Accelerated Transferrin Degradation in HFE-Deficient Mice Is Associated with Increased Transferrin Saturation[1]

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Abstract

HFE, a major histocompatibility complex class I-related protein, is implicated in the iron overload disease, hereditary hemochromatosis. Whereas patients with hereditary hemochromatosis have low serum transferrin levels, little is known about transferrin turnover in HFE deficiency states. We injected mice intravenously with radioiodinated transferrin and compared plasma transferrin decay and steady-state endogenous transferrin concentration in the plasma between HFE-deficient and wild-type C57BL/6 mouse strains. HFE-deficient mice degraded transferrin faster than normal ($P < 0.001$) and had lower plasma transferrin concentrations ($P < 0.001$). Both HFE-deficient and wild-type mice were then fed diets with 3 different iron concentrations that we designated deficient (2–5 mg/kg of iron), control (0.2 g/kg), and overload (20 g/kg) for 6 wk immediately after weaning to create a range of serum iron concentrations and resultant transferrin saturations ranging from 16 to 78%. We found an inverse correlation between transferrin saturation and transferrin half-life ($P < 0.0001$, $r = -0.839$) for both HFE-deficient and wild-type mice, which suggests that HFE does not have a direct effect on transferrin catabolism; rather, HFE may influence transferrin half-life indirectly through its effect on transferrin saturation, which in turn enhances transferrin decay in HFE-deficient mice. J. Nutr. 136: 2993–2998, 2006.

Introduction

Mutations in the HFE gene are responsible for most cases of hereditary hemochromatosis (HH),[7] a common iron overload disease characterized by an inability to check absorption of dietary iron from the gut, increased iron deposition in parenchymal cells, and resultant damage of specific vital organs. The most frequent HFE mutation, C282Y, reduces expression and function of HFE, a nonclassical major histocompatibility complex class I (MHC-I) molecule that is expressed with β2-microglobulin (B2m) as a heterodimeric integral membrane glycoprotein. Although it is known that HFE/B2m binds as a second ligand to the transferrin receptor 1 (TfR1) to form a ternary complex consisting of HFE/B2m, TfR1, and transferrin [see recent reviews (1,2)] and that it competes with transferrin for binding to TfR1 (3), the precise role of HFE in iron metabolism has not been defined.

In the course of studying protein decay in the B2m-deficient homozygous knockout (KO) mouse strain we noted that both B2m-deficient (KO) and HFE-KO mice degraded transferrin more rapidly than normal mice. To our knowledge, despite considerable study of the relation between HFE and transferrin, rapid transferrin degradation in the absence of HFE was never before noted. Nor was a shortened transferrin half-life ($t_{1/2}$) seen in HH patients, although we found a single HH patient whose transferrin turnover had been reported as normal (4). However, for 40 y, reports of serum transferrin concentrations in patients with HH have been low (5–8), with, for example, the median serum transferrin concentration being 57% of the control population in one large study of 162 HH patients (9). Upon recognizing the difficulty of distinguishing increased degradation from decreased production due to liver disease, workers assessed HH patients without evidence of iron overload (9) as well as healthy relatives of HH patients (8), presuming that some would have subclinical HH, and found that these individuals also have low plasma transferrin concentrations. These low transferrin concentrations in HH patients, however, have captured little investigative attention, perhaps because transferrin saturation (TS) was recognized early on as a far superior diagnostic criterion and because of the difficulty in excluding associated liver disease.

To examine how HFE might influence transferrin catabolism, we studied HFE-deficient mice. B2m-KO and HFE-KO mice rapidly degraded transferrin and the rapid degradation in HFE-KO mice was reflected in lower serum transferrin concentrations. Rapid transferrin degradation, however, was most likely not a direct effect of HFE on transferrin turnover (10–13); rather, it

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[6] C. Chaudhury and J. Kim contributed equally to this work.
[7] Abbreviations used: B2m, β2-microglobulin; HH, hereditary hemochromatosis; KO, homozygous knockout; MHC-I, major histocompatibility complex class I; TfR1, transferrin receptor 1; TS, transferrin saturation (%); WT, wild-type.
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was likely associated with the increased TS caused by HFE-deficiency.

