# Inactivation of the Hemochromatosis Gene Differentially Regulates Duodenal Expression of Iron-Related mRNAs Between Mouse Strains

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***Background & Aims:*** ***Hfe*** **knockout mice, like patients with hereditary hemochromatosis, have augmented duodenal iron absorption and increased iron deposition in hepatic parenchymal cells. The goals of the present study were to gain further insight into the control of iron absorption by comparing the transcript levels of iron-related genes in the duodenum of DBA/2** ***Hfe***$^{-/-}$ **mice, susceptible to iron loading, and wild-type controls, and to test whether variations in the duodenal expression of these messengers contribute to the DBA/2 and C57BL/6 strain differences in the severity of hepatic iron loading.** ***Methods:*** **Expression of the different transcripts was quantified by real-time polymerase chain reaction.** ***Results:*** **The 2 strains differ strikingly, not only in the severity of hepatic iron loading, but also in the duodenal expression of iron-related genes. In DBA/2** ***Hfe***$^{-/-}$ **mice, increased intestinal iron absorption results from the concomitant up-regulation of the Dcytb, DMT1, and FPN1 messengers. No increase in the expression of these messengers is seen in C57BL/6** ***Hfe***$^{-/-}$ **mice.** ***Conclusions:*** **The up-regulation of these transcripts suggests that an inappropriate iron-deficiency signal is sensed by the duodenal enterocytes, leading to an enhanced ferric reductase activity and the increase of duodenal iron uptake and transfer to the circulation. The genes modifying the hemochromatosis phenotype probably act by modifying the expression of these 3 messengers.**

Hereditary hemochromatosis (HH) is a common autosomal-recessive disorder of iron homeostasis characterized by increased dietary iron absorption and progressive iron accumulation, mainly in the liver. If untreated, iron accumulation can result in tissue damage, with clinical manifestations that include cirrhosis, hepatic carcinoma, congestive heart failure, and premature death.[1] The hemochromatosis gene, now called HFE, was isolated in 1996. Most patients with HH carry the same mutation, resulting in a change from cysteine to tyrosine at position 282 (C282Y) in the HFE protein.[2] Formal proof that mutations in HFE result in iron loading was provided by disrupting *Hfe* in mice.[3–5] *Hfe* knockout (*Hfe*$^{-/-}$) mice, like HH patients, have augmented duodenal iron absorption, abnormally high plasma transferrin saturations, and increased iron deposition in hepatic parenchymal cells. However, despite our understanding of the genetic basis of HH, the exact function of HFE in iron homeostasis remains unknown. The finding that wild-type HFE, but not the C282Y mutant HFE, binds to transferrin receptor (TfR) and reduces its affinity for transferrin[6] has led to various hypotheses of how HFE-TfR interactions establish a set point to regulate intestinal iron absorption,[7–9] but none of them has been formally proved.

Nonheme dietary iron, mostly in the form of ferric iron complexes, is first converted to a transportable form by duodenal cytochrome b (Dcytb), a putative brush-border, surface ferric reductase.[10] Ferrous iron is then supplied to divalent metal transporter 1 (DMT1), formerly called Nramp2 or DCT1, an apical transmembrane iron transporter that actively transports reduced dietary iron into intestinal enterocytes.[11,12] Iron traverses the epithelial cell and is exported through the basolateral membrane by a process that involves a second transmembrane iron transporter, ferroportin 1 (FPN1, also known as IREG1 or MTP1),[13–15] and requires the transmembrane-bound multicopper ferroxidase, hephaestin.[16] Another protein, called stimulator of Fe transport (SFT), has been described recently.[17] SFT, which is expressed in

*Abbreviations used in this paper:* Dcytb, duodenal cytochrome b; DMT1, divalent-metal transporter 1; FPN1, ferroportin 1; *Hfe*$^{-/-}$, *Hfe*-null mice; *Hfe*$^{+/+}$, wild-type controls; HH, hereditary hemochromatosis; PCR, polymerase chain reaction; SFT, stimulator of Fe transport; TfR, transferrin receptor; TIBC, total iron binding capacity.


