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Impact of epidermal desquamation on tissue stores of iron

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Abstract

Background—Although several billion corneocytes are shed from human skin daily, metabolic studies from 50 years ago led to the conclusion that corneocyte desquamation had no measurable impact on systemic protein or iron status in humans.

Objective—To measure iron content of internal organs after introducing local genetic changes in epidermis that alter iron metabolism in skin.

Methods—Iron was measured in tissues and blood from groups of animals 7 weeks after weaning in three different mouse models expressing a transgene in epidermis: a hyperproliferation model in which the HPV16 E7 gene causes a 3-fold increase in epidermal turnover; an epidermal iron sink model in which overexpression of the transferrin receptor causes a 3–4 fold increase of iron in epidermis; a systemic hemochromatosis knockout model that has been crossed with the epidermal iron sink model.

Results—In the hemochromatosis model with the iron sink transgene in epidermis, there was a statistically significant reduction in non-heme iron in serum and in the liver and kidney. In all models there was a statistically significant reduction in non-heme iron in the kidney.

Conclusion—Local changes in iron metabolism in epidermis can have a measurable impact on systemic iron metabolism. By implication, disruptions in epidermal homeostasis might affect systemic levels of trace nutrients, and circulating toxins might be remediated by sequestering them in epidermis.

Keywords

epidermis; systemic disease; metabolism; iron; toxicology; hepcidin

INTRODUCTION

There is a long history of studies concerning nutrient loss through human skin. Early studies focused on electrolyte loss through sweat and adaptive responses that minimize losses [1]. Radioactive precursor labeling studies in the 1950s allowed accurate measurement of

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epidermal proliferation and turnover and highlighted the extent to which hyperproliferation and accelerated turnover can occur in diseased epidermis [2, 3]. Appreciation of altered dynamics in skin disease led to studies of protein and iron loss in psoriasis and ichthyosis [4, 5]. Such studies fostered the generally accepted view that, even though nutrients were lost through epidermal desquamation, altered epidermal dynamics in disease had no significant impact on systemic metabolism. More recently, and in contrast, the general failure-to-thrive experienced by many neonates with ichthyosis is now attributed to diseased epidermis, in which impaired epidermal barrier function and accelerated turnover lead to clinically relevant calorie wasting [6].

Iron is essential for cell growth and many metabolic processes, but it also can be toxic. Even though the body has limited capacity to rid itself of excess iron, tight regulation of iron stores usually prevents iron accumulation. This homeostasis is accomplished primarily by constant reutilization of the 2.5–3.0 gm of total body iron [7]. Daily absorption of approximately 1mg of dietary iron through the gut is balanced by an equal amount excreted through the stool (0.6mg/d), urine (0.15–0.2mg/d) and skin (0.2–0.25mg/d)[8]. Desquamation of keratinocytes accounts for the 20–25% of absorbed iron that is lost through skin [9–11]; very little is lost through sweat [12, 13]. Total body iron content is normally regulated at the level of absorption from the gut: absorption is increased when body stores are low, limited when body stores are high. Hepatic stores of iron appear to regulate the production of hepcidin, which in turn regulates iron uptake by the enterocyte [14, 15]. It is generally believed that loss through urine is relatively fixed and that increased loss through feces and skin can compensate for only small increases in body stores [8]. Increased loss of iron in hyperproliferative epidermal disease, such as psoriasis, have been thought to have little effect on iron homeostasis [4].

We have reexamined the ability of epidermis to affect iron metabolism in transgenic mouse models, which permit both rigid controls and tissue sampling that were impossible in older human studies. We hypothesized that if epidermal renewal and desquamation could be accelerated or if epidermis could be made to act as an iron sink by accumulating iron from the circulation, then increased amounts of iron would be eliminated from the body by desquamation and, as a consequence, stores of iron in internal organs would be reduced. To test this, we took advantage of the fact that iron gets into epidermis mostly by transferrin receptor-mediated uptake of Fe-transferrin into the proliferative basal layer. We tested the hypothesis in lines of transgenic mice: one had a hyperproliferative epidermis due to expression of a human papilloma virus (HPV) E7 transgene in basal keratinocytes [16] and the others accumulated excess iron in epidermis due to expression of a human transferrin receptor transgene in basal keratinocytes [17].