Materials and Methods

Animals. We used 4 strains of male mice: 3 KO strains and the relevant wild-type control strain (C57BL/6). The KO strains were HFE-KO [*Hfe*^{tm1Gfn}; (14)], FcRn α-chain KO [FcRn-KO; B6.129 × 1/SvJ*Fcgrt*^{Tm1Dcr}; (15)], and β2-microglobulin KO [B2m-KO; B6.129P2-*B2m*^{tm1Unc}; (16)]. The FcRn-KO, B2m-KO, and their corresponding WT control strain were obtained from Jackson Laboratories, whereas the HFE-KO and the corresponding WT strain were obtained from our in-house breeding colony using breeders obtained from Jackson Laboratories. All KO mice used in the experiments were backcrossed to the background strain >11 times. Except for the mice fed different iron diets as indicated, the mice were fed a standard diet (catalogue 8640, containing 0.349 g/kg of iron) obtained from Harlan Teklad. The Ohio State University Institutional Laboratory Animal Care and Use Committee approved all animal studies.

Proteins and radioiodination. Transferrin from pooled mouse serum (catalogue 55943) and IgA myeloma protein TEPC15 (catalogue 50326) were obtained from ICN Pharmaceuticals after being purified by multistep procedures including salt fractionation, size exclusion, ionic exchange chromatography, and immunoabsorption. A high degree of purity of transferrin (>97%) was confirmed by SDS-gel electrophoresis and immunoblot (J. Kim, data not shown). These proteins were dialyzed against pH 7.2 buffer containing 50 mmol/L NaCl and 100 mmol/L phosphate and were radioiodinated by the chloroglycouril method (17) using 5–10 μg chloroglycouril (Iodogen, Pierce) per 50–100 μL protein solution. Transferrin was labeled with ^{125}I and IgA with ^{131}I at molar substitution ratios of 0.2 atoms of ^{125}I or ^{131}I per molecule of protein. It is presumed that radioiodinated transferrin instantaneously equilibrated with circulating transferrin.

Creating transferrin saturation range in mice. Both WT and HFE-KO mice were fed diets with 3 different iron contents deemed deficient (catalogue TD 80396 containing 2–5 mg/kg of iron), control (catalogue TD 01583 containing 0.2 g/kg of iron), and overload (catalogue TD 03025 containing 20 g/kg of iron as carbonyl iron from Sigma), all obtained from Harlan Teklad (**Table 1**). Specifically, we divided 3-wk–old, age-matched WT and HFE-KO mice into 3 groups each that were designated deficient, control, and overload. For 6 wk mice ate diets containing different amounts of iron to create a range of serum iron concentrations and resultant TS ranging from 16 to 78% (18).

Kinetic measurements of transferrin decay. Mice were infused via tail veins with 150 μL of a mixture of ^{125}I-labeled transferrin and ^{131}I-labeled IgA (561–1403 kBq of each ^{125}I and ^{131}I per mouse; specific activity of 26.3 kBq/pmol transferrin and 23.1 kBq/pmol IgA) diluted in PBS containing 10% normal mouse serum (ICN Biomedicals). In the dosing solution, transferrin saturation by iron was 15% and the amount of radiolabeled transferrin was <0.1% of vascular endogenous transferrin in the mouse. Within 2 min of infusion (time zero) and daily through 120 h, 40 μL blood was sampled via heparinized tubes from the retro-orbital plexus. Plasma was harvested, the proteins in 15 μL plasma were precipitated in 12.5% trichloroacetic acid, and radiolabeled proteins in the pelleted precipitates were counted in a γ-counter and were always >95% of the total radioactivity. The plasma radioactivity, normalized for dose, was plotted on a log scale vs. time, and the half-lives were calculated from the β- or elimination-phase of decay using the formula $t_{1/2} = \ln(2)/\beta$, where β was the slope of a line fitted by the least squares regression analysis (WinNonlin, Pharsight, version 4.0) to a plot of the log concentration–time values that appeared to fall along a straight line. In the present study, we assumed that the distribution of transferrin from the vascular to the extravascular space was not affected by the presence of HFE or different iron levels. Therefore, the α- or distribution-phase of decay prior to 48 h was ignored. After the distribution phase, a decrease in ^{125}I-transferrin plasma concentration was directly related to an increase in its degradation as judged by an increase in the level of urinary excretion of radioiodide (4). Further, because none of the mouse strains was proteinuric or transferrinuric (details below and in Results), the terminal phase decay likely represented transferrin degradation. The harmonic mean $t_{1/2}$ and its SD were calculated using the jackknife technique as described by others (19).

