Serum transferrin receptors are decreased in the presence of iron overload

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To test the hypothesis that the quantities of circulating transferrin receptors are reduced in iron overload, we studied serum transferrin receptors and indirect measures of iron status in 150 subjects from rural Zimbabwe. We found significant inverse correlations between serum concentrations of transferrin receptors and ferritin, the ratio of ferritin to aspartate aminotransferase, and transferrin saturation ($r \geq 0.44$; $P < 0.001$). The mean ± SD concentration of serum transferrin receptors in 23 subjects classified as having iron overload (ferritin >300 μg/L and transferrin saturation $>60\%$) was 1.55 ± 0.61 mg/L, significantly lower than the 2.50 ± 0.62 mg/L in 75 subjects with normal iron stores (ferritin 20–300 μg/L and transferrin saturation 15–55%; $P < 0.0005$) and the 2.83 ± 1.14 mg/L in 8 subjects with iron deficiency (ferritin <20 μg/L; $P = 0.001$). In keeping with the regulation of transferrin receptor expression at the cellular level, our findings suggest that serum transferrin receptors are decreased in the presence of iron overload.

Iron balance in humans is regulated fundamentally by the rate of erythropoiesis and by the size of the iron stores [1]. The concentration of soluble transferrin receptors in the plasma reflects the degree of erythropoiesis, being increased in states of enhanced erythropoiesis and decreased with reduced erythropoiesis [2]. Circulating transferrin receptors also reflect the body's iron status, in that the concentration of transferrin receptors is increased in the presence of iron-deficiency anemia [3–5], but whether serum transferrin receptors are decreased with iron overload states is not clear. On one hand, Huebers et al. [6] reported serum transferrin receptor concentrations within the normal reference interval in 7 treated patients with idiopathic hemochromatosis, and Baynes et al. [7] found normal concentrations in 14 patients with hereditary hemochromatosis and 49 black Africans with dietary iron overload. On the other hand, Thorstensen and Romslo [8] reported low serum transferrin receptor concentrations in 19 men with increased transferrin saturations in comparison with that in 800 men with normal saturation values, and Ledue and Craig [9] observed low concentrations of receptor in 7 subjects with hereditary hemochromatosis. Furthermore, Centis et al. [10] found a significant negative correlation between serum transferrin receptor concentrations and ferritin concentrations in 230 patients who had been cured of thalassemia by bone marrow transplantation.

Circulating transferrin receptors are derived by proteolytic cleavage from transferrin receptors expressed on the cell surface [11]. On the basis of current understanding of regulation of transferrin receptor expression at the cellular level, we hypothesized that serum transferrin receptor concentrations would be reduced in iron overload states. Intracellular iron influences the translation of ferritin mRNA and the stability of transferrin receptor mRNA [12, 13]. This regulation occurs by means of an interaction between the iron regulatory protein (IRP),[5] a molecule that senses changes in the chelatable intracellular iron pool [14–16], and iron-responsive elements (IREs) located on untranslated regions of ferritin and transferrin receptor mRNAs [16–18]. When intracellular iron is ample, IRP has aconitase activity and does not bind to the IREs; this results in increased ferritin mRNA translation and increased transferrin receptor mRNA degradation. Conversely, in iron deprivation, IRP loses aconitase activity and binds to IREs, causing a repression in ferritin mRNA translation and increased transferrin receptor mRNA stability [19]. Thus at the cellular level, transferrin receptor

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[5] Nonstandard abbreviations: IRP, iron regulatory protein; IRE, iron-responsive element; AST, aspartate aminotransferase.

expression is decreased when iron supply is ample and increased when iron supplies are curtailed [15, 20, 21]. In keeping with these observations in vitro, patients with secondary iron overload and with hereditary hemochromatosis have been determined to have decreased transferrin receptor expression in hepatocytes and other cells [22–24].

The present study was designed to examine the relation of soluble transferrin receptors to iron status in a group of subjects whose iron status ranged from iron deficient to iron loaded. Our objective was to determine if, as predicted from experiments at the cellular level, serum transferrin receptors are reduced in the presence of dietary iron overload.

