{mol/L whole blood/d}) \times \frac{(200 + 2.2S)}{(200 + 6.4S)}
\]

where S is the percentage of transferrin saturation.

**RESULTS**

In eight subjects whose mean plasma iron was 90 ± 22 μg/dL and whose transferrin saturation was 31% ± 6%, the \( t/2 \) disappearance of a trace amount of predominantly monoferric transferrin radioiron was 95 ± 35 minutes, whereas the clearance of a trace amount of diferric transferrin radioiron was 68 ± 20 minutes (\( P < .001 \)). Red cell utilization from these two forms of transferrin showed no significant difference; the value at two weeks for the predominantly monoferric tag was 90% ± 8% and for the diferric form, 87% ± 6% (\( P > .05 \)).

Results of plasma iron turnover measurements in 53 normal subjects are shown in Table 2. Within this group there was a close correlation between plasma iron and PIT (\( r = .81, P < .001 \)) and between transferrin saturation and PIT (\( r = .77, P < .001 \)). Red cell utilization averaged 85% ± 5% and was independent of both plasma iron (\( r = -.14, P > .05 \)) and transferrin saturation (\( r = -.21, P > .05 \)).

Twenty-five subjects were studied after their plasma had been elevated by the intravenous injection of cold iron. At an average transferrin saturation of 83% ± 11%, their PIT was 1.01 ± 0.11 mg/dL whole blood/d. Sixteen of these subjects had a prior turnover performed immediately before under basal conditions (Fig 1A). The initial saturation was 34% ± 12% and the PIT was 0.71 ± 0.15 mg/dL whole blood/d. During the second PIT, transferrin saturation had a mean value of 81% ± 11%, with a mean PIT of 1.01 ± 0.11 mg/dL whole blood/d. These values were significantly different (\( P < .001 \)) from basal values. On the other hand, red cell utilization at the elevated plasma iron concentration averaged 88% ± 6%, not significantly different from the mean basal measurement of 86% ± 5% (\( P > .05 \)). The iron infusion did disturb the normal equilibrium and there was a subsequent average fall in plasma iron concentration averaging 10 μg/dL/h. In order to minimize this, six subjects undergoing tandem studies were given a continued iron infusion to maintain their elevated plasma iron. Their plasma iron was stable with an average change of 0 ± 10 μg/dL plasma/h, and their plasma iron turnover was 1.01 ± 0.13 μg/dL whole blood/d. In the other 19 subjects who had an average decrease of 13 μg/dL plasma/h, the plasma iron turnover was 1.00 ± 0.11 mg/dL whole blood/d.

An additional five subjects with early idiopathic hemochromatosis showed a continued elevation of transferrin

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<td><strong>Plasma Iron (μg/dL)</strong></td>
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*Red cell utilization of radioiron was studied only in some subjects (numbers within parentheses).*
saturation and had a mean PIT of 1.01 ± 0.13 mg/dL whole blood/d as shown in Table 2. Red cell utilization of radioiron was normal (80% and 89%) in the two subjects who were studied until the 14th day, indicating no change in initial tissue iron distribution.

The data of all three groups of subjects studied are displayed in Fig 2A. It is apparent that the regression of PIT on transferrin saturation had a curvilinear trend approaching a maximum (r = .85, P < .001).

These measurements of PIT, converted from iron turnover to transferrin uptake by membrane receptors (transferrin receptor turnover) are displayed in Fig 1B and 2B. There was no significant difference (P > .05) between the basal transferrin uptake of 71 ± 11 μmol/L whole blood/d and the value of 72 ± 11 μmol/L whole blood/d obtained after iron loading in the group of 16 subjects who underwent tandem ferrokinetic studies (Fig 1B). Similarly, no significant difference was observed in the rate of transferrin uptake between the 53 normal subjects studied under basal conditions (71 ± 13 μmol/L whole blood/d) and the 25 subjects studied at elevated plasma iron levels (72 ± 12 μmol/L whole blood/d). Subjects with early idiopathic hemochromatosis also showed values for transferrin uptake in the normal range. Figure 2B shows the independence of the transferrin receptor turnover on transferrin saturation (P > .05).