TABLE 1 Composition of experimental diets

Ingredient	Iron-deficient	Control	Iron-overload
		g/kg	
Casein, high protein	200	200	200
DL-Methionine	3	3	3
Sucrose	550	549	530
Corn starch	150	150	150
Corn oil	50	50	50
Mineral mix, iron deficient[1]	35	35	35
Iron	0[3]	1[4]	20[5]
Vitamin mix[2]	10	10	10
Choline bitartrate	2	2	2
Antioxidant	0.01[6]	0.01[7]	0.01[6]

[1] Minerals supplemented (per kg diet): calcium phosphate, dibasic, 17.5 g; sodium chloride, 2.6 g; potassium citrate, monohydrate, 7.7 g; potassium sulfate, 1.8 g; magnesium oxide, 0.8 g; manganous carbonate, 0.12 g; zinc carbonate, 0.06 g; cupric carbonate, 0.01 g; potassium iodate, 0.35 mg; sodium selenite, 0.35 mg; chromium potassium sulfate, 0.02 g.
[2] Vitamins supplemented (per kg diet): thiamin HCl, 6 mg; riboflavin, 6 mg; pyridoxine HCl, 7 mg; niacin, 0.03 g; calcium pantothenate, 0.16 g; folic acid, 2 mg; biotin, 0.2 mg; vitamin B-12, 0.1 g; vitamin A palmitate, 4000 U; vitamin E, 50 U; cholecalciferol, 1000 U; menadione sodium bisulfite, 1.5 mg.
[3] No iron added.
[4] Ferrous sulfate, $FeSO_4 \cdot 7H_2O$.
[5] Carbonyl iron.
[6] Ethoxyquin.
[7] Tert-butylhydroquinone.

Determination of urinary proteins. Urinary protein concentration was measured in all 4 strains of mice by the Bradford method. None of the strains was proteinuric and all had <0.5 g/L of urinary protein, with no differences among them (20).

Determination of steady-state plasma protein concentrations. Plasma samples were obtained from 12 to 16-wk–old, untreated mice. Steady-state plasma concentrations of endogenous mouse transferrin, IgA, and serum albumin were measured using sandwich ELISA, with affinity-purified polyclonal goat antibody against mouse transferrin, IgA, or albumin (Bethyl Laboratories) as a coating antibody and horseradish peroxidase-conjugated goat antibody (Bethyl Laboratories) as a conjugate antibody with reference to standard concentrations of purified proteins for mouse transferrin, IgA, and albumin. Absolute standard concentrations were determined by UV absorption at 280 nm using the known extinction coefficient of the proteins: $E_{280}^{0.1\%} = 1.08$ for mouse transferrin, $E_{280}^{0.1\%} = 0.58$ for mouse albumin (21), and $E_{280}^{0.1\%} = 1.4$ for IgA myeloma protein. The extinction coefficients of ferric-transferrin and apotransferrin are different (22,23), but because of little available information about the distribution of transferrin species (apo-, monoferric-, and diferric-transferrin) in the plasma and serum, we arbitrarily used the extinction coefficient for apotransferrin. Thus, although "absolute" transferrin concentrations were not obtainable, our comparisons of the relative ratios of transferrin plasma concentration among mouse strains were nevertheless valid. The ELISA dilution buffer was 50 mmol/L Tris, 140 mmol/L NaCl, 1% BSA, 0.05% Tween 20, pH 8.0.