MATERIALS AND METHODS

Animals

The K14-hTfR mice previously derived by the Yale Animal Research Core transgenic facility in C57Bl/6JxSJL/J background [17] were bred to 129/S6/SvEvTac (Taconic, Germantown, NY) mice and then backcrossed to 129/S6/SvEvTac for more than 12 generations. Those transferrin receptor transgenics were bred to hemochromatosis mice [129/S6/SvEvTac *Hfe*^{-/-} [18]] and the K14-hTfR/*Hfe*^{+/-} offspring backcrossed to *Hfe*^{-/-} mice to give hemochromatosis mice with the transferrin receptor transgene, which we refer to as K14-hTfR x *Hfe*^{-/-}. Litters of experimental mice were obtained by crossing K14-hTfR x *Hfe*^{-/-} to 129/SV *Hfe*^{-/-}. Mice that express the HPV-16 E7 gene in basal keratinocytes [16] in the FVB background were a gift from Paul Lambert, University of Wisconsin and are designated K14-E7. All mouse production and experimentation was performed in compliance with guidelines of the Yale Institutional Animal Care and Use

Committee. Animals were fed mouse chow containing 226mg/kg iron (Harlan Teklad, TD2018, Madison, Wisconsin) during breeding. Experimental animals were fed a low iron diet (78mg/kg, Harlan Teklad, TD97184,) starting at the time of weaning.

Genotyping

Wild type *Hfe* was distinguished from the disrupted *Hfe*⁻ by 30 cycles of PCR using primers 5'-ATCAAATGGCATCTCTGGCA-3' and 5'-GTGGCGAGTCACTTTCACCA-3' that produce a 202 bp wild-type band and primers 5'-CTAGCTTCGGCCGTGACG-3' and 5'-AGTTGGGAGTGGTGTCCGA-3' that produce a 190 bp band of the disrupted gene [19]. The hTfR transgene was detected using primers for the hTfR coding sequence 5'-CTGCTATGGGACTATTGCTGTG-3' and 5'-CCGACAACCTTCTCTTCAGGTC-3' that do not amplify murine DNA [17]. The K14-E7 transgene was identified using primers 5'-CAGCTCAGAGGAGGAGGATG-3' and 5'-GCCCATTAACAGGTCTTCCA-3' [16].

Iron measurement

Non-heme iron in tissues was measured using the Torrance and Bothwell bathophenanthroline sulfonate (BPS, Sigma, St. Louis, MO) dye binding assay adapted to a microtiterplate format [20]. All tissue manipulations were done using iron-free titanium instruments. Tissues were blotted dry, weighed and frozen in tared microfuge tubes until analysis. Epidermis was separated from dermis using Dispase [20]. Serum iron, iron-binding capacity and transferrin saturation measurements were performed by the Clinical Pathology laboratory at Yale-New Haven Hospital.

Histology and immunohistochemistry

Tissues were fixed in Bouin's solution, embedded in paraffin, sectioned at 5 microns, deparaffinized and stained with H&E or an anti-mouse Ki67 antibody (#15580) and a peroxidase secondary antibody (#6721), both from Abcam, Cambridge, MA.

Quantitative RT PCR

Approximately 100mg of tissue was harvested, snap frozen on dry ice, homogenized in 1ml Trizol (Invitrogen, Carlsbad, CA). Total RNA was recovered and treated with DNase I (Roche, Indianapolis, IN) and then purified using Total RNA MinElute Cleanup Kit from Qiagen (Valencia, CA). 50ng RNA was reverse transcribed using iScript cDNA kit (Bio-Rad, Hercules, CA). Real time quantification of hepcidin and β -actin mRNA transcript levels was performed using Power SYBRgreen (ABI Applied Biosystems, Foster City, CA) in an ABI 7500 thermocycler. For each sample triplicate measurements of Δ Ct for hepcidin relative to β -actin mRNA were averaged and then expressed as a ratio relative to a non-transgenic control hepcidin/actin measurement in each plate, for which the Δ Ct was assigned the value 1. Hepcidin primers are: 5' -CTGAGCAGCACCATCTCTC -3' and 5' -TGGCTCTAGGCTATGTTTTGC -3'; β -actin cDNA primers are: 5' -ACCCACACTGTGCCCATCTA -3' and 5' -CACGCTCGGTCAGGATCTTC -3', as described [21].