Materials and Methods

Study subjects. We determined the iron status of 150 subjects—146 adults (ages 18 years and above) and 4 teenagers (between ages 12 and 18)—from rural Zimbabwe from 1993 to 1995. The investigation was approved by the Medical Research Council of Zimbabwe, and written informed consent was obtained from each subject. Eighty-eight of the subjects were enrolled as part of a study of families with African iron overload, and 62 subjects were enrolled as part of a study of community members with a history of traditional beer consumption. Iron overload is common in rural Africa [25] and is related to the consumption of traditional home-brewed beer, rich in bioavailable iron [26]. Each subject was asked to abstain from drinking any alcoholic beverage for at least 48 h before fasting morning blood samples were drawn on two different days. Because serum concentrations of iron and ferritin may be inappropriately lowered in the presence of iron overload and vitamin C deficiency [27, 28], each subject was also given 2.0 g of vitamin C orally 24 h before each blood sample was collected.

Analysis of blood samples. Serum transferrin receptor concentrations were measured with the Quintikine enzyme immunoassay (R&D Systems, Minneapolis, MN). The reference limits for the transferrin receptor assay were 0.85–3.05 mg/L, as recommended by the manufacturer. Serum ferritin concentrations were measured with the Spectroferritin enzyme immunoassay (Ramco Labs. Houston, TX). A modification to the methods recommended by the International Committee for Standardization in Haematology [29, 30] was used to determine serum iron and total iron-binding capacity. Transferrin saturation was calculated by dividing the serum iron by the total iron-binding capacity and multiplying by 100. Liver function tests were determined with an automated Cobas Bio analyzer and use of Roche Diagnostics reagents. Complete blood counts were determined with an automated cell counter (Coulter Electronics). Erythrocyte sedimentation rates were determined by the Westergren method [30a].

Indirect measures of iron status. For the purposes of this study, values of serum transferrin receptor, serum ferritin, and transferrin saturation were the mean of determinations performed on fasting blood samples obtained on two different days, each after supplementation with vitamin C and after abstention from alcohol. Because 71% of our study subjects had a history of consumption of an alcoholic beverage, we used the ratio of serum ferritin to aspartate aminotransferase (AST) to adjust the serum ferritin results for possible effects of hepatocellular damage related to alcohol. This ratio correlates well with the hepatic iron concentration [31] and is constant in a given patient, both in the setting of acute alcohol ingestion and after prolonged abstention from alcohol [32]. To obtain the ratio of ferritin to AST, we divided the serum ferritin concentration of each subject by the AST determined on the same sample, setting the minimum value for AST at 30 U/L, the upper limit of normal in our assay.

Assignment of iron status. According to the manufacturer of the ferritin assay kit used in this study, a low serum ferritin is <20 μg/L and a high value is >300 μg/L. In a recent survey of 500 Zimbabwean adults who had normal dietary iron content, we confirmed that the reference interval for serum ferritin falls between these values (Gomo et al., unpublished observations, 1997). Because serum ferritin may be increased by inflammation, we assigned a subset of 106 subjects to three categories of iron status, confirmed both by the serum ferritin and the transferrin saturation: iron deficiency, serum ferritin <20 μg/L; normal iron status, ferritin 20–300 μg/L and transferrin saturation 15–50%; iron overload, ferritin >300 μg/L and transferrin saturation >60%.

Statistical analysis. Baseline characteristics were compared according to iron category of serum ferritin by using analysis of variance for continuous variables that followed a gaussian distribution and the Kruskal–Wallis test for continuous variables that were skewed. Pearson's χ^2 was used to evaluate the effect of sex, if any, according to category of serum ferritin. Spearman's correlation was used to examine the relation of serum transferrin receptors to serum ferritin, the ratio of ferritin to AST, and transferrin saturation. We also compared concentrations of serum transferrin receptors according to classification of iron status, using analysis of variance.

Results

The clinical characteristics of the study population are summarized in Table 1. Indeed, 35% of the subjects had serum ferritin concentrations >300 μg/L, and only 5% had ferritin <20 μg/L. As Table 1 shows, increased serum ferritin was associated with older age, male sex, longer history of traditional beer consumption, higher liver function test results, higher indirect measures of iron status and lower concentrations of circulating transferrin receptors.

Table 1. Baseline clinical characteristics of the study subjects.