Determination of serum iron concentration and transferrin saturation. Sera were obtained from 16-wk–old untreated mice (for comparison among mouse strains) and from the same 9-wk–old mice fed different iron diets in which the transferrin half-life was determined after a radioiodinated transferrin injection. Serum iron was measured by the

Ferene method-based colorimetric assay (Diagnostic Chemicals) (24–26) and transferrin saturation was calculated.

Statistical analyses. The 2-tailed Student's t test was applied to compare the variables between the KO and WT mice. For experiments where 3 different iron diet groups were compared, 2-way ANOVA and the Tukey's multiple comparison test (18) were performed by SPSS (SPSS, version 14.0). Regression analysis was performed using SigmaPlot (Systat Software, version 9.0). Differences among strains or the iron diet groups were considered significant at $P < 0.05$. Data are presented as means ± SD.

Results

We assessed the rate of transferrin degradation in the B2m-deficient strain, which is presumably deficient in all B2m-associated molecules, by infusing radioiodinated mouse transferrin intravenously and measuring its plasma decay over the course of 5 d. Transferrin β-phase decay on a semilogarithmic scale was linear (1st-order decay) with the rate of decay faster in B2m-KO (~21%, $P < 0.0001$) than in the WT strain, $t_{1/2}$ for a duration of 31 and 40 h, respectively (**Table 2**). The rate of IgA decay in the B2m-KO strain, however, did not differ from normal (Table 2). When we measured the half-life in HFE-deficient mouse strain, we found the transferrin $t_{1/2}$ to be ~37 h, faster by 12% ($P = 0.0003$) compared with the $t_{1/2}$ of ~41 h in the WT strain (Table 2). The decay of radioiodinated mouse IgA was also faster (7%, $P = 0.029$) in HFE-KO strain (Table 2). Measuring protein decay in a mouse strain deficient only in the α-chain of FcRn, a molecule structurally similar to HFE but with no known relevance to iron metabolism, we found that the rate of decay in both transferrin and IgA did not differ between the FcRn α-chain KO and WT strains (Table 2). We obtained similar results when the experiments were repeated with a sample size of 5 for both transferrin and IgA for all 4 strains of mice (not shown). None of these strains was proteinuric (see Materials and Methods) and the liver-to-body weight ratios did not differ among the 4 strains (**Table 3**).

Plasma transferrin concentrations in the HFE-KO strain were 76% of WT ($P < 0.001$), those in the FcRn-KO strain were 125% of WT ($P < 0.001$), and those in the B2m-KO strain did not differ from those in WT mice. Plasma IgA concentrations, in contrast, did not differ from WT in in any of the 3 KO strains. Plasma albumin concentrations in both FcRn-KO and B2m-KO strains were about one-half of that in the WT strain (12–16 wk of age), due to a lack of FcRn-mediated albumin recycling (27), whereas in the HFE-KO strain, the albumin concentration did not differ from the WT strain (Table 3).

TABLE 2 Half-lives of transferrin and IgA in HFE-KO, B2m-KO, FcRn-KO, and WT mice fed a diet with standard concentration of iron[1,2]

Strain and protein	KO	WT
	h	
HFE		
Transferrin	36.8 ± 2.0**	41.1 ± 1.7
IgA	29.2 ± 2.0*	31.3 ± 1.5
B2m		
Transferrin	31.4 ± 1.4**	39.8 ± 2.1
IgA	28.5 ± 1.4	29.1 ± 1.7
FcRn		
Transferrin	43.0 ± 4.9	42.1 ± 2.7
IgA	31.7 ± 4.2	30.2 ± 2.2

[1] Values are harmonic means ± SD; $n = 7$–10. *Different from WT, $P < 0.05$; **$P < 0.001$.

