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Is the Iron Donor Lipocalin 2 Implicated in the Pathophysiology of Hereditary Hemochromatosis?

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Abstract

Under normal conditions, iron is taken up by the cells through the transferrin-mediated pathway. However, in hereditary hemochromatosis, a common iron-overloading disorder associated with mutations in the *HFE* gene, iron in plasma exceeds transferrin-binding capacity, and non–transferrin-bound iron (NTBI) appears in the circulation of patients with iron overload. NTBI can be taken up by hepatocytes through a transferrin-independent pathway. Lipocalin 2 (Lcn2), a secreted protein of the lipocalin family, has emerged as the mediator of an alternative, transferrin-independent pathway for cellular iron delivery. To evaluate the importance of Lcn2 in the pathogenesis of hepatic iron loading in *Hfe* knockout mice, we generated *HfeLcn2* double-deficient mice. Our studies revealed that deletion of Lcn2 in *Hfe*-knockout mice does not influence hepatic iron accumulation in $Hfe^{-/-}$ mice, or their response to iron loading, as the phenotype of $HfeLcn2^{-/-}$ mice remained indistinguishable from that of $Hfe^{-/-}$ mice.

Conclusion—Lcn2 is not essential for iron delivery to hepatocytes in hemochromatosis.

Under normal conditions, the major pathway for cellular iron uptake is through internalization of the complex of iron-bound transferrin and the transferrin receptor. Thereafter, iron is released from transferrin as the result of acidic pH in endosomes and is transported to the cytosol by divalent metal ion transporter 1 (also known as SLC11A2).[1,2] However, there is convincing evidence that, in situations of disrupted iron homeostasis, iron can also be delivered to cells by alternative, transferrin-independent mechanisms. For example, mice and humans lacking transferrin, while anemic, develop iron overload in the liver, suggesting that iron is being delivered to hepatocytes by transferrin-independent pathways.[3,4] Likewise, in *HFE*-related hereditary hemochromatosis (HH), a common disorder of iron homeostasis characterized by excessive iron deposition principally in the liver,[5,6] iron overload exceeds the iron-binding capacity of serum transferrin,[7,8] and this non–transferrin-bound iron (NTBI) is cleared from the circulation and deposited into

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hepatocytes.[9] This alternate iron uptake pathway is further brought into evidence by studies in *Dmt1*$^{-/-}$ knockout mice.[10] In fact, despite the presence of a disrupted transferrin cycle, *Dmt1*$^{-/-}$ mice have abnormally high iron liver stores. Moreover, iron loading with iron dextran leads to significant accumulation of iron in *Dmt1*$^{-/-}$ hepatocytes, further indicating that the liver has alternate mechanisms than the transferrin cycle for iron uptake.[10] The identification of the components of NTBI, however, remains elusive.

Lipocalin 2 (Lcn2), also called neutrophil gelatinase-associated lipocalin, or 24p3,[11] has been proposed to be a mediator of the transferrin-independent iron delivery pathway[12] after it was found that Lcn2 can bind bacterial ferric siderophores,[13] and that Lcn2–siderophore–iron complexes can transport iron into cells during kidney development.[14]

Lcn2 has been found to exert a broad range of biological activities, which seems to depend on whether Lcn2 is bound to iron-laden siderophores or not, implicating iron-loaded Lcn2-siderophore as an iron donor and, conversely, iron-free Lcn2-siderophore as an iron chelator.[15,16] As such, iron-loaded Lcn2-siderophore (Holo-Lcn2) has been shown to be required for mesenchymal–epithelial transition of embryonic kidney[14] and oncogene Ras-transformed epithelial cells,[17] as well as for kidney protection from renal failure,[18,19] and delivery of ferric ion to mouse spermatozoa.[20] In contrast, the Lcn2–siderophore complex without iron[21] and Lcn2 (Apo-Lcn2)[22] chelates iron from cells and, through iron deprivation, can induce apoptosis of pro-B cells[22] and inhibit bacterial growth[13,23,24] and erythropoiesis.[25,26] Additional evidence that Lcn2 may assist cellular iron trafficking is provided by the demonstration that iron delivered through Lcn2 has been shown to regulate iron-sensitive genes[21] and the identification of one of its receptors as being megalin.[27] Megalin is also known to bind another iron-binding protein, namely lactoferrin.[28,29] Thus, Lcn2 has emerged as a possible candidate involved in NTBI uptake under iron overload conditions and, as such, is possibly implicated in the pathophysiology of HH.[12, 30]