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various organs, including the liver and the duodenum, has been found to facilitate both transferrin- and non–transferrin-bound iron uptake[17] and its enhanced expression in the liver of HH patients might contribute to the disease pathogenesis.[18]

Interestingly, $Hfe^{-/-}$ mice that carry mutations in DMT1 do not develop hepatic iron overload.[19] However, whether the increased intestinal iron absorption of $Hfe^{-/-}$ mice and HH patients results from up-regulated DMT1 is still controversial. Fleming et al.[20] reported that $Hfe^{-/-}$ mice have greater duodenal DMT1 messenger RNA (mRNA) levels than $Hfe^{+/+}$ mice, whereas a similar investigation found no differences in DMT1 mRNA and protein levels between knockout mice and controls.[21] The possibility that the increased intestinal absorption observed in $Hfe^{-/-}$ mice and HH patients might result from up-regulation of molecules other than or in addition to DMT1 has not been tested so far.

Another question of great interest is understanding why the severity of iron loading and clinical disease is variable in human patients with identical HFE genotypes, with some individuals homozygous for the C282Y mutation who do not show iron overload.[22–24] In the mouse, marked differences in the severity of hepatic iron loading in response to *Hfe* disruption have also been observed between the C57BL/6 and AKR strains, suggesting the existence of genes that modify the gravity of iron accumulation in this murine model of HH.[25] We observed that, compared with the C57BL/6 strain, the DBA/2 strain was also particularly susceptible to iron loading in response to *Hfe* disruption.

The first objective of the present study was thus to get more insight into the control of iron absorption by comparing the transcript levels of the recently described molecules participating in this process, Dcytb, DMT1, FPN1, hephaestin, and SFT, in the duodenum of DBA/2 $Hfe^{-/-}$ mice, susceptible to iron loading, and DBA/2 $Hfe^{+/+}$ controls. The second objective was to test whether variations in either the iron-reducing step, the apical-uptake step into the enterocyte, or the basolateral-transfer step might contribute to the mouse strain differences observed in the severity of iron accumulation by comparing the transcript levels of the molecules participating in these different steps in the duodenum of C57BL/6 and DBA/2 $Hfe^{-/-}$ mice.

## Materials and Methods

### Mice

The disrupted *Hfe* allele, initially on a mixed background (129/Ola × C57BL/6),[3] was bred by successive crosses for 9 generations onto the DBA/2 and C57BL/6 backgrounds. Mice heterozygous for the disrupted allele were then mated to produce *Hfe*-null ($Hfe^{-/-}$) mice. DBA/2 and C57BL/6 mice were purchased from the Centre d'Elevage Robert Janvier (Le Genest St-Isle, France) and used as wild-type ($Hfe^{+/+}$) controls. The studied DBA/2 population consisted of 8 male and 8 female $Hfe^{+/+}$ mice, and 12 male and 11 female $Hfe^{-/-}$ mice. The studied C57BL/6 population consisted of 5 male and 6 female $Hfe^{+/+}$ and 16 male and 10 female $Hfe^{-/-}$ mice. $Hfe^{-/-}$ and wild-type mice were housed in the IFR30 animal facility and had free access to water and R03 diet (UAR, Epinay-sur-Orge, France) containing 280 mg Fe/kg. All mice were analyzed between 6 and 7 weeks of age and fasted for 14 hours before blood sampling. After blood was obtained, the mice were killed and the duodenum (2-cm length of small intestine distal to the pylorus) was dissected for RNA isolation. Mucosa samples were scraped with a glass slide. Experimental protocols were approved by the IFR30 Animal Care and Use Committee.

