Expression of Stimulator of Fe Transport Is Not Enhanced in *Hfe* Knockout Mice[1,2]

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ABSTRACT *Hfe* knockout (−/−) mice recapitulate many of the biochemical abnormalities of hereditary hemochromatosis (HH), but the molecular mechanisms involved in the etiology of iron overload in HH remain poorly understood. It was found previously that livers of patients with HH contained 5-fold higher SFT (stimulator of Fe transport) mRNA levels relative to subjects without HH. Because this observation suggests a possible role for SFT in HH, we investigated SFT mRNA expression in $Hfe^{-/-}$ mice. The 4- and 10-wk-old $Hfe^{-/-}$ mice do not have elevated levels of hepatic SFT transcripts relative to age-matched $Hfe^{+/+}$ mice, despite having 2.2- and 3.3-fold greater hepatic nonheme iron concentrations, respectively. Northern blot analyses of various mouse tissues revealed that SFT is widely expressed. The novel observation that SFT transcripts are abundant in brain prompted a comparison of SFT transcript levels and nonheme iron levels in the brains of $Hfe^{+/+}$ and $Hfe^{-/-}$ mice. Neither SFT mRNA levels nor nonheme iron levels differed between groups. Further comparisons of $Hfe^{-/-}$ and $Hfe^{+/+}$ mouse tissues revealed no significant differences in SFT mRNA levels in duodenum, the site of increased iron absorption in HH. Important distinctions between $Hfe^{-/-}$ mice and HH patients include not only differences in the relative rate and magnitude of iron loading but also the lack of fibrosis and phlebotomy treatment in the knockout animals. J. Nutr. 131: 1459–1464, 2001.

KEY WORDS: • *stimulator of Fe transport* • HFE • *hemochromatosis* • *brain* • *mice*

Hereditary hemochromatosis (HH)[4] is a common genetic 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 (Bothwell and MacPhail 1998).

Recently, Feder et al. (1996) identified a candidate gene for HH called *HFE*. Two missense mutations have been found within HFE in HH patients, but only one, a Cys-to-Tyr substitution at amino acid 282 of HFE (C282Y), is associated with phenotypic expression of HH (Feder et al. 1996). Formal proof that mutations in *HFE* result in iron loading was provided by disrupting *Hfe* in mice (Bahram et al. 1999, Levy et al. 1999, Zhou et al. 1998). *Hfe* knockout ($Hfe^{-/-}$) mice, like HH patients, have augmented duodenal iron absorption, abnormally high plasma transferrin saturations and increased deposition of iron in hepatic parenchymal cells. Mice homozygous for the C282Y mutation also incur hepatic iron loading but less so than in mice with the null mutation (Levy et al. 1999). However, despite our understanding of the genetic basis of HH, the exact function of *HFE* in iron homeostasis remains unknown (Powell et al. 2000). The finding that wild-type HFE, but not HFE (C282Y), binds to transferrin receptor (TFR) and reduces its affinity for transferrin (Feder et al. 1998) has led to various hypotheses of how HFE-TFR interactions establish a set point to regulate intestinal iron absorption (Levy et al. 2000, Roy et al. 2000, Waheed et al. 1999).

Dietary nonheme iron is absorbed by intestinal epithelial cells via DMT1 (divalent metal transporter 1), an apical transmembrane protein (Fleming et al. 1997, Gunshin et al. 1997). $Hfe^{-/-}$ mice that carry mutations in DMT1 do not develop hepatic iron overload (Levy et al. 1999). However, whether the increased intestinal iron absorption of $Hfe^{-/-}$

[1] Presented in part at Experimental Biology '00, April 2000, San Diego, CA [Knutson, M. D., Levy, J. L., Andrews, N. C. & Wessling-Resnick, M. (2000) Stimulator of Fe transport expression in *HFE*-knockout mice. FASEB J. 14: A731 (abs.); and at American Society for Biochemistry and Molecular Biology, June 2000, Boston [Knutson, M. D., Levy, J. L., Andrews, N. C. & Wessling-Resnick, M. (2000) Expression of stimulator of Fe transport mRNA in *HFE*-knockout mice. FASEB J. 14: A1328 (abs.)].

[2] Supported by National Institutes of Health Grants DK07703 and DK09998 (M.D.K), DK56160 (M.W.R.), HL03503 (J.E.L.) and HL51057 (N.C.A). M.W.-R. is an Established Investigator of the American Heart Association. N.C.A. is an Associate Investigator of the Howard Hughes Medical Institute.