In this study, we investigated whether Lcn2, as a component of an alternative iron delivery system, may contribute to the pathophysiology of HH. For this purpose, we generated and then characterized iron metabolism in *HfeLcn2* double mutant mice.

Materials and Methods

Animals

All procedures were performed in accordance with Canadian Council on Animal Care guidelines and approved by our institution's Animal Care Committee. Control, wild-type mice were C57BL/6 female mice purchased from Charles River Laboratories, Inc. (Wilmington, MA). *Hfe*$^{-/-}$ mice were kindly provided by Dr. Nancy C. Andrews, Howard Hughes Medical Institute and Harvard Medical School, Children's Hospital (Boston, MA)[31] in the 129/SvEvTac background and were back-crossed onto the C57BL/6 (B6) background for 10 generations.[32] *Lcn2*$^{-/-}$ mice generated in the C57BL/6 (B6) background have been described.[23] Compound mutants (*HfeLcn2*$^{-/-}$) were obtained by interbreeding *Hfe*$^{-/-}$ and *Lcn2*$^{-/-}$ mice and were genotyped via polymerase chain reaction (PCR) (Supplementary Fig. 1A,B). The following primers were used: Hfe, 5′-AGTTGG-GAGTGGTGTCCGA-3′, 5′-TGGCTACAGTGTGAG-AGGC-3′, and 5′CTAGCTTCGGCCGTGACG-3′; Lcn2, 5′-

CCTCAAGGACGACAACATCA-3′, 5′-ACCCATT-CAGTTGTCAATGC-3′, 5′-TTGGGT-GGAGAGGC-TATTC-3′, and 5′-AGGTGAGATGACAGGAGATC-3′. All mice used in the experiments were genotyped via PCR assay performed on DNA prepared from mouse tails. Absence of Lcn2 expression in *Lcn2*$^{-/-}$ and *HfeLcn2*$^{-/-}$ mice was confirmed via western blotting using a monoclonal antimouse Lcn2/neutrophil gelatinase-associated lipocalin antibody (R&D Systems, Inc., Minneapolis, MN) (Supplementary Fig. 1C).

Diets

Mice were given a commercial diet (Harlan Teklad, Madison, WI) or, when indicated, an iron-supplemented diet containing 2.5% (wt/wt) carbonyl iron (Sigma Immunochemicals, St. Louis, MO).

Measurement of Serum Iron, Transferrin Saturation, and Tissue Iron Concentration

Serum iron, total iron-binding capacity, and transferrin saturation were assessed via colorimetric assay.[33] Iron levels in the liver, spleen, heart, and kidneys were measured via acid digestion of tissue samples followed by iron quantification via atomic absorption spectroscopy.[33]

Quantitative Reverse-Transcription PCR

Total RNA was isolated with Trizol reagent (Invitrogen, Burlington, Ontario, Canada), and reverse-transcription was performed with the Thermoscript RT-PCR system (Invitrogen). Hepcidin 1 (*Hamp1*) and *β-actin* messenger RNA levels were measured via real-time PCR using a Rotor Gene 3000™ Real Time DNA Detection System (Montreal Biotech, Inc., Kirkland, Quebec, Canada) with the QuantiTect SYBRGreen I PCR kit (Qiagen, Mississauga, Ontario, Canada) as described,[33] and expression levels of hepcidin were normalized to the housekeeping gene *β-actin*. The primers employed were as follows: *β-actin*, 5′-TGTTACCAACTGGGACGACA-3′ and 5′-GGTGTTGAAGGTCTCAAA-3′; *Hamp 1*, 5′AGAGC-TGCAGCCTTTGCAC3′ and 5′GAAGATGCAGATG-GGGAAGT3′.