[2] Half-lives of transferrin and IgA were determined from semilog plots of mean concentrations of plasma radioactivity remaining vs. time after intravenous injection of ^{125}I-labeled transferrin and ^{131}I-labeled IgA in 12 to 16-wk-old HFE-KO, B2m-KO, FcRn-KO, and their WT control mice.

TABLE 3 Steady-state plasma transferrin, IgA, and albumin concentrations, transferrin saturation, and relative liver weights of untreated HFE-KO, B2M-KO, FcRn-KO, and WT mice fed a diet with standard iron concentrations[1]

	HFE-KO	B2M-KO	FcRn-KO	WT
Transferrin, *μmol/L*	85 ± 3**	109 ± 8	139 ± 8**	112 ± 12
% of WT	76	98	125	100
IgA, *nmol/L*	40 ± 7	37 ± 4	46 ± 10	38 ± 6
% of WT	103	95	119	100
Albumin, *μmol/L*	545 ± 19	287 ± 24**	314 ± 18**	542 ± 18
% of WT	101	53	58	100
Transferrin saturation, %	73.4 ± 5.5**	nd	33.7 ± 4.9	37.5 ± 5.0
% of WT	196	nd	90	100
Liver-to-body wt, %	4.24 ± 0.14	4.17 ± 0.30	4.24 ± 0.16	4.43 ± 0.27
% of WT	96	94	96	100

[1] Values are arithmetic means ± SD; $n = 5$–8.** Different from WT, $P < 0.001$.

Transferrin saturation in the HFE-KO strain was almost 2-fold of that in the WT strain (Table 3), whereas the FcRn-KO strain did not differ from the WT mice. We had no age-matched B2m-KO mice for this purpose. However, in a separate group of 8-wk–old B2m-KO mice, the serum iron concentration and transferrin saturation were 35 and 20% higher, respectively, than age-matched HFE-KO mice (J. Kim, unpublished data). The much higher iron overload was associated with faster transferrin catabolism in the B2m-KO strain compared with the HFE-KO strain (Tables 2 and 3).

To test whether the shortened transferrin $t_{1/2}$ in the HFE-deficient mice would result from the increased TS seen with HFE deficiency, we altered the TS in WT and HFE-KO mice and measured transferrin $t_{1/2}$. As expected, different iron diets generated different levels of TS; i.e., 16–18% for deficient, 25–42% for control, and 77–78% for overload. Serum iron concentrations in these mice were directly related to the TS values (**Table 4**). The presence or absence of HFE expression, however, was not associated with a TS difference in 2 of 3 groups, specifically, the iron-deficient and iron-overload groups. Only in the group fed the control iron diet did we see an HFE deficiency-dependent increase in TS. The relative difference in iron concentration between WT and HFE-KO strains in this study differed from those in an earlier study (18), but in that study, plasma of female mice was assayed, whereas the present study assayed the serum of males. Serum iron concentrations differ between males and females, in HH patients (9). Plasma tends to give a high background (28), so serum is typically used. In addition, Chua et al. (29) reported TS of 40 and 77% in WT and HFE-KO C57B6 mice, respectively.

When we assessed the rate of transferrin degradation in WT and HFE-KO mice fed different diets (Table 4), we found that the serum iron level rather than the presence of HFE was associated with the rate of transferrin degradation. Specifically, compared with control group, we found that transferrin $t_{1/2}$ was 8% longer (37 h; $P = 0.02$) and 15% shorter (29 h; $P = 0.002$) in WT mice fed the deficient and overload diets, respectively. Transferrin $t_{1/2}$ in WT mice fed iron-deficient diet was 22% longer

TABLE 4 Serum iron concentration, TS, and trasferrin and IgA half-lives in HFE-KO and WT mice fed diets with different iron concentrations for 6 wk[1]