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[4] Abbreviations used: $B2m^{-/-}$, β-2 microglobulin knockout; $B2m^{+/+}$, β-2 microglobulin wild type; DMT1, divalent metal transporter 1; EST, expressed sequence tag; $Hfe^{-/-}$, *Hfe* knockout; $Hfe^{+/+}$, *Hfe* wild type; HH, hereditary hemochromatosis; NTBI, non–transferrin-bound iron; SFT, stimulator of iron transport; TFR, transferrin receptor; $Trf^{hpx/hpx}$, homozygous hypotransferrinemic; $Trf^{+/?}$, either *Trf* wild type ($Trf^{+/+}$) or heterozygous for the hypotransferrinemic mutation ($Trf^{+/hpx}$).

Manuscript received 18 September 2000. Initial review completed 14 November 2000. Revision accepted 21 February 2001.

mice (Bahram et al. 1999) and HH patients (Powell et al. 1970) results from up-regulated DMT1 is controversial. Fleming et al. (1999) reported that $Hfe^{-/-}$ mice have greater duodenal DMT1 mRNA levels than $Hfe^{+/+}$ mice, whereas a similar investigation found no differences in DMT1 mRNA and protein levels between knockouts and controls (Cannone-Hergaux et al. 2001). Studies of mice carrying mutations that impair normal iron metabolism indicate that a number of other genes most likely influence intestinal iron absorption in $Hfe^{-/-}$ mice (Levy et al. 2000).

The mechanisms responsible for the hepatic iron loading in HH are less clear. The liver normally takes up the majority of its iron via TFR (Bonkovsky 1991), but under conditions of high intracellular iron, TFR expression becomes down-regulated (Hubert et al. 1993). Consequently, TFR expression is virtually undetectable in livers of HH patients (Sciot et al. 1987) and $Hfe^{-/-}$ mice (Fleming et al. 2000). Other iron transport mechanisms therefore appear to be responsible for the progressive hepatic iron accumulation in HH. One pathway involves the uptake of non–transferrin-bound iron (NTBI), which is detected in the plasma of HH patients when transferrin becomes highly saturated (Aruoma et al. 1988). NTBI undergoes a rapid first-pass extraction by the liver (Wright et al. 1986) and thus can contribute to hepatic iron loading in HH. How the liver takes up NTBI is unknown, but some evidence suggests that hepatic iron uptake is affected by SFT (stimulator of Fe transport). Cells transfected with SFT cDNA exhibit increased uptake of NTBI and transferrin-bound Fe (Gutierrez et al. 1997). Moreover, iron-dependent modulation of SFT mRNA levels has been observed in human liver HepG2 cells (Yu et al. 1998). Importantly, we have found that liver samples from HH patients contain 5-fold higher levels of SFT mRNA relative to subjects without HH (Yu et al. 1998). This latter finding, which suggests a role for SFT in the etiology of HH, motivated us to investigate SFT expression in a mouse model of the human disease. The main objective of the present study was to determine whether SFT levels are up-regulated in the livers of $Hfe^{-/-}$ mice. In addition, SFT expression in various tissues was examined, and SFT transcript levels in the duodenums and brains of $Hfe^{-/-}$ and $Hfe^{+/+}$ mice were compared.

MATERIALS AND METHODS

Animals. *Hfe*-null ($Hfe^{-/-}$) mice were bred and genotyped as described previously (Levy et al. 1999). $Hfe^{-/-}$ and wild-type ($Hfe^{+/+}$) mice (129/SVEvTac background) were housed in the barrier facility at Children's Hospital and had free access to water and Prolab RMH3000 diet (PMI Nutrition International, Richmond, IN; http://www.labdiet.com/5p00.htm). Animal protocols were approved by the Children's Hospital Animal Care and Use Committee. Non–food-deprived male and female mice of ages 4 and 10 wk were killed by exposure to carbon dioxide, and tissues were collected and snap-frozen in liquid nitrogen. To stabilize tissue RNA after excision, duodenum (~1.5-cm length of small intestine distal to the pylorus) was submerged in RNAlater (Ambion, Austin, TX) and stored overnight at 4°C before RNA isolation. Liver samples from $Trf^{hpx/hpx}$ mice were kindly provided by Dr. Mark Fleming (Harvard Medical School, Boston, MA). $Trf^{hpx/hpx}$ mice were treated with 6 mg transferrin on d 1, 8, 15 and 22 of life.

Identification of murine SFT cDNA. Searches of the murine EST database for SFT sequences identified an EST clone (GenBank accession no. AA178012) with identity to human SFT (GenBank accession no. AF020761). Digestion of the EST clone (I.M.A.G.E. Clone I.D. 620233; Genome Systems, St. Louis, MO) with *Not*I and *Eco*RI produced a fragment of ~0.9 kb that was sequenced (Molecular Medicine Unit, Beth Israel Deaconess Medical Center, Boston, MA). The fragment contains 879 nucleotides with 83% identity to the human orthologue (data not shown). This cDNA was used as the template for the SFT probe in Northern blot analysis.