Characteristics	All subjects	Serum ferritin, μg/L			
		>300	20–300	<20	P
n	150	53	89	8	
Age, years (mean $\pm$ SD)	48 $\pm$ 19	59 $\pm$ 13	44 $\pm$ 19	29 $\pm$ 7	<0.0005
Sex, male:female	75:75	39:14	34:55	2:6	<0.0005
Years of beer consumption (median and range)	19 (0–63)[a]	32 (0–63)[b]	6 (0–61)	3 (0–19)	<0.0005
Hemoglobin, g/L (mean $\pm$ SD)	141 $\pm$ 16[c]	144 $\pm$ 16[d]	140 $\pm$ 15[e]	131 $\pm$ 18	0.056
Erythrocyte sedimentation rate, mm/h (median and range)	16 (0–120)[c]	17 (0–115)[d]	15 (0–120)[e]	24 (2–90)	0.8
Alanine aminotransferase, U/L (median and range)	19 (6–169)[f]	25 (11–169)	17 (6–94)[g]	16 (11–23)	<0.0005
[Gamma]-Glutamyltransferase, U/L (median and range)	20 (3–661)[h]	30 (10–661)	16 (4–125)[i]	10 (3–24)	<0.0005
AST, U/L (median and range)	26 (7–179)[a]	32 (16–179)	23 (7–118)[e]	28 (19–50)	<0.0005
Serum ferritin, μg/L (median and range)	148 (4–12 485)	920 (303–12 485)	73 (20–295)	12 (4–18)	—
Ferritin:AST ratio, μg/U (median and range)	3.9 (0.1–416.2)[f]	25.2 (2.3–416.2)	2.3 (0.4–9.8)[g]	0.4 (0.1–0.6)	<0.0005
Transferrin saturation, % (mean $\pm$ SD)	43 $\pm$ 25	60 $\pm$ 30	35 $\pm$ 14	21 $\pm$ 8	<0.0005
Serum transferrin receptors, mg/L (mean $\pm$ SD)	2.26 $\pm$ 0.72	1.87 $\pm$ 0.67	2.45 $\pm$ 0.60	2.83 $\pm$ 1.14	<0.0005

[a] n = 149; [b] n = 52; [c] n = 146; [d] n = 50; [e] n = 88; [f] n = 148; [g] n = 87; [h] n = 147; [i] n = 86; [j] n = 147.

Table 2 shows the significant inverse Spearman correlations of circulating transferrin receptor with serum ferritin, the ratio of ferritin to AST, and transferrin saturation ($r \geq 0.44$, $P < 0.001$).

Figure 1 shows the serum transferrin receptor concentrations in 106 subjects who were assigned to the category of iron overload, iron deficiency, or normal iron status. (Forty-four subjects had discordant values for ferritin and percent transferrin saturation, and could not be assigned to any iron status category.) As determined by analysis of variance and Bonferroni adjusted pairwise comparisons, subjects with iron overload had significantly lower concentrations of serum transferrin receptors than did the normal subjects ($P < 0.0005$) and the iron-deficient subjects ($P = 0.001$).
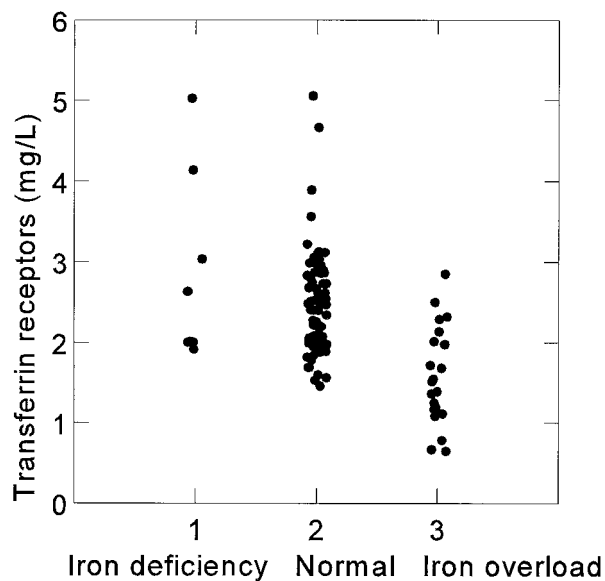

Fig. 1. Serum transferrin receptor concentrations according to assignment of iron status in 106 subjects: mean $\pm$ SD transferrin receptor concentration = 2.83 $\pm$ 1.41 mg/L in subjects with iron deficiency (n = 8), 2.50 $\pm$ 0.62 mg/L in normal iron status (n = 75), and 1.55 $\pm$ 0.61 mg/L in iron overload (n = 23).