	Deficient, 2–5 mg/kg		Control, 0.2 g/kg		Overload, 20 g/kg	
	HFE-KO	WT	HFE-KO	WT	HFE-KO	WT
Serum iron, *μmol/L*	17 ± 4[a]	15 ± 2[a]	38 ± 15[b]	23 ± 10[b]	87 ± 22[c]	78 ± 12[c]
Transferrin saturation, *%*	18 ± 6[a]	16 ± 2[a]	42 ± 11[b*]	25 ± 8[b]	78 ± 8[c]	77 ± 6[c]
Half-lives, *h*						
Transferrin	37.3 ± 1.0[c]	37.0 ± 2.0[c]	34.0 ± 2.3[b]	34.0 ± 2.0[b]	30.4 ± 1.6[a]	28.9 ± 2.3[a]
IgA	35.5 ± 3.2	34.1 ± 2.3	34.8 ± 1.7	33.9 ± 1.1	35.3 ± 3.1	33.7 ± 1.3

[1] Values are means ± SD; $n = 5$–7. Means in a row with superscripts without a common letter differ, $P < 0.01$. *Different from WT fed that diet.

than WT mice fed the overload diet ($P < 0.001$). In the absence of HFE, the iron-dependent transferrin half-lives differed among the 3 diet groups in a similar fashion to the WT strain. The decay of IgA, however, did not differ among the 3 diet groups for either WT or KO strain. Also, the decay of both transferrin and IgA did not differ between the WT and HFE-KO strains within any diet group (Table 4).

Discussion

HFE deficiency was associated with an accelerated rate of degradation of transferrin. Mice deficient in B2m expression degraded transferrin ~21% more rapidly than did WT mice (Table 2). HFE-KO mice also showed a rapid transferrin degradation but of lesser magnitude than in the B2m-KO strain. This difference in the 2 strains was consistent with the more pronounced HH phenotype reported in the B2m-KO compared with the HFE-KO strain (30,31). In contrast, a KO strain missing a different member of the nonclassical MHC-I family, namely, FcRn, degraded transferrin at a normal rate, as expected.

In addition to a shorter half-life, the HFE-deficient strain had a plasma transferrin concentration that was ~24% lower than in WT mice. Similar observations have been reported (29). The lower plasma transferrin concentration likely was not due to diminished liver function resulting from iron storage, because the plasma albumin concentration, an indicator of liver function, did not differ between the HFE-KO and WT strains. Moreover, liver size was not appreciably different between HFE-KO and WT strains.

It is possible that the shortened half-life of transferrin in the HFE-KO strain could be caused by reduced distribution or uptake by peripheral tissues without changing degradation. To address this issue, we calculated the volume of distribution of the extravascular space (peripheral tissues), a measure of the extent of peripheral transferrin distribution based on pharmacokinetic theory (32). First, using either noncompartmental analysis or 2-compartmental analysis (32), we calculated the volume of distribution at steady-state (Vss), which equals the sum of vascular and extravascular volumes of distribution. Second, for plasma proteins the vascular volume of distribution is typically the plasma volume (~1 mL/25 g body wt; the strains did not differ in body wt). We then computed the extravascular volume of distribution of transferrin (Vss − vascular volume), which did not differ between WT (1.00 ± 0.11) and HFE-KO mice (1.00 ± 0.15 mL/25 g; $P = 0.465$). This analysis indicates that tissue uptake of transferrin in HFE-KO strain and WT strain was similar, and that, therefore, the half-life difference mainly reflected the degradation of transferrin rather than its distribution.