### Measurement of Serum Iron, Transferrin Saturation, and Tissue Iron Content

Blood was obtained by puncture of the inferior vena cava. Serum iron and total iron binding capacity (TIBC) were assessed on a Roche/Hitachi 717 Automatic Analyzer (Roche Diagnostics, Meylan, France), by using a method based on ascorbic acid as reducing agent and ferrozine as chromagen. TIBC was measured after saturation of the transferrin by an iron solution and absorption of the excess iron on magnesium hydroxycarbonate, according to the TIBC kit protocol (ABX Diagnostics, Montpellier, France). Transferrin saturation was calculated as (serum iron/TIBC) × 100%. Hepatic iron content was evaluated as described previously.[26] Briefly, the liver specimens (0.5–4 mg) were first desiccated for 24 hours at 120°C in a ventilated oven. Thereafter, the dried samples were weighed and mineralized by strong acid digestion and heating. Iron was then complexed to the bathophenantroline sulfonate chromogen and absorbance measured at 535 nm. For histologic assessment of nonheme iron deposition, tissue sections were stained with Perl's Prussian blue, counterstained with nuclear fast red, and examined by light microscopy.

### Quantification of Duodenal Transcripts Through Real-Time Polymerase Chain Reaction

Expression of duodenum-specific transcripts was analyzed by a 2-step reverse-transcription polymerase chain reaction (PCR). Total RNA from mouse duodenum was isolated by using the SV Total RNA Isolation System (Promega, Charbonnières, France). RNA was then reverse-transcribed into complementary DNA with the M-MLV reverse transcriptase RNase H− (Promega). Quantification of the different mRNAs was performed through real-time PCR on a 5700 Sequence Detection System (Applied Biosystems, Courtaboeuf, France). The PCR reaction mix contained the complementary DNA quantity equivalent to 75 ng RNA, 0.3 μmol/L of each primer (sequences on Table 1), $4 \times 10^{-5}$X SYBR Green I (Sigma-Aldrich, Saint-Quentin Fallavier, France), 1X ROX (Life

**Table 1.** Primer Pairs Used to Quantify Iron-Related Transcripts Expressed in the Duodenum

| Gene | Sense and antisense primers | GenBank accession number (position)[a] |
|---|---|---|
| Dcytb | 5′-GCAGCGGGCTCGAGTTTA-3′ | AF354666 (137-235) |
| | 5′-TTCCAGGTCCATGGCAGTCT-3′ | |
| DMT1 | 5′-GGCTTTCTTATGAGCATTGCCTA-3′ | L33415 (289-385) |
| | 5′-GGAGCACCCAGAGCAGCTTA-3′ | |
| FPN1 | 5′-TTGCAGGAGTCATTGCTGCTA-3′ | AF226613 (1670-1789) |
| | 5′-TGGAGTTCTGCACACCATTGAT-3′ | |
| Hephaestin | 5′-TTGTCTCATGAAGAACATTACAGCAC-3′ | AF082567 (3803-3963) |
| | 5′-CATATGGCAATCAAAGCAGAAGA-3′ | |
| SFT | 5′-CTGTGCTCATTGAAGAGGACCTT-3′ | AA178012 (232-329) |
| | 5′-TCTGGTTGCTTTCTCAGTCACG-3′ | |
| β-Actin | 5′-GACGGCCAAGTCATCACTATTG-3′ | M12481 (652-740) |
| | 5′-CCACAGGATTCCATACCCAAGA-3′ | |

[a]All these primers map to gene coding sequences.

Technologies, Cergy Pontoise, France) to normalize for non–PCR-related fluctuations in fluorescence signal, and 1X Platinum Quantitative PCR SuperMix-UDG (Life Technologies). PCR amplification began with 1 cycle of 50°C for 2 minutes (UDG-PCR carry-over decontamination) and 95°C for 10 minutes, followed with 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 60 seconds. Direct detection of PCR products was monitored by measuring the increase in fluorescence caused by the binding of SYBR Green to double-stranded DNA. All experiments were performed in duplicate. Quantification was obtained by comparing the threshold cycles of unknown samples against standard curves with known copy numbers.