Statistics

Groups of 12–16 animals were used in each experiment based on a power calculation using the variance of iron measurements in livers of *Hfe*^{-/-} animals and the desire to detect a 20% difference in iron between experimental groups. Differences between transgenic and control mice for both males and females were evaluated using analysis of covariance models for tissue iron, blood measurements and body weight. These models included fixed effects for transgene, gender, experiment and the interaction of transgene and gender. Interactions of transgene with experiment were not significant indicating that transgene effects were not

heterogeneous across experiment and were therefore excluded from the models. Comparisons were performed within males and females because of the known influence of sex on tissue iron stores [22]. Data are presented as least squares means $\pm$ S.E.M. and *p* values were estimated by linear contrasts.

RESULTS

Effect of the epidermal hyperproliferation model on tissue iron

Transgenic mice that express the HPV E7 gene in basal keratinocytes (K14-E7 mice) have a non-inflammatory, hyperproliferative epidermis [16]. Consistent with those previous findings, we found that the acanthosis and hyperkeratosis were different for different regions of epidermis and was most striking in the ear, which had a 5-fold increase in Ki67 positive keratinocytes compared to non-transgenic littermates (Figure 1). At ten weeks of age non-heme iron was dramatically reduced in the kidneys of K14-E7 transgenic animals compared to non-transgenic littermates, 30% in males and 29% in females (Table 1). In livers of females there was a greater absolute reduction in nonheme iron (46 μ g/gm) than in kidneys, but this did not achieve statistical significance. In other tissues of the E7 transgenics, non-heme iron was equal to, or marginally lower than, that in the non-transgenic littermate controls. Serum iron and transferrin saturation were unaffected by the transgene. In both transgenic and non-transgenic littermates, iron in liver and kidney of males was lower than that in tissues of females, as commonly observed in other strains of mice [23].

Effect of the epidermal iron sink model on tissue iron

The cDNA lacking 5' and 3' non-coding sequences of the human transferrin receptor (hTfR) is insensitive to regulation by local iron [24]. We previously showed that C57Bl/6JxSJL/J mice in which hTfR is overexpressed in basal keratinocytes (K14-hTfR mice) have 3–4 times more iron in epidermis than their non-transgenic littermates and that animals with this transgene are phenotypically normal and their epidermis is microscopically indistinguishable from controls [17]. When the K14-hTfR transgene was backcrossed into a 129/SV background (to prepare for the next series of breeding experiments), iron in epidermis remained 3–4 times greater in the transgenics compared to non-transgenic littermates (Figure 2), and the animals remained phenotypically normal and their epidermis was microscopically indistinguishable from controls. In the K14-hTfR transgenic animals at ten weeks of age, the greatest proportionate change was in the kidneys where non-heme iron was significantly reduced compared to non-transgenic littermates, 25% in males and 30% in females (Table 2). In females carrying the K14-hTfR transgene, non-heme iron was significantly lower in liver, and serum iron and transferrin saturation were also reduced.

Effect of the epidermal iron sink model on tissue iron in a mouse model of hemochromatosis

The previous experiments indicated that altering iron elimination through epidermis has an impact on iron in internal organs in animals that should have otherwise intact homeostatic mechanisms controlling iron metabolism. We wondered whether the loss of excess iron through epidermis might protect internal organs from iron overload in animals with genetic hemochromatosis and disrupted iron homeostasis. Animals lacking a functional *Hfe* gene have the same amount of iron in epidermis as *Hfe*^{+/+} littermates, and when the K14-hTfR transgene was bred into those hemochromatosis (*Hfe*^{-/-}) animals there continued to be 3–4 times more iron in epidermis than in non-transgenic littermates (Figure 2). The effect of the K14-hTfR transgene was measured in groups of animals with genetic hemochromatosis (*Hfe*^{-/-}). At ten weeks of age, animals with or without the K14-hTfR transgene had no gross or microscopic cutaneous phenotype. In four separate experiments in which males and females were analyzed separately, iron in kidney was always lower in the animals with the

K14-hTfR transgene compared to littermates lacking the K14-hTfR transgene. Iron in the liver was lower in 9 of 10 groups of transgenic animals compared to controls and serum iron was lower in 7 of 8 groups of transgenics. Pooled data from a total of five experiments are shown in Table 3. For the entire data set, tissue iron reductions were statistically significant in livers and kidneys of males and females. The average reductions were: kidney - 17% for males and 20% for females; liver - 10% for males and 9% for females. While the serum iron was reduced 9% for males and 5% for females, the iron sink transgene had no significant effect on transferrin saturation and no effect on iron content of the heart or pancreas or on the hematocrit in these animals with hemochromatosis.