Statistical Analysis

All statistics were calculated using SigmaStat 3.1 (Systat Software, Richmond, CA). All values are expressed as the mean ± standard deviation. Multiple comparisons were evaluated statistically via one-way analysis of variance, followed by the Bonferroni multiple comparison test.

Results

Hfe$^{-/-}$ mice develop iron overload characterized by high circulating iron levels and deposition of excess iron in the liver, but with resistance to iron loading in the spleen due to deficient iron storage in macrophages.[31] To determine whether Lcn2 participates in iron delivery to the liver in Hfe-deficient mice, we interbred *Hfe*$^{-/-}$ and *Lcn2*$^{-/-}$ mice. Circulating iron levels, assessed by measuring serum iron and transferrin saturation as well as iron deposition in the liver and spleen, were analyzed at 10 and 20 weeks of age (Fig. 1). Iron parameters in Lcn2 single knockout mice were similar to those in wild-type mice (B6), whereas *Hfe*$^{-/-}$ and *HfeLcn2* double mutants had higher amounts of circulating and liver

iron than B6 mice (≈1.5- to 2-fold higher [$P < 0.05$]) (Fig. 1A–C). In contrast, spleen iron content, though slightly increased with age, was lower in 10-week-old *Hfe*$^{-/-}$ and *HfeLcn2*$^{-/-}$ mice compared with B6 (31% lower [$P < 0.05$]) or *Lcn2*$^{-/-}$ mice (Fig. 1D). No significant differences were found in heart iron (Fig. 1E), whereas iron levels in kidneys were slightly increased in both *Hfe*$^{-/-}$ and *HfeLcn2*$^{-/-}$ mice compared with B6 mice (Fig. 1F). Thus, we found no significant differences between *Hfe*$^{-/-}$ and *HfeLcn2*$^{-/-}$ mice regarding iron parameters. These results indicate that iron accumulation in the livers of *Hfe*$^{-/-}$ mice did not improve in the absence of Lcn2, suggesting that Lcn2-mediated cellular iron delivery is not essential in iron uptake by the liver in *Hfe*$^{-/-}$ mice.

To further investigate the responses to dietary iron loading in compound mutants, we challenged the mice with a 2.5% wt/wt carbonyl iron-supplemented diet for 2 weeks (Fig. 2). B6 and *Lcn2*$^{-/-}$ mice fed the iron-enriched diet showed significant increments of serum iron, transferrin saturation (>50% increase [$P < 0.01$]), hepatic iron content (>4-fold rise [$P < 0.001$]) and spleen iron content (>2.5-fold elevation [$P < 0.001$]) compared with mice on the control diet (Fig. 2A–C). Whereas *Hfe*$^{-/-}$ and *HfeLcn2*$^{-/-}$ double knockout mice also manifested heightened liver iron content over the already-elevated levels observed on the control diet in response to dietary iron loading (>2-fold rise; $P < 0.001$), the increase in iron loading of the spleen was considerably more modest than what was seen in B6 ($P < 0.01$) or *Lcn2*$^{-/-}$ mice on the iron-supplemented diet (Fig. 2D).

Because augmented iron absorption in *Hfe*$^{-/-}$ mice has been related to inappropriate expression levels of hepcidin,[34] the principal regulator of systemic iron homeostasis, we also measured hepatic hepcidin 1 messenger RNA. As illustrated in Fig. 2E, we found lower amounts of hepcidin in both *Hfe*$^{-/-}$ and *HfeLcn2* double mutants than in B6 and *Lcn2*$^{-/-}$ mice on the control diet (≈30% decrease). Hepcidin levels rose approximately 2.5- to 3.5-fold in response to iron loading in all mouse strains ($P < 0.01$) but remained significantly lower in *Hfe*$^{-/-}$ and *HfeLcn2*$^{-/-}$ mice compared with B6 ($P < 0.05$) or *Lcn2*$^{-/-}$ mice. Because the response to iron loading was indistinguishable between *Hfe*$^{-/-}$ and *HfeLcn2*$^{-/-}$ mice, these results further confirm that Lcn2 is not essential for iron delivery to the liver and the regulation of hepcidin in HH.