Northern blot analyses. Total cellular RNA was isolated using RNAzol B (Tel-Test, Friendswood, TX) according to the manufacturer's protocol. RNA was electrophoresed in 1.2% agarose gel containing 2.2 mol formaldehyde/L, transferred to Nytran N membranes (Schleicher & Schuell, Keene, NH) using the Turboblotter transfer system (Schleicher & Schuell) and immobilized by UV cross-linking. Blots were prehybridized at 42°C for 4 h in 50% formamide (750 mol NaCl, 150 mol Tris, 113 mol Na_2HPO_4, 45 mol NaH_2PO_4 and 4 mol $Na_4P_2O_7$ per L, pH 7.4), 10× Denhardt's reagent, 10 mol ETDA/L, 0.1% SDS and 200 mg denatured salmon sperm DNA/L. Blots were then hybridized for 24–36 h at 42°C in prehybridization solution containing 10% dextran sulfate and a randomly primed ^{32}P-labeled murine SFT probe. After washing in 0.1% SDS and 0.1× SSC at room temperature for 1 h and at 60°C for 30 min, radioactivity was detected by autoradiography and PhosphorImaging (Personal FX; Bio-Rad, Hercules, CA). Blots were subsequently rehybridized with a randomly primed ^{32}P-labeled probe for mouse β-actin (DECAtemplate; Ambion, Austin, TX). Signal intensities were quantified using Quantity One software (Bio-Rad), and values obtained for SFT transcripts were normalized to those obtained for β-actin. To confirm that the β-actin reference transcript did not differ between $Hfe^{+/+}$ and $Hfe^{-/-}$ mice, blots were stripped and reprobed to determine mRNA levels of a second reference transcript, the ribosomal phosphoprotein 36B4 (Laborda 1991). The analytical error of Northern blot analysis was assessed by routine determination of samples in duplicate, and the mean difference in values for normalized SFT mRNA was 8.4% (n = 17, range 1–18%), indicating relatively low analytical variability.

Measurement of tissue nonheme iron concentration. Hepatic nonheme iron was measured colorimetrically after acid digestion of ~100 mg of tissue (Torrance and Bothwell 1968). Total brain nonheme iron was measured after the entire brain was homogenized using a ground glass pestle and tube. To prevent RNase activity, the homogenization buffer (PBS, pH 7.4) contained 4× 10^4 U RNasin/L (Promega, Madison, WI). One aliquot of the homogenate was used for RNA isolation as described above. Brain nonheme iron concentration was measured colorimetrically after acid digestion of ~one-half total homogenate.

Statistical analyses. Values are expressed as mean ± SE. Differences between means were determined using unpaired Student's t test ($P < 0.05$).

RESULTS

Tissue distribution of SFT mRNA. The pattern of SFT expression in various mouse tissues was determined using Northern blot analysis; the 1.4-kb SFT transcript is widely expressed (**Fig. 1**). A faint band of ~2.4 kb also can be seen in some tissues, as has been reported for human tissues (Gutierrez et al. 1997). Previous Northern blot analyses of human tissues revealed SFT expression in spleen, small intestine, colon, thymus, prostate, testis, ovary and peripheral blood leukocytes (PBL), with the highest levels in PBL and the spleen (Gutierrez et al. 1997). Here we found that SFT transcripts are also found in mouse spleen, small intestine (duodenum) and colon, as well as in mouse liver, kidney, lung, heart and brain. Of particular interest for this study were the confirmation that SFT transcripts are expressed in liver and duodenum of mice and the observation that SFT transcripts are particularly abundant in brain.

Nonheme iron levels in liver of Hfe$^{-/-}$ mice. The 4-wk-old $Hfe^{-/-}$ mice had 2.2-fold higher ($P < 0.05$) levels of hepatic nonheme iron than $Hfe^{+/+}$ mice; by 10 wk of age, these values were 3.3-fold higher ($P < 0.001$) (**Fig. 2**). The measured elevations in hepatic nonheme iron concentrations are within the range of values previously reported for similarly aged $Hfe^{-/-}$ mice (Bahram et al. 1999, Fleming et al. 1999 and 2000, Levy et al. 1999).