Effect of epidermal transgenes on hepcidin in liver

Overproduction of hepcidin in our models would reduce iron absorption and might account for our observations. We used quantitative RTPCR to measure hepcidin (HAMP) mRNA levels in the skin and liver of K14-E7 animals, in K14-hTfR animals and in K14-hTfR×Hfe^{-/-} animals. No HAMP transcripts were detected in the skin of control or transgenic animals (data not shown). In none of the models did the epidermal transgene increase hepatic *Hamp* mRNA compared to non-transgenic littermates (Table 4).

DISCUSSION

We have shown that perturbations in the quality and quantity of squames shed from epidermis have measurable effects on systemic iron metabolism and storage. In our three transgenic models, the greatest absolute effects were on iron in the liver and the greatest proportional effects on iron in the kidney. This unprecedented systemic effect of epidermal desquamation warrants a reconsideration of epidermal turnover itself. The epidermis normally functions as a barrier, most importantly to prevent water loss. Mammals maintain and repair the barrier function by a steady-state renewal process that matches desquamation, the loss of effete cells and barrier remnants from the surface, with equal regeneration of cells from the basal layer [25]. The epidermis is morphologically and functionally stratified. The proliferating cells that drive epidermal renewal are limited to the innermost (basal) layer; after progressively differentiating and traversing the spinous layer, cells are lost into the environment (exfoliated or desquamated) from the outermost layer, the stratum corneum. It normally takes 28 days for a basal cell to traverse the epidermis and to desquamate in humans, 6–8 days in mice. There is a widely accepted, though poorly documented, view that individual epidermal keratinocytes are parsimonious but that epidermis as a whole is not: except for a few actively secreted hormones and cytokines, anything that gets into a basal keratinocyte stays there and is ultimately lost through desquamation.

Advances in genetic manipulation of epidermis in animal models have allowed us to reconsider the role of epidermis in metabolism and nutrient homeostasis under carefully controlled circumstances. We chose to study iron for a number of reasons. First, iron loss through epidermal desquamation is established to be quantitatively important in iron metabolism, accounting for 20–25% of daily iron loss. Second, local factors have been shown to change the amount of iron in epidermis: epidermal iron is increased in skin disease such as psoriasis [26, 27] and atopic eczema [27]; there is 3 times as much iron in sun-exposed compared to sun-shielded skin in humans [28]. Third, advances in understanding regulation of epidermal proliferation and epidermal transport of iron provided the rationale and molecular tools with which to proceed. Based on that published quantitative data, we hypothesized that a 3–4-fold increase in epidermal turnover or a similar increase in epidermal iron content should have a detectable effect on iron homeostasis and relevance to human physiology and disease. We took advantage of transgenic mice that provided either a kinetic or a metabolic mechanism for increasing iron loss through epidermal desquamation.

Both the hyperproliferation and iron sink models had a clear impact on internal tissue stores of iron. Hyperproliferation and increased epidermal turnover account for reduced tissue iron in the K14-E7 model. The HPV-16 E7 gene is overexpressed in basal keratinocytes in this model, preventing the retinoblastoma protein from inhibiting cell cycle progression and resulting in increased proliferating cells in the suprabasal as well as the basal cell layers [16]. K14-E7 lines of varying phenotypic severity were originally described by Heber et al [16]; the line we used has a mild phenotype with epidermal thickening most pronounced in muzzle and ear skin (Figure 1), but is otherwise phenotypically normal. In this mildly affected line, animals grow normally and rarely develop skin tumors [16]. At ten weeks of age these transgenic animals weigh the same as non-transgenic littermates, implying that impaired dietary intake of iron does not account for our observations. Unlike the hyperproliferative epidermis seen in skin diseases such as psoriasis and ichthyosis, the skin in these animals is not red and shows no histological evidence of inflammation. Epidermal proliferation in these animals was increased 5-fold in the ear but only 2-fold in the back and tail epidermis compared to non-transgenics. This variability across the epidermis may account for the less robust effects in this model compared to the iron sink model where the transgene has more uniform action over the entire integument (see below).