### Data Analyses

Raw values for Dcytb, DMT1, FPN1, hephaestin, and SFT were first normalized to the β-actin endogenous control to account for variability in the initial concentration and quality of the total RNA and in the conversion efficiency of the reverse-transcription reaction. Briefly, for each experimental sample, the amounts of target and β-actin mRNAs were determined from the appropriate standard curves and $10^6 \times$ the target amount was divided by the endogenous reference amount to obtain a normalized target value. Results were expressed as mean ± SEM. As observed previously,[27] most of the mRNA distributions had a high degree of skewness. Therefore, only nonparametric tests or parametric tests after logarithmic transformations of the variables were applied. The distributions of transferrin saturation and liver iron content as well as the distributions of the mRNA quantities obtained in DBA/2 *Hfe*$^{+/+}$ and *Hfe*$^{-/-}$ mice were compared by nonparametric Wilcoxon tests. Correlations between transcript levels were evaluated by Spearman's rank-order correlation tests. For strain comparisons, mean values for transferrin saturation, liver iron concentration, and the mRNA quantities (after logarithmic transformation) were compared across mice from the different strains and with the different genotypes by 1-way analyses of variance and subsequent Duncan's multiple-range tests.

## Results

### Transferrin Saturation and Hepatic Iron Concentration in DBA/2 *Hfe*$^{-/-}$ Mice

Twenty-three DBA/2 *Hfe*$^{-/-}$ mice and 16 *Hfe*$^{+/+}$ mice of the same genetic background were analyzed at 6–7 weeks of age. Compared with the *Hfe*$^{+/+}$ controls, the *Hfe*$^{-/-}$ mice had highly saturated serum transferrin levels (86.5% ± 2.2% vs. 45.9% ± 2.4%; $P < 0.0001$) and 6.4-fold higher levels of hepatic iron (2420 ± 155 vs. 377 ± 32 μg Fe/g dry liver; $P < 0.0001$) (Figure 1*A*). The measured elevations in hepatic iron concentrations are within the range of values previously reported for similarly aged *Hfe*$^{-/-}$ mice.[3–5] The deposition of liver iron was predominantly in hepatocytes and showed the typical zonal gradient seen in patients with HH (not shown).

### Duodenal Expression of the Dcytb, DMT1, and SFT mRNAs in DBA/2 *Hfe*$^{-/-}$ Mice

Transcripts of the molecules involved in reducing dietary iron to a transportable form (Dcytb) and transporting reduced iron from the intestinal lumen into the enterocytes (DMT1) were examined first. As indicated in the Materials and Methods section, all the transcript amounts reported in this study are values normalized to the β-actin endogenous control. As shown in Figures 1*B* and 1*C*, the level of duodenal expression of the Dcytb transcript was increased 6.1-fold on average (1295 ± 203 vs. 212 ± 46; $P < 0.0001$) and that of DMT1 5.1-fold (3577 ± 1179 vs. 707 ± 113; $P < 0.0001$) in the *Hfe*$^{-/-}$ mice relative to the *Hfe*$^{+/+}$ mice of identical genetic background. Thus, despite elevated serum iron saturation and liver iron content in *Hfe*$^{-/-}$ mice, the expression of the duodenal Dcytb and DMT1 transcripts is up-regulated. Dcytb and DMT1 transcript levels were more significantly correlated in *Hfe*$^{-/-}$ mice (Spearman rank-correlation coefficient $R = 0.72$; $P < 0.0001$) than