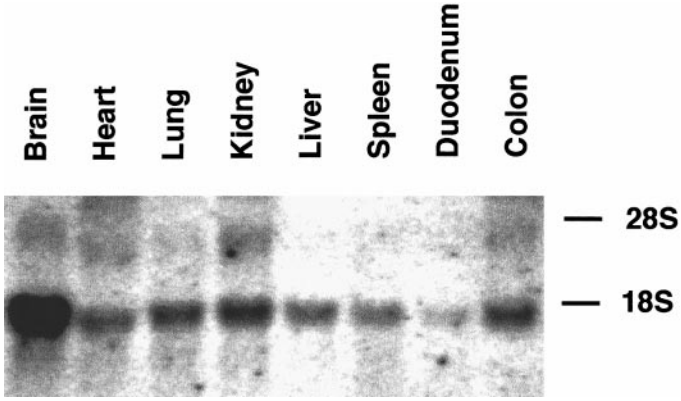

FIGURE 1 Northern blot analysis of SFT expression in various mouse tissues. Twenty micrograms of total RNA from the mouse tissues indicated were electrophoresed and transferred to a Nytran N membrane, as described in Materials and Methods. Equal loading of RNA in different lanes was confirmed by ethidium bromide staining (data not shown). The Northern blot was hybridized with ^{32}P-labeled probe for murine SFT and subjected to autoradiography. Shown is a 4-d exposure using two intensifying screens.

SFT mRNA levels in liver of* Hfe$^{-/-}$ *mice. To determine whether hepatic SFT expression is up-regulated in *Hfe*$^{-/-}$ mice, SFT mRNA levels were compared in *Hfe*$^{-/-}$ and *Hfe*$^{+/+}$ mice at 4 and 10 wk of age (**Fig. 3**, *A* and *B*, respectively). No differences in hepatic SFT mRNA levels were observed between groups at either age. The finding that SFT expression is not higher in the *Hfe*$^{-/-}$ mice contrasts markedly with the observed up-regulation of SFT mRNA in HH patients (Yu et al. 1998). To confirm that the apparent lack of SFT up-regulation did not reflect differences in β-actin expression between *Hfe*$^{+/+}$ and *Hfe*$^{-/-}$ mice, blots were reprobed to measure levels of a second reference transcript, 36B4 (Laborda 1991). For the Northern blot shown in Fig. 3A, the R^2 between β-actin and 36B4 was 0.90; for Fig. 3B, R^2 was 0.84. Excellent correlations between β-actin and 36B4 mRNA levels and the lack of any difference in SFT expression normalized to either reference transcript strengthen the conclusion that SFT mRNA levels are not enhanced in *Hfe*$^{-/-}$ mice.

SFT mRNA in duodenum of* Hfe$^{-/-}$ *mice. Because *Hfe*$^{-/-}$ mice have increased absorption of dietary iron by the small intestine (Bahram et al. 1999), duodenal SFT expression was also evaluated. SFT transcript levels did not differ significantly between groups at 4 or 10 wk of age (**Fig. 4**). It therefore seems unlikely that SFT is responsible for the increased intestinal iron absorption observed in these animals (Bahram et al. 1999).

SFT mRNA and nonheme iron levels in brain of* Hfe$^{-/-}$ *mice. The observation that SFT transcripts are abundant in brain prompted further comparison of SFT transcript and nonheme iron levels in total brain homogenate of *Hfe*$^{+/+}$ and *Hfe*$^{-/-}$ mice. SFT mRNA levels did not differ between groups at either age (**Fig. 5**). Total brain nonheme iron concentrations for 4-wk-old *Hfe*$^{+/+}$ and *Hfe*$^{-/-}$ mice were 0.32 ± 0.08 and 0.25 ± 0.02 μmol/g wet brain, respectively; in 10-wk-old mice, values were 0.27 ± 0.01 and 0.30 ± 0.01 μmol/g wet brain. Total brain iron levels did not differ. The lack of iron accumulation in the brain, despite significant hepatic iron loading, is consistent with studies of other animal models of iron overload (Papanastasiou et al. 2000).

SFT mRNA and nonheme iron levels in liver of hypotransferrinemic (Trf$^{hpx/hpx}$) mice. The observation that *Hfe*$^{-/-}$ mice do not have elevated levels of hepatic SFT transcripts motivated us to examine hepatic SFT mRNA levels in *Trf*$^{hpx/hpx}$ mice (Bernstein 1987). When maintained without continuous transferrin or transfusional therapy, *Trf*$^{hpx/hpx}$ mice absorb excessive amounts of dietary iron and accumulate greater levels of hepatic iron in parenchymal cells than do *Hfe*$^{-/-}$ mice (Trenor et al. 2000). The 4- to 5-wk-old *Trf*$^{hpx/hpx}$ mice had 2.6-fold higher ($P < 0.05$) hepatic nonheme iron concentrations than *Trf*$^{+/?}$ mice; at 10 wk, these levels were 22-fold higher ($P < 0.0001$) (**Fig. 6**). [*Trf*$^{+/?}$ denotes that the mice were either wild-type (*Trf*$^{+/+}$) or heterozygous for the hpx mutation (*Trf*$^{+/hpx}$). *Trf*$^{+/hpx}$ mice do not accumulate significantly more hepatic iron than do *Trf*$^{+/+}$ mice (Trenor et al. 2000).] However, hepatic SFT mRNA levels did not differ significantly between *Trf*$^{hpx/hpx}$ and *Trf*$^{+/?}$ mice at 4 wk of age (**Fig. 7**). Northern blot analysis of 10-wk-old *Trf*$^{+/?}$ and *Trf*$^{hpx/hpx}$ mice indicated a similar lack of hepatic SFT up-regulation in severely iron-loaded animals (data not shown).