In the iron sink model there was a large proportionate decrease in iron in the kidneys and, to a lesser extent, in liver and serum. As previously shown for the iron sink founder line [17] and confirmed in these K14-hTfR transgenic animals in the 129SV background, iron was increased 3–4-fold in ear, back and tail epidermis and 2-fold in hair. This was accompanied by a great increase in ferritin, the cellular storage protein for iron, which is barely detectable in normal murine epidermis by Western blot [17]. We believe that this somewhat artificial iron sink mouse model has relevance to human physiology since a comparable 2–3-fold increase in epidermal iron has been reported in psoriasis [26] and in human skin following chronic ultraviolet radiation [28], though the mechanism(s) for the iron increase in those circumstances has not been elucidated.

The K14-hTfR transgene increased iron in epidermis of animals with genetic hemochromatosis to the same extent as it did in wild-type animals. The data show that absence of a functional *Hfe* protein did not impair the ability of the transferrin receptor transgene to transport iron into epidermis, despite the physical association of those membrane proteins in other tissues [29] and the role of that association in regulating iron metabolism [21]. It may be relevant that *Hfe* mRNA is undetectable in mouse epidermis by PCR (Adams and Milstone, unpublished). We previously showed, and confirm here, that genetic hemochromatosis by itself has no effect on iron in epidermis [20].

The *Hfe* deficient mouse is generally considered to be a good model of the human disease, yet it is clear that different strains of mice have different amounts of tissue iron and different quantitative responses to *Hfe* deficiency [30]. In the 129SV line, *Hfe* deficiency greatly increases hepatic iron (which is why we chose that line for these studies), but has little effect on pancreas or heart, which are affected in human hemochromatosis. At ten weeks of age, animals with genetic hemochromatosis had 5 times as much iron in liver as animals with the wild-type *Hfe* gene but the *Hfe*^{-/-} genotype had little effect on iron in pancreas, kidney or heart. The iron sink model reduced tissue iron even more convincingly in animals with hemochromatosis than it did in animals without hemochromatosis. The consistently lower serum iron in *Hfe*^{-/-} animals carrying the hTfR transgene suggests that removal of iron from blood into epidermis by the transgene accounts for reduced iron in internal tissues. The indirect effect of the K14-hTfR transgene on iron loading into liver was larger and more consistent in animals with hemochromatosis than in mice with the wild-type *Hfe* gene. This suggests that using epidermis as a sink to alter systemic homeostasis works best in the setting of already disordered homeostasis.

Do the data presented support the idea that iron loss through epidermis might account for the hypochromic anemia seen in patients with extensive or erythrodermic psoriasis and a four to six-fold accelerated rate of epidermal turnover? This possibility was considered many years ago after radioactive iron studies had established that iron loss through skin occurred mostly through epidermal desquamation, not through sweat [11]. Radioactive iron balance studies in individuals with extensive psoriasis and anemia confirmed that patients with psoriasis lost more iron through the skin than did normal individuals. The authors noted that the anemia had many similarities to that seen in infection and concluded that increased iron loss through epidermal desquamation in psoriasis generally did not account for the anemia in those patients [4]. Our data support that conclusion since the increased iron lost through desquamation in our models had a measurable effect on iron in kidneys and liver but never caused anemia. Today, it could be argued that the anemia accompanying extensive psoriasis might be caused by chronic inflammation and increased IL-6 production in skin [31], which in turn would mediate a hepcidin-induced “anemia of chronic disease” [32]. To the best of our knowledge, hepcidin has not been measured in inflammatory skin diseases. Even though we could detect no inflammation in the skin of our animals, concern that our results might be explained by an unexpected role for hepcidin led us to measure hepcidin mRNA in skin and liver and to show that it was unaffected in each of our models.