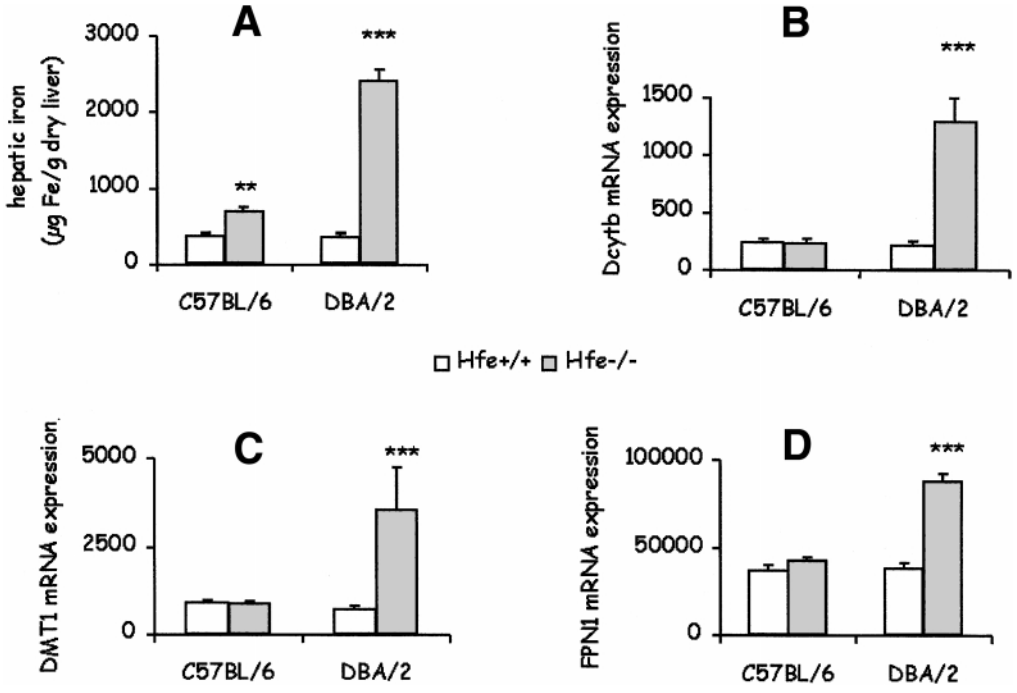


**Figure 1.** Effect of strain differences and Hfe genotype on hepatic iron concentration and on the duodenal expression of the Dcytb, DMT1, and FPN1 messengers. The amounts of Dcytb, DMT1, and FPN1 mRNAs were normalized by dividing raw values by the amount of beta-actin mRNA and expressed as the normalized values per $10^6$ beta-actin molecules. Data are presented as mean ± SEM. $***P < 0.0001$; $**P = 0.003$ (when $Hfe^{-/-}$ mice were compared with $Hfe^{+/+}$ mice of the same genetic background).

in $Hfe^{+/+}$ mice (R = 0.60; $P = 0.01$). The transcript levels of the stimulator of Fe transport, SFT, previously shown to be localized in the subapical part of the villus cells,[17] were not significantly increased in the duodenum of the $Hfe^{-/-}$ mice, compared with the wild-type $Hfe^{+/+}$ mice (2645 ± 370 vs. 2578 ± 165; $P = 0.07$).

### Duodenal Expression of the FPN1 and Hephaestin mRNAs in DBA/2 $Hfe^{-/-}$ Mice

Transcripts of the molecules involved in the export of iron through the basolateral membrane of the villus enterocytes, FPN1 and hephaestin, were examined next. As shown in Figure 1*D*, the level of duodenal expression of the FPN1 transcript was increased 2.3-fold (88,028 ± 4272 vs. 38,400 ± 2805; $P < 0.0001$) in the $Hfe^{-/-}$ mice relative to the $Hfe^{+/+}$ mice. The level of duodenal expression of the hephaestin transcript was increased 1.2-fold, a difference that is not significant after correction for multiple testing (7695 ± 510 vs. 6568 ± 846; $P = 0.03$). A significant correlation between the FPN1 and hephaestin transcripts was also observed in $Hfe^{-/-}$ (R = 0.60; $P = 0.003$), but not in $Hfe^{+/+}$ mice (R = 0.46; $P = 0.07$).