DISCUSSION

The observation that livers of HH patients contain 5-fold higher SFT mRNA levels (Yu et al. 1998) suggests that enhanced SFT expression contributes to the etiology of the disease. Thus, the role of SFT in HH was evaluated by examining mRNA levels in the *Hfe*$^{-/-}$ mouse, an animal model of HH. The most important finding from this study is that 4- and 10-wk-old *Hfe*$^{-/-}$ mice do not have elevated hepatic SFT mRNA levels. This observation, which contrasts sharply with that of highly elevated levels of SFT transcripts in the livers of HH patients, indicates that hepatic iron loading can occur without up-regulation of hepatic SFT expression.

Different responses of liver SFT mRNA levels obviously may reflect species differences. For example, most HH patients accrue iron gradually, with clinical manifestations usually appearing around age 50 (Edwards 1999), whereas *Hfe*$^{-/-}$ mice rapidly develop hepatic iron overload relatively early (i.e., by 4–10 wk of age). Another consideration is that hepatic nonheme iron concentrations in HH patients are often >10-fold higher than normal (Edwards 1999, Jandl 1996), whereas the 10-wk-old *Hfe*$^{-/-}$ mice in the present study had only 3.3-fold higher hepatic iron levels than age-matched controls. However, the observation that SFT levels are not elevated in *Trf*$^{hpx/hpx}$ mice, which have up to 22-fold higher hepatic iron

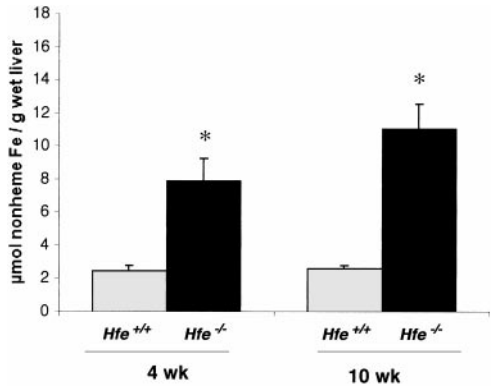

FIGURE 2 Hepatic nonheme iron concentrations in *Hfe*$^{+/+}$ and *Hfe*$^{-/-}$ mice. Liver was excised from mice at 4 wk (n = 3) and 10 wk (n = 6) of age. Iron concentration (μmol nonheme Fe/g wet liver) was determined by the method of Torrance and Bothwell (1968). The values shown are means ± SE. *Significantly different at same age ($P < 0.02$ at 4 wk; $P < 0.001$ at 10 wk).