Our experimental results may have implications for patients with widespread skin disease or as a novel approach to remediating systemic toxins. The body store of iron is large relative to the daily intake and elimination of iron, therefore epidermal hyperproliferation or creation of an epidermal iron sink are not likely to have a clinically significant impact on established hemochromatosis. But what about trace elements or toxins for which large stores and tight homeostatic mechanisms do not exist? Under circumstances of marginal nutrition or malabsorption it would be difficult to argue that epidermal desquamation could not contribute to nutritional deficiency. If a systemic toxin is known to be partially eliminated through skin, it is reasonable to suggest that accelerated epidermal turnover might speed the elimination of that toxin. Indeed it has been suggested that epithelial exfoliation might be the first line of defense against environmental toxins [33], and partial elimination through epidermis of at least one toxin, TCDD, has been demonstrated [34]. Should patients with hyperproliferative epidermis, such as those with ichthyosis or extensive psoriasis, be monitored for trace element deficiency? There is a growing literature suggesting that chronic hyperproliferative skin diseases, such as psoriasis, are associated with increased risk for cardiac or other internal disease [35]. Should micronutrient loss through desquamation be added to genetics and inflammation as a possible mechanism to explain such co-morbidities? Taken together, our results demonstrate that epidermal desquamation has a measurable impact on systemic metabolism and justify reconsideration of circumstances in which desquamation might have clinical significance beyond the skin.

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Abbreviations

hTfR	human transferrin receptor
HPV	human papilloma virus

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Figure 1.

Tissue sections from 10 week old FVB mice, all at same magnification, are stained with H&E (A-D) or antibody to Ki67 (E,F). A and B from tail. C-F from ear.

Figure 2. Iron in epidermis of mice with the K14-hTfR transgene

Tail epidermis was harvested at 10 weeks. **a.** Iron in epidermis from 6–10 mice with the K14-hTfR transgene in a normal 129SV background (open bars) and from mice with the K14-hTfR transgene in a 129SV hemochromatosis (*Hfe*^{-/-}) background (shaded bars) was compared to iron in littermates lacking the K14-hTfR transgene. The K14-hTfR transgene increases epidermal iron 3–4-fold, but the hemochromatosis genotype has no effect on epidermal iron.

Table 1

Effect of the epidermal hyperproliferation model on tissue iron

K14-E7 mice were compared to non-transgenic controls 7 weeks after weaning. Data shown are means and S.E.M. from a single experiment with a minimum of 12 animals in each group.

K14-E7	males		females	
	control	transgenic	control	transgenic
Tissue iron (µg/g wet wt)				
liver	376 +/- 18.5	380.4 +/- 20	468.6 +/- 31.6	422.6 +/- 20.5
kidney	73.8 +/- 2.1	51.9 +/- 1.6	108.5 +/- 5	76.7 +/- 3.1
pancreas	27 +/- 1.3	23.2 +/- 0.8	23.6 +/- 0.9	22.4 +/- 0.9
heart	48.2 +/- 0.5	47.4	49.9 +/- 0.7	47 +/- 0.8
ear epidermis	15.4 +/- 1.7	12.4 +/- 0.9	14.3 +/- 2.1	11 +/- 0.8
Blood measurements				
serum iron (µg/ml)	318.2 +/- 8.3	314.8 +/- 10.3	353.5 +/- 11.1	331.3 +/- 7.7
TIBC (µg/ml)	399.6 +/- 8.7	397.3 +/- 8.6	382.8 +/- 8.5	373 +/- 7.6
% saturation	79.7 +/- 2.2	79.1 +/- 5.5	92.4 +/- 2.6	89 +/- 6.2
Body Weight	25.6 +/- 0.5	24.9 +/- 0.5	20.9 +/- 0.3	21.4 +/- 0.2
				0.28

Table 2

Effect of the epidermal iron sink model on tissue iron

K14-hTfR mice were compared to non-transgenic controls 7 weeks after weaning. Data were pooled from two separate experiments in which there were approximately 12 animals in each group. Numbers shown are least square means for each group $\pm$ S.E.M. The p value is based on the estimate of the difference between least square means of transgenic and control groups.