### Simultaneous Up-Regulation of the Dcytb, DMT1, and FPN1 Transcripts in DBA/2 $Hfe^{-/-}$ Mice

For each mouse, knockout or wild-type, the quantities of the 3 transcripts that were significantly upregulated as a consequence of *Hfe* disruption (Dcytb, DMT1, and FPN1) were plotted on a 3-dimensional graph. As can be seen in Figure 2, overall, the 3 processes of reduction, uptake at the apical membrane, and transport through the basolateral membrane work in unison,

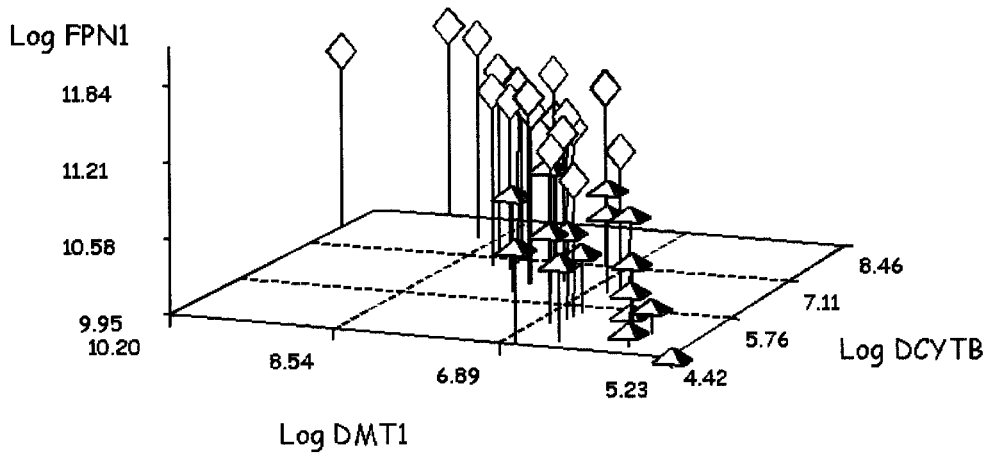


**Figure 2.** Simultaneous variations of the Dcytb, DMT1, and FPN1 transcripts in DBA/2 wild-type and knockout mice. The natural logarithm of the transcript quantities were used here to induce normality of the distributions. Simultaneous variations of the transcripts are shown in DBA/2 $Hfe^{+/+}$ (*pyramid*) and $Hfe^{-/-}$ (*diamond*) mice.

both in *Hfe*$^{-/-}$ and in *Hfe*$^{+/+}$ mice. However, the transcript levels of the 2 iron transporters, DMT1 and FPN1, are more significantly correlated in *Hfe*$^{+/+}$ mice ($R = 0.78$; $P = 0.0004$) than in *Hfe*$^{-/-}$ mice ($R = 0.47$; $P = 0.03$).

### Expression of the Dcytb, DMT1, and FPN1 mRNAs in C57BL/6 Hfe$^{-/-}$ Mice

The expression of the 3 transcripts significantly up-regulated in DBA/2 *Hfe*$^{-/-}$ mice were then measured in 26 C57BL/6 *Hfe*$^{-/-}$ mice and 11 *Hfe*$^{+/+}$ mice of the same genetic background. Mean transferrin saturations, mean concentrations of hepatic iron, and mean levels of the 3 duodenal transcripts differed significantly across mice from the different strains and with the different genotypes (1-way analyses of variance all significant at $P < 0.0001$). Within each strain, the *Hfe*$^{-/-}$ mice had significantly higher transferrin saturations and liver iron concentrations than *Hfe*$^{+/+}$ mice. However, the increase in transferrin saturation was only 1.3-fold in the C57BL/6 strain (63.6% ± 1.8% vs. 48.0% ± 3.5%), whereas it was 1.9-fold in the DBA/2 strain (86.5% ± 2.2% vs. 45.9% ± 2.4%). Moreover, as shown in Figure 1*A*, the increase in liver iron content was only 1.9-fold in the C57BL/6 strain (711 ± 54 vs. 382 ± 24 μg Fe/g dry liver; $P = 0.003$), whereas it was previously shown to be 6.4-fold in the DBA/2 strain. These results indicate that the DBA/2 strain has a much greater propensity for liver iron loading in response to *Hfe* disruption than the C57BL/6 strain, and confirm that the latter strain is almost resistant.[25] The mean levels of the Dcytb, DMT1, and FPN1 messengers were not significantly different between C57BL/6 *Hfe*$^{+/+}$ mice, C57BL/6 *Hfe*$^{-/-}$ mice, and DBA/2 *Hfe*$^{+/+}$ mice, but significantly differed from the mean levels in the DBA/2 *Hfe*$^{-/-}$ mice (Figures 1*B–D*).