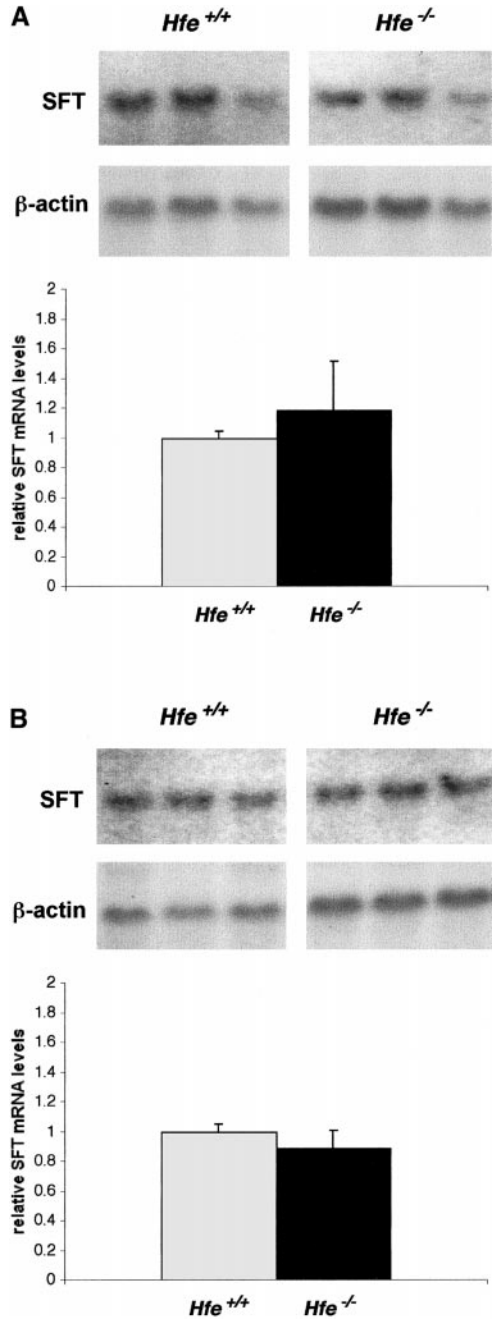

FIGURE 3 Northern blot analysis of SFT expression in liver of $Hfe^{+/+}$ and $Hfe^{-/-}$ mice. *A*, Northern analysis of SFT expression in livers of 4-wk-old mice ($n = 6$; three representative samples are shown). Total RNA (50 μg) from liver was electrophoresed and transferred to a Nytran N membrane. The Northern blot was hybridized with ^{32}P-labeled murine SFT probe and then stripped and reprobed with β-actin. After exposure to autoradiographic film, SFT transcript levels were quantified using densitometry and normalized to those obtained for β-actin. *B*, Northern analysis of SFT expression in livers from 10-wk-old mice ($n = 6$; three representative samples shown). Samples in *B* were exposed to a phosphor screen and quantified using PhosphorImaging (Quantity One software; Bio-Rad). The values shown are means ± SE.

concentrations relative to $Trf^{+/?}$ controls, suggests that the lack of up-regulated SFT mRNA in $Hfe^{-/-}$ mice is not due to a lower magnitude of iron loading in the knockout mice relative to HH patients. One caveat is that $Trf^{hpx/hpx}$ mice cannot export iron from liver. Thus, although the $Trf^{hpx/hpx}$ mice load iron in hepatic parenchymal cells similar to HH patients (Kaplan et al. 1988), aspects of their iron metabolism differ.

Two additional important distinctions between the $Hfe^{-/-}$ mice and HH patients merit further attention. First, the knockout mice do not develop hepatic fibrosis (J.E.L. and N.C.A., personal observations), a condition commonly seen in

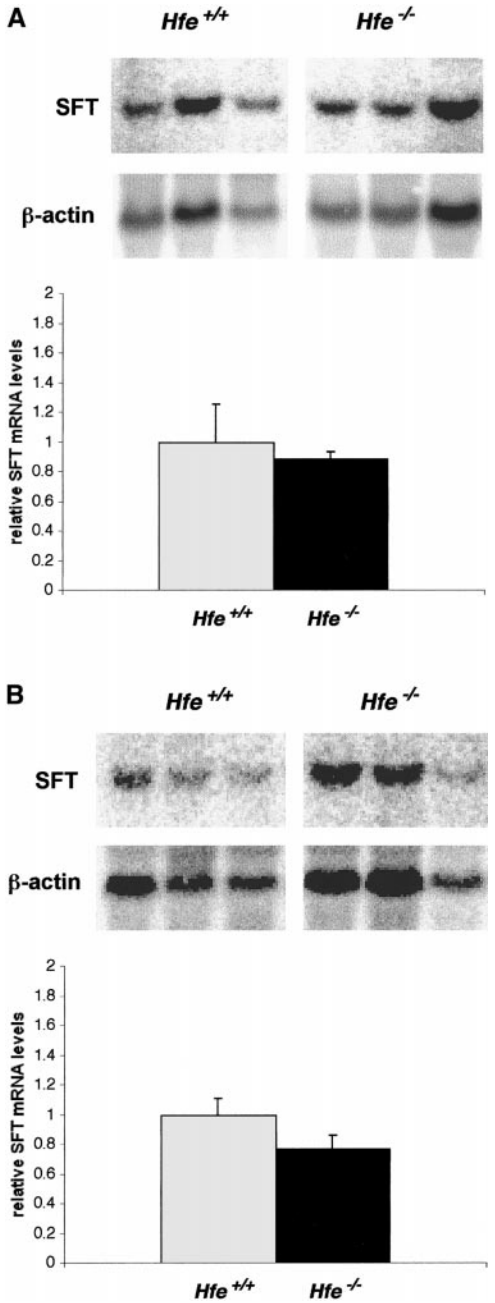

FIGURE 4 Northern blot analysis of SFT expression in duodenum of $Hfe^{+/+}$ and $Hfe^{-/-}$ mice. *A*, Northern analysis of SFT expression in duodenum of 4-wk-old mice ($n = 3$). Total RNA from duodenum (~1.5-cm length of small intestine distal to the pylorus) (50 μg) was electrophoresed and transferred to a Nytran N membrane. The Northern blot was hybridized with ^{32}P-labeled murine SFT probe and then stripped and reprobed with β-actin. After exposure to phosphor screen (Bio-Rad), SFT transcript levels were quantified using PhosphorImaging (Quantity One software, Bio-Rad) and normalized to those obtained for β-actin. *B*, Northern analysis of SFT expression in duodenum of 10-wk-old mice ($n = 6$; three representative samples are shown). The values shown are means ± SE.