For this study, iron deficiency was defined as serum ferritin <20 μg/L; normal iron status as serum ferritin 20–300 μg/L and transferrin saturation 15–50%; and iron overload as serum ferritin >300 μg/L and transferrin saturation >60%. Analysis of variance: $df = 2$, F-ratio = 14.6, $P < 0.0005$.

Table 2. Spearman correlations of circulating transferrin receptors with indirect measures of iron status in 150 subjects.

Measure of iron status	n	Inverse correlation with transferrin receptor	
		r	P
Ferritin	150	0.468	<0.001
Ferritin:AST ratio	148	0.440	<0.001
Transferrin saturation	150	0.491	<0.001

Discussion

In studies of cell cultures, a reduction in the supply of iron from the media is associated with a sharp increase in transferrin receptor synthesis. Conversely, when exogenous iron is supplied to cultured cells, the number of transferrin receptors decreases [15, 20, 21], apparently as a way of reducing further uptake of iron. Consistent with these cell-culture findings, circulating transferrin receptors are increased in subjects with iron deficiency [3, 4, 38] but, surprisingly, some investigators have reported that concentrations of soluble transferrin receptors are normal in subjects with African dietary iron overload and in patients with hereditary hemochromatosis [6]. Circulating transferrin receptors have also been reported at normal concentrations in patients who had been treated for hereditary hemochromatosis [7]. Serum concentrations of ferritin and transferrin receptor apparently reflect the intracellular synthesis of these molecules [2, 33, 34], and a reciprocal relation exists between ferritin and transferrin receptors intracellularly [13, 23].

Circulating transferrin receptors are derived by proteolytic cleavage from transferrin receptors, which are transmembrane proteins with two identical polypeptide chains expressed on the cell surface [11]. Cleavage occurs on the extracellular domain of the dimeric tissue receptor and results in formation of a soluble truncated monomer that can be measured in serum or plasma [35]. All iron-requiring cells express transferrin receptors, and 80% of tissue receptors are found on erythroid progenitor cells (but not on mature erythrocytes) [36]. The amount of tissue transferrin receptors is proportional to the concentration of transferrin receptors in serum [37].

In the present study, we provide evidence that circulating transferrin receptor concentrations are inversely and significantly proportional to indirect measures of the size of the body's iron stores, and that receptor concentrations are significantly less in subjects with African iron overload than in normal and iron-deficient subjects. The difference between our findings and those of Baynes et al. [7] may be explained because we compared the transferrin receptor concentrations in subjects with African iron overload with those in normal subjects from the same population, whereas Baynes et al. compared the receptor concentrations in iron-loaded subjects with the recommended normal range for their assay [7]. The mean serum transferrin receptor concentration in the iron-loaded subjects in our study was also within the recommended normal range for our assay but was significantly lower than the mean in the normal subjects from our study population. In summary, our findings provide further evidence that the magnitude of the systemic iron stores is correlated with transferrin receptor expression [1].

Our study is limited, in that iron status was determined indirectly by serum measurements of iron status. Several factors may perturb these measurements. Serum ferritin values may be increased because of inflammation, malignancy, and liver disease and may not accurately reflect iron stores in these settings [31, 39, 40]. Transferrin saturations may be reduced in inflammation and malignancy and increased in liver disease. To minimize the influence of the disorders affecting measurement of ferritin and transferrin saturation, we asked our subjects to refrain from the ingestion of any alcoholic beverage for 48 h before venisection. We also corrected for any alcohol-related hepatocellular damage by using the ferritin/AST ratio as an indirect measure of iron status. Finally, we analyzed serum transferrin receptors in a subgroup of subjects whose iron status had been assigned fairly rigorously according to both serum ferritin and transferrin saturation (Fig. 1). In this subgroup, the reduction in serum transferrin receptors with iron overload was statistically highly significant.

In conclusion, we note that our findings do not suggest that serum transferrin receptor concentrations will be useful for the diagnosis of iron overload, because there is considerable overlap in the values between subjects with iron overload and those with normal iron stores (Fig. 1). Nevertheless, this work strongly supports the conclusion that circulating transferrin receptors reflect the body's iron status, and that the inverse relationship between transferrin receptor expression and ferritin expression demonstrated in many studies at the cellular level is also detectable systemically. Although our finding of decreased concentrations of circulating transferrin receptors is at odds with some previous studies [7], our observation is consistent with the model for regulation of iron metabolism that has developed over the past two decades.

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