## Discussion

The up-regulation of the Dcytb (6.1-fold), DMT1 (5.1-fold), and FPN1 (2.3-fold) transcripts observed in the DBA/2 *Hfe*$^{-/-}$ mice relative to the *Hfe*$^{+/+}$ mice of the same genetic background is reminiscent of the increase in expression of the Dcytb, DMT1 (≈15-fold each), and FPN1 (≈2.5-fold) transcripts observed in wild-type mice fed an iron-deficient diet for 2 weeks relative to control mice fed an iron-balanced diet (data submitted for publication). This up-regulation thus very much resembles the adaptive response of the duodenum to iron deficiency and suggests that, in DBA/2 *Hfe*$^{-/-}$ mice, an inappropriate iron-deficiency signal is sensed by the duodenal enterocyte despite the considerably increased iron stores (6.4-fold higher levels of hepatic nonheme iron). Although it is possible that increased mRNA production does not result in increased protein production, this hypothesis is unlikely. Indeed, McKie et al.[10] observed that iron deficiency obtained by feeding mice an iron-deficient diet strongly induced both Dcytb mRNA levels and protein expression in duodenal extracts, and that ferric reductase activity was significantly higher in membranes prepared from Caco-2 cells transfected with Dcytb than in membranes prepared from untransfected cells. Furthermore, Zoller et al.[27] recently showed that, in humans, differences in DMT1 and FPN1 mRNA expression well reflected the corresponding changes in the expression of the respective proteins in the duodenum.

In the mouse, mucosal Fe(III) reduction rates, though quantitatively higher than uptake rates, paralleled changes in the iron uptake rates induced by iron deficiency.[28] This provides strong evidence for a simultaneous up-regulation of both the reduction and uptake processes at the intestinal level in response to the body's needs for iron. The correlated up-regulation of both Dcytb and DMT1 mRNAs observed in DBA/2 *Hfe*$^{-/-}$ mice further shows the close connection between the reduction and the uptake processes in another pathologic condition, targeted disruption of the *Hfe* gene. The lower degree of significance of this correlation in *Hfe*$^{+/+}$ mice may reflect a regulation taking place specifically when an iron-deficiency signal, either legitimate or aberrant, as in hemochromatosis, is relayed to the enterocyte. Of interest, patients with HH show a significant increase in the duodenal mucosal Fe(III) reducing activity compared with normal controls. This increase in reducing activity also correlates with the changes in Fe uptake rates and is site-specific because no enhancement in reducing activity was seen with gastric samples.[29] The observation that duodenums of HH patients have higher ferric reductase activity suggests that the enhanced Dcytb mRNA expression observed here in the DBA/2 strain correlates with the conversion of a higher amount of ferric iron to the ferrous form taken over by the up-regulated DMT1 transporter and thus contributes to the disease etiology.

Other groups have reported increases in duodenal DMT1 mRNA levels in *Hfe* knockout mice[20] and in human patients with HH.[30] In addition, Griffiths et al.[31] clearly showed that disruption of the *Hfe* gene in mice of the 129/SvJ background up-regulated functional DMT1 transporters and enhanced uptake of ferrous iron by this mechanism. These results were recently questioned by Canonne-Hergaux et al.[21] Our data in C57BL/6 *Hfe*$^{-/-}$ mice suggest that the source of the discrepancy could be attributed to strain-to-strain variations between the mice analyzed in the different studies. Our observations that

DMT1 and FPN1 mRNA levels are unidirectionally up-regulated in the DBA/2 *Hfe*$^{-/-}$ mice are also in agreement with the recent findings made in HH patients[27] and very likely mean that increased duodenal iron uptake, as indicated by high DMT1 mRNA/protein expression, is associated with increased iron export from enterocytes into the circulation, as reflected by high FPN1 mRNA-protein levels.