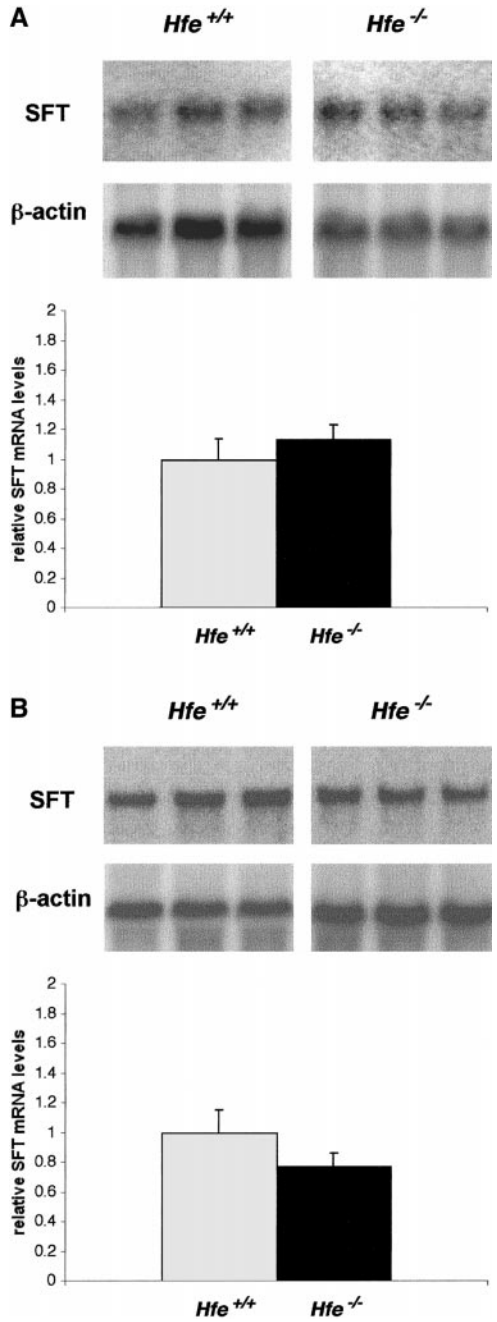

FIGURE 5 Northern blot analysis of SFT expression in brain of $Hfe^{+/+}$ and $Hfe^{-/-}$ mice. *A*, Northern analysis of SFT expression in brains of 4-wk-old mice brain ($n = 3$). Total RNA (20 μg) from whole brain homogenate was electrophoresed and transferred to a Nytran N membrane. The Northern blot was hybridized with ^{32}P-labeled murine SFT probe and then stripped and reprobed with β-actin. After exposure to phosphor screen (Bio-Rad), SFT transcript levels were quantitated using PhosphorImaging (Quantity One software; Bio-Rad) and normalized to those obtained for β-actin. *B*, Northern analysis of SFT expression in brains of 10-wk-old mice ($n = 3$). The values shown are means ± SE.

HH patients with >80 μmol Fe/g wet liver (Bassett et al. 1986). Although this difference might relate to lower hepatic iron loads in $Hfe^{-/-}$ mice (~12 μmol Fe/g wet liver), the lack of detectable fibrosis in 9-mo-old $Trf^{hpx/hpx}$ mice with massive iron loads (287 μmol Fe/g wet liver) suggests that mouse liver is intrinsically more resistant to liver damage (Trenor et al. 2000). Pathology reports for the HH liver samples in which

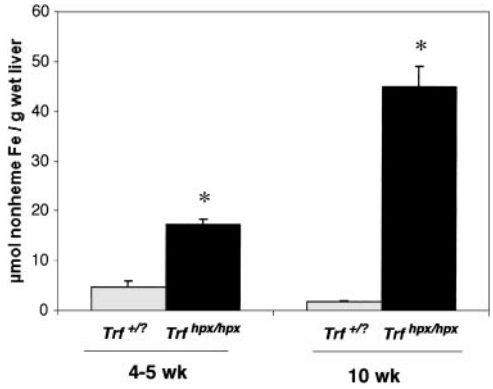

FIGURE 6 Hepatic nonheme iron concentrations in hypotransferrinemic ($Trf^{hpx/hpx}$) mice and ($Trf^{+/?}$) controls. Liver was excised from mice at 4–5 wk ($n = 4$) and 10 wk ($n = 3$) of age. Iron concentration (μmol nonheme Fe/g wet liver) was determined by the method of Torrance and Bothwell (1968). The values shown are means ± SE. *Significantly different at same age ($P < 0.001$ at 4–5 wk; $P < 0.0001$ at 10 wk).