We found that the plasma transferrin concentration in the B2m-KO strain, unlike the HFE-KO strain, was normal, in agreement with other reports (33). When we calculated transferrin production by a mass balance analysis using transferrin concentrations and catabolic rates, we found that transferrin production was decreased in HFE-KO mice but did not differ from normal in the B2m-KO strain. In contrast, the FcRn-KO strain manifested increased production rates of both transferrin and albumin (~20%), which compensated in part for the low oncotic pressure associated with hypercatabolic hypoalbuminemia that resulted from the absence of FcRn-mediated recycling of albumin (27). With transferrin production accelerated and the rate of degradation unchanged from normal, the FcRn-KO strain showed an increased transferrin steady-state concentration. Likewise, the B2m-KO strain, lacking FcRn, was severely hypoalbuminemic (Table 3), and like the FcRn-KO strain, was expected to manifest an upregulation of transferrin production. However, this anticipated ~20% upregulation of transferrin production due to FcRn deficiency in B2m-KO mice was offset by a 21% increase in transferrin catabolism due to HFE-deficiency, resulting in steady-state transferrin concentration that did not differ from normal (13). In contrast, normoalbuminemic HFE-KO mice (Table 3) likely were not stimulated to enhance their transferrin production rate. Further, we infer that HFE-KO mice would have ~12% decrease in the transferrin production rate based on 12% hypercatabolism and a ~24% decrease in the steady-state concentration of transferrin. These data might suggest that HFE alone upregulates transferrin production by a direct mechanism rather than indirectly via iron overload, because iron-overloaded B2m-KO mice do not have diminished transferrin production. Moreover, we also calculated transferrin production rates in WT and HFE-KO mice fed different amounts of iron; the HFE-KO strain still appeared to have lower production than the WT strain, especially for the iron-overload groups (not shown). Although transferrin production is modulated by iron status, the mechanism is not yet clear. Our production rate calculation was indirect because it was based on radioiodinated transferrin catabolism and endogenous transferrin concentration. Because the transferrin species (apo-, monoferric, and diferric) varies depending on the iron saturation, and radioiodinated transferrin may not perfectly behave as endogenous transferrin, more direct evidence is required for actual quantification. Among several observations, it is notable that iron-overload decreases transferrin production through a translational mechanism (34), whereas iron-deficiency enhances the transferrin synthesis at the transcription level (35). A more direct and systematic study of iron- and/or HFE-related transferrin synthesis is warranted.

The rapid decay and lower plasma concentration of transferrin in HFE-KO mice could be a direct effect of HFE-deficiency on transferrin turnover or it could be an effect secondary to the elevated TS seen with HFE-deficiency. We tested the latter

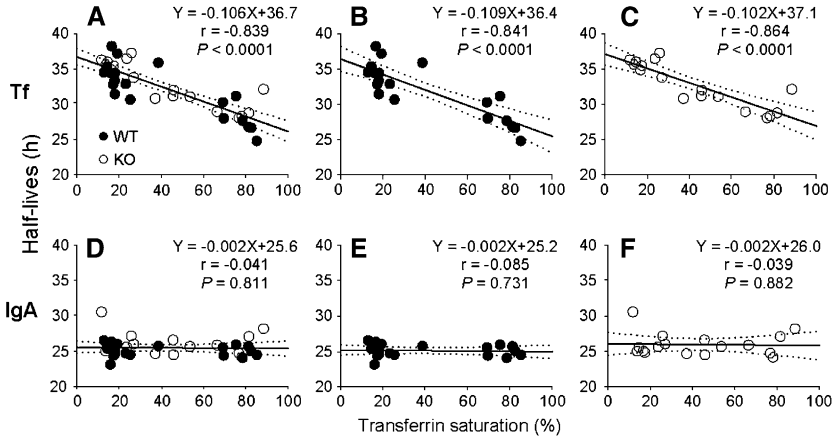

Figure 1 HFE does not directly influence the inverse relation between transferrin half-life and TS in WT and HFE-KO mice fed diets with different iron concentrations for 6 wk. Plots of the half-lives of radioiodinated transferrin (panels *A*, *B*, and *C*) and IgA (*D*, *E*, and *F*) against TS based on the results of the iron-level experiment shown in Table 4; Combined strains (both WT and HFE-KO strains; panels *A* and *D*), WT strain only (●; panels *B* and *E*), and HFE-KO strain only (○; *C* and *F*). Upper and lower dotted lines represent the 95% CI, whereas straight solid lines are the correlation lines for which equations are shown inside the panels. Tf, transferrin.