The nonsignificant changes in hephaestin mRNA levels in *Hfe*$^{-/-}$ mice suggest that there is no need for further enhancement of hephaestin mRNA synthesis to allow the export of the excess of iron transported from the intestinal lumen into the enterocytes. Finally, though the stimulator of Fe transport SFT was shown to be mildly increased in the duodenum of HH patients,[32] its mRNA expression did not significantly vary in the DBA/2 *Hfe*$^{-/-}$ mice analyzed in this study or in the 129/Sv *Hfe*$^{-/-}$ mice previously studied,[33] making unlikely the hypothesis that SFT is responsible for the increased intestinal iron absorption observed in these animals.

The mechanism by which the expression of Dcytb, DMT1, and FPN1 is regulated in response to the needs of the body for iron and the reasons why their expression appears unrelated to these needs in the DBA/2 *Hfe* knockout mice or the HH patients remain to be determined. Up-regulation of the 3 transcripts in DBA/2 *Hfe* knockout mice suggests that an inappropriate iron-deficiency signal is sensed by the duodenal enterocytes. This hypothesis is supported by the finding of increased iron regulatory protein 1 activity and high TfR mRNA expression, both indicative of intracellular deprivation, in duodenal biopsy specimens of patients with HH.[34] This inappropriate signal could result from the alteration of a putative sensor for iron homeostasis whose existence has been suspected for a long time.[9] The nature of this sensor is still unknown, but the hepcidin peptide that is secreted in plasma after synthesis and maturation in the liver was recently suggested to fulfill this important role. Indeed, Pigeon et al.[35] showed that accumulation of iron in the liver up-regulated hepcidin expression, whereas Nicolas et al.[36] clearly showed that a complete defect in hepcidin expression was responsible for progressive tissue iron overload. The similarities of the alterations in iron metabolism between *Hfe*$^{-/-}$ mice and *Usf2*$^{-/-}$ hepcidin-deficient mice[36] suggest that hepcidin may function in the same regulatory pathway as HFE. The requirement of a functional HFE for hepcidin activity would explain why DBA/2 *Hfe* knockout mice incorrectly perceive the body's iron needs and up-regulate the transcripts of the molecules involved in duodenal iron reduction, uptake, and transfer to the circulation, as shown in this study.

The present work shows that the 2 C57BL/6 and DBA/2 strains differ strikingly, not only in the severity of hepatic iron loading in response to *Hfe* disruption but also in the duodenal expression of iron-related genes. In DBA/2 *Hfe*$^{-/-}$ mice, increased intestinal iron absorption results not only from the up-regulation of DMT1, but also from that of Dcytb and FPN1. On the contrary, C57BL/6 *Hfe*$^{-/-}$ mice do not differ in their duodenal expression of these mRNAs from C57BL/6 *Hfe*$^{+/+}$ and DBA/2 *Hfe*$^{+/+}$ mice. These observations cannot be accounted for by the relatively young age of the mice analyzed in this study (7 weeks). Indeed, virtually no hepatic iron staining was detectable in C57BL/6 *Hfe*$^{-/-}$ mice maintained up to 42–45 weeks. The marked phenotypic differences between the C57BL/6 and DBA/2 *Hfe*$^{-/-}$ strains suggest the existence of genes other than HFE that modify the murine HH phenotype. These genes probably act by modifying the expression of the messengers involved in each of the 3 steps of iron reduction, transport through the apical membrane, and export through the basolateral membrane of the enterocyte. Their identification, theoretically possible by carrying a genome-wide search in the F2 generation, would be of great practical importance because their human homologues may potentially be involved in the variable susceptibility to iron loading observed in individuals homozygous for the HFE C282Y mutation. In any case, they will add to our understanding of the regulation of iron homeostasis.

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