SFT was elevated (Yu et al. 1998) reveal that four of six were fibrotic (pathology reports were not available for the other two, but all samples were taken from liver transplant recipients). Further studies could assess the effect of fibrosis and/or cirrhosis on hepatic SFT mRNA levels. The second notable distinction arises from the fact that HH patients are usually treated by phlebotomy. In response to acute blood loss, erythropoiesis is stimulated and iron absorption is increased. Thus, if the HH patients who had elevated hepatic SFT mRNA levels had been bled (detailed clinical histories are not avail-

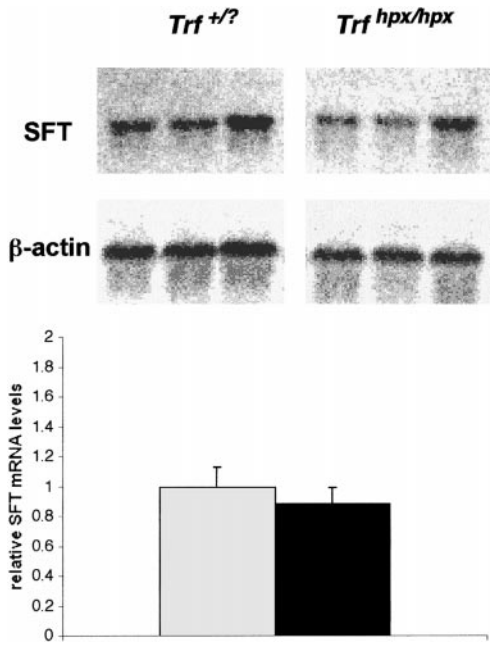

FIGURE 7 Northern blot analysis of SFT expression in liver of $Trf^{hpx/hpx}$ and $Trf^{+/?}$ mice. Northern analysis is presented of SFT expression in livers of 4- to 5-wk-old mice ($n = 4$; three representative samples shown). Total RNA (50 μg) from liver was electrophoresed and transferred to a Nytran N membrane. The Northern blot was hybridized with ^{32}P-labeled murine SFT probe and then stripped and reprobed with β-actin. After exposure to phosphor screen (Bio-Rad), SFT transcript levels were quantified using PhosphorImaging (Quantity One software; Bio-Rad) and normalized to those obtained for β-actin. Values are means ± SE.

able) (Yu et al. 1998), it is conceivable that phlebotomy promoted the effect. This hypothesis can be tested using the *Hfe*$^{-/-}$ mouse as a model to study the response to blood loss.

SFT mRNA is particularly abundant in mouse brain, suggesting a role in brain iron metabolism. The mechanisms that govern the uptake and distribution of iron in the brain are still poorly understood (Malecki et al. 1999). Nonheme iron is unevenly distributed in the brain; it is found predominantly in oligodendrocytes and is particularly concentrated in the globus pallidus, caudate nucleus, putamen and substantia nigra (Beard et al. 1993). Future studies on the regional distribution and cellular localization of SFT mRNA in the brain may help to determine whether SFT plays a role in brain iron homeostasis. Total brain iron concentrations do not differ between *Hfe*$^{-/-}$ and *Hfe*$^{+/+}$, consistent with studies of β-2 microglobulin knockout [B2m$^{-/-}$] mice (Moos et al. 2000), another animal model of HH. Moreover, the distributions of ferric iron, ferritin and transferrin in brain are indistinguishable between the B2m$^{-/-}$ and B2m$^{+/+}$ mice. Taken together, our results and those obtained by Moos et al. (2000) indicate that mouse brain appears to be protected from iron overload resulting from a lack of functional *Hfe*.

In conclusion, this study demonstrates that SFT is widely expressed in mouse tissues and that its expression levels in duodenum, brain and liver do not differ between *Hfe*$^{-/-}$ and *Hfe*$^{+/+}$ mice. The lack of elevated hepatic SFT mRNA levels in *Hfe*$^{-/-}$ mice suggests that SFT is not responsible for the hepatic iron loading observed in these animals. However, these data do not exclude the possibility that SFT may be a potential modifier gene of the hemochromatosis phenotype, as has been reported recently for β-2 microglobulin, DMT1, hephaestin and transferrin receptor (Levy et al. 2000).

ACKNOWLEDGMENTS

We thank Mark Fleming and Lynne Montross for kindly providing the liver samples and iron analyses, respectively, from the hypotransferrinemic mice.

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