**DISCUSSION**

A number of elegant models of iron kinetics have been developed which are designed to take into consideration the amount of iron going through erythroid and nonerythroid tissues and various refluxes of iron from those tissues. These measurements, while they may accurately reflect iron turnover, are determined not only by the number of tissue receptors which take up the transferrin iron complex but also by the plasma iron supply as illustrated by the data presented. In order to quantitate the tissue receptor number, or more specifically, erythropoietic capacity, it is necessary to remove the effect of plasma iron or transferrin saturation.

A relationship between plasma iron concentration and plasma iron turnover was appreciated sometime ago by Cook et al, but was ascribed to a change in nonerythroid iron turnover. While there have been earlier reports that the loading of transferrin’s two iron-binding sites in vitro is neither random nor determined by relative binding strengths of the iron-binding sites, recent work in rats, rabbits, and human beings is consistent with the concept that the binding of iron to transferrin conforms to simple probability rules (random loading model). It has also been shown that tissue distribution from the two sites of transferrin and from monoferric and diferric forms is identical. The preferential uptake of diferric transferrin by tissue receptors then explains the progressive increase in plasma iron turnover which occurs in both animals and humans as plasma iron and transferrin saturation increase.

There are differences between a simple in vitro model and in vivo kinetics. First of all, there is an extravascular flux in vivo whereby transferrin and its iron leave the vascular system and return through the lymphatics. This reflux, described by Morgan et al and Cook et al, has been shown to vary in relation to plasma iron concentration. In the second place, there is a continuous release of tissue iron to circulating transferrin, which does not occur in the in vitro system. Iron entering the vascular system would, therefore, transform a certain proportion of monoferric to diferric transferrin, thereby changing its intravascular kinetics. This has been previously examined by us in a detailed study in the rabbit, where direct evidence of the expected conversion has been obtained. Since the in vivo results with rabbits approximated those obtained from incubation of rabbit transferrin with rabbit reticulocytes, it seemed appropriate to use a formula based on the 4.2 advantage of diferric over monoferric transferrin derived by human reticulocyte incubation. By such calculations, a curve has been defined expressing the mono/diferric effect, from which the rate of uptake of iron-bearing transferrin can be calculated.

The estimate of transferrin uptake was independent of changes in plasma iron and transferrin saturation as shown by the similar values obtained in normal subjects under basal
conditions and with artificially elevated plasma iron. Likewise, individuals with constant hyperferremia due to an iron-loading state, although slightly higher, were within normal limits. Thus, while the number of iron atoms delivered to tissues increases with increasing plasma iron and transferrin saturation, the number of iron-bearing transferrin molecules binding to tissue receptors is relatively constant.

These observations have certain practical implications. First of all, if radioiron is to be cleared in a fashion similar to that of cold iron in patients, ie, if the true iron turnover of the patient is to be measured, the amount of labeled monoferric and diferric transferrin must be made identical to that in the patient's circulating plasma. The manner in which this is achieved is unimportant and several suitable ways have been described. In the present study, in vitro tagging resulted in an increase in saturation of 2% to 6%. Passage of iron through a column to remove unbound iron seems unnecessary and provides an additional opportunity for increasing the transferrin saturation.

The other implication concerns the interpretation of ferrokinetic data. While it is a simple matter to determine plasma iron turnover provided radioiron is added properly, it is another matter to evaluate tissue capacity to assimilate iron. A near doubling of iron turnover (and erythroid marrow uptake since utilization remains constant) can be obtained merely by increasing the plasma iron concentration. This does not imply that the number of tissue receptors for transferrin has been altered or that erythropoiesis has been increased, but rather that the amount of iron taken up by receptors is proportionately greater. Since the major objective of ferrokinetic measurements is to characterize erythropoiesis, it becomes important to separate the plasma transferrin effect on turnover from the intrinsic marrow capacity to assimilate iron—the latter reflecting the functional capacity of the marrow.

REFERENCES

Transferrin saturation, plasma iron turnover, and transferrin uptake in normal humans

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