possibility and compared the rate of decay of transferrin in both WT and HFE-KO mice fed different iron diets to create a range of TS levels. We found that transferrin $t_{1/2}$ was similar in the WT and HFE-KO strains within the same diet group, which suggests that HFE did not directly affect transferrin decay; instead, enhanced transferrin decay resulted from the TS level, which in turn was due to HFE-deficiency. These data indicate an inverse correlation between the percentage of TS and transferrin $t_{1/2}$ (**Fig. 1***A*) in both the WT (Fig. 1*B*) and HFE-KO mice (Fig. 1*C*). Moreover, because this inverse correlation was observed for both WT and HFE-KO mice, we inferred that HFE does not have a direct effect on transferrin $t_{1/2}$; rather, HFE affects transferrin $t_{1/2}$ indirectly through its effect on TS, which in turn enhances transferrin decay in HFE-KO mice (Table 2). The IgA $t_{1/2}$, however, was not correlated with the percentage of TS (Fig. 1*D*) for either WT (Fig. 1*E*) or HFE-KO mice (Fig. 1*F*), suggesting that iron level induced hypercatabolism of transferrin but not of IgA or other plasma proteins.

We noted (Table 2) that the half-life of IgA in the HFE-KO strain was significantly shorter than normal; its $t_{1/2}$ difference between HFE-KO and WT mice was approximately half the transferrin $t_{1/2}$ difference between the two strains. However, the IgA $t_{1/2}$ in all experiments with the B2m-KO strain did not differ from normal. The reason for the shortened IgA $t_{1/2}$ in this single set of experiments is obscure. The difference from normal was small and of borderline significance ($P = 0.029$), but it was not seen in the B2m-KO strain, and there was no evidence that HFE deficiency led to decreased steady-state IgA concentrations. Furthermore, after we corrected the noted transferrin decay rate in the HFE-KO strain by dividing by the IgA decay rate, we found that the transferrin decay rate was still faster in the HFE-KO strain than the WT strain (4.4%, $P = 0.014$).

Although TS was significantly higher in the HFE-KO strain fed control iron diet compared with the WT strain (Table 4), transferrin decay did not differ between the 2 strains (Table 4). The likely reason for this paradox is that the mice of the HFE-KO strain were young (9 wk of age) and had accumulated insufficient iron to show detectable transferrin hypercatabolism. Compared with WT mice, the serum iron concentration in the HFE-KO strain was not higher ($P = 0.143$), although TS was modestly elevated (25 vs. 42%; $P = 0.021$; Table 4). In contrast, older HFE-KO mice (12–16 wk) showed considerable transferrin hypercatabolism (Table 2) and a much greater TS (73%) compared with WT mice (37%, Table 3). Nonetheless, the results of Table 4 clearly demonstrate that serum iron concentrations and the resultant TS, regardless of the presence of HFE, are associated with altered rates of transferrin catabolism.

As a possible explanation for the inverse correlation between transferrin $t_{1/2}$ and TS, we suggest that increased TS due to the iron overload of HFE-deficiency results in a downregulation of TfR1 expression (5–7). Fewer TfR1 would decrease the fraction of transferrin taken up and recycled by the TfR1-dependent pathway and would thereby increase the fraction of transferrin taken up by constitutive TfR1-independent pinocytosis (36,37). The TfR1-independent pathway (low affinity, high capacity) is responsible for transferrin degradation (10% of uptake is degraded), whereas the TfR1-dependent pathway (high affinity, low capacity) recycles almost all of endocytosed transferrin (37). Moreover, along with the downregulation of TfR1, the ligand (i.e., transferrin) concentration is increased due to the greater fraction of ferric-transferrin that results from the iron overload of HFE-deficiency; both cases favor the high degree of saturation of TfR1, which leads to an increase in the fraction of transferrin moving through the TfR1-independent pathway. These iron level–related changes in TfR1 and transferrin would result in an increased rate of transferrin decay and a lower plasma transferrin concentration. The inverse correlation between transferrin $t_{1/2}$ and TS, regardless of the presence of HFE, suggests that HFE is not directly involved in transferrin catabolism.

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