K14-hTfR	males		p	females		p
	control	transgenic		control	transgenic	
Tissue iron (μg/g wet wt)						
liver	186.7 +/- 10.1	188.5 +/- 10.1	.90	225.9 +/- 9.3	199 +/- 9.3	.04
kidney	40.6 +/- 1.4	30.4 +/- 1.4	<.0001	55.4 +/- 1.3	38.8 +/- 1.3	<.0001
pancreas	25 +/- 2.2	28.9 +/- 2.2	.21	27.4 +/- 2	24.1 +/- 2	.25
heart	42.5 +/- 0.4	43.2 +/- 0.4	.28	44.5 +/- 0.4	45.7 +/- 0.4	.04
Blood measurements						
serum iron (μg/ml)	315.8 +/- 11.4	321 +/- 11.4	.75	330 +/- 10.8	300.6 +/- 10.8	.06
TIBC (μg/ml)	370.7 +/- 10.8	358.1 +/- 10.8	.41	396.4 +/- 10.3	397.8 +/- 10.3	.93
% saturation	85.1 +/- 2.1	89.4 +/- 2.1	.16	83.7 +/- 2	76.2 +/- 2	.01
Weight	21.3 +/- 0.3	19.7 +/- 0.3	.0008	17.1 +/- 0.3	16.7 +/- 0.3	.38

Table 3
Effect of the epidermal iron sink model on tissue iron in a mouse model of hemochromatosis

Hfe^{-/-} mice with the K14-hTfR transgene were compared to *Hfe*^{-/-} mice without the K14-hTfR transgene 7 weeks after weaning. Data taken from five separate experiments in which there were approximately 12 animals in each group. Numbers shown are least square means for each group +/- S.E.M. The p value is based on the estimate of the difference between least square means of transgenic and control groups.

K14-hTfR <i>xHfe</i> ^{-/-}	males		females		p
	control	transgenic	control	transgenic	
Tissue iron (µg/g wet wt)					
Liver (5 ^a)	853.8 +/- 28.5	771.9 +/- 28.8	1193.3 +/- 27.6	1084 +/- 28.2	.006
Kidney (4)	38.2 +/- 1.1	31.8 +/- 1	50.1 +/- 1	40.7 +/- 1.1	<.0001
Pancreas (3)	27.2 +/- 1	27.7 +/- 1	30.4 +/- 1	28.9 +/- 1	.31
Heart (2)	45.9 +/- 0.7	47.8 +/- 0.7	49.1 +/- 0.8	48.8 +/- 0.8	.84
Blood measurements					
Hematocrit (%) (2)	58.5 +/- 0.8	59.1 +/- 0.7	58.8 +/- 0.7	58.4 +/- 0.6	.7
serum iron (µg/ml)(4)	363.8 +/- 4.5	331.1 +/- 4.4	370.7 +/- 4.7	353.6 +/- 4.5	.01
TIBC (µg/ml) (4)	384.7 +/- 5.2	358.5 +/- 5.1	391.5 +/- 5.4	377.9 +/- 5.4	.08
% saturation (4)	94.6 +/- 0.7	92.7 +/- 0.7	94.6 +/- 0.7	93.6 +/- 0.7	.35
Weight (5)	25.7 +/- 0.3	24.2 +/- 0.3	20.2 +/- 0.3	19.7 +/- 0.3	.2

^aNumber of experiments used for each data set

Effect of epidermal transgenes on hepcidin in liver

Livers were harvested 7 weeks after weaning. The hepcidin (*Hamp*) mRNA data are $\Delta\Delta C_T$ values relative to β -actin and as a ratio to a non-transgenic sample.

Transgenic line	<i>Hamp</i> mRNA in Liver				
	males		females		
	control	transgenic	p	control	transgenic
K14-E7	1.0 +/- 0.1 (9) ^a	1.0 +/- 0.1 (8)	0.82	1.4 +/- 0.2 (14)	1.8 +/- 0.2 (13)
K14-hTTR	1.1 +/- 0.1 (13)	1.0 +/- 0.1 (14)	0.31	1.2 +/- 0.1 (17)	1.1 +/- 0.2 (17)
K14-hTTRxHfe ^{-/-}	1.1 +/- 0.1 (28)	1.5 +/- 0.2 (24)	0.11	1.4 +/- 0.2 (28)	1.3 +/- 0.2 (23)

^a number of animals measured