Discussion

Although under normal conditions hepatocytes acquire iron mostly through the transferrin-receptor pathway, the existence of uptake of NTBI in HH is now well established. Several candidates have been proposed, including Lcn2, but clear demonstration of its participation and importance in HH has not been yet provided.

We set out to determine whether Lcn2 represents a physiologically relevant mechanism of iron uptake by the liver in HH, an iron-overloading disease. For this purpose, we generated and characterized iron metabolism in *HfeLcn2* double knockout mice. The *Hfe*$^{-/-}$ mice have increased plasma NTBI levels and, importantly, hepatocytes from *Hfe*$^{-/-}$ mice have been shown to uptake significantly more NTBI than control mice.[8] We found that basal iron status and iron metabolism changes induced by oral iron supplementation were indistinguishable between *Hfe* single knockout and *HfeLcn2* double knockout mice. Our results indicate that

Lcn2 is dispensable for NTBI uptake by hepatocytes in HH. However, they do not exclude that the Lcn2-mediated iron delivery pathway may be involved in other pathologies.

The identification of the components of NTBI delivery pathways remains important for the understanding of the patophysiology of HH and other iron overload diseases such as hypotransferrinemia and thalassemia, because in these situations plasma iron exceeds the binding capacity of transferrin and uptake of NTBI significantly contributes to iron accumulation in the liver, pancreas, and heart.[35]

NTBI uptake mechanisms have been described in a variety of cell lines,[36,37] including hepatocytes.[38] In hepatocytes, this system requires the reduction of Fe^{3+} to Fe^{2+}.[38] Iron salts (such as iron ascorbate, citrate, and nitrilotriacetate) have been suggested as candidates for low molecular-weight NTBI carriers, but the transporters of NTBI have not yet been identified. In addition to Lcn2, which the present study suggests is redundant, potential candidates include L-type voltage-dependent Ca2+ channels (LVDCCs)[39] and Zip14.[40]

LVDCCs have been identified as key transporters of iron into cardiomyocytes and neuronal cells under iron overload conditions.[39, 41] Further support for a role for cardiac LVDCCs in myocardial NTBI uptake under conditions of iron overload comes from the demonstration that LVDCC blockers are protective and able to attenuate myocardial iron accumulation in iron-overloaded mice.[42]

Zip14 is a zinc transporter and member of the SLC39A metal ion transporter family[43] that is highly expressed in hepatocytes. Recent studies have shown that mouse Zip14 transports both iron and zinc in cultured hepatocytes.[40]

In conclusion, the work presented here shows that Lcn2 is dispensable for iron delivery to hepatocytes in the context of *Hfe* deficiency. Further studies will be necessary to establish whether other candidates—namely LVDCCs and Zip14 —are involved in the uptake of NTBI by hepatocytes under iron overload conditions such as HH.

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Abbreviations

HH	hereditary hemochromatosis
Lcn2	lipocalin 2
LVDCC	L-type voltage-dependent Ca2+ channel
NTBI	non–transferrin-bound iron
PCR	polymerase chain reaction

References

1. Ponka P, Lok CN. The transferrin receptor: role in health and disease. Int J Biochem Cell Biol. 1999; 31:1111–1137. [PubMed: 10582342]
2. Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. Proc Natl Acad Sci U S A. 1998; 95:1148–1153. [PubMed: 9448300]
3. Craven CM, Alexander J, Eldridge M, Kushner JP, Bernstein S, Kaplan J. Tissue distribution and clearance kinetics of non-transferrin-bound iron in the hypotransferrinemic mouse: a rodent model for hemochromatosis. Proc Natl Acad Sci U S A. 1987; 84:3457–3461. [PubMed: 3472216]
4. Sheth S, Brittenham GM. Genetic disorders affecting proteins of iron metabolism: clinical implications. Ann Rev Med. 2000; 51:443–464. [PubMed: 10774476]
5. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996; 13:399–408. [PubMed: 8696333]
6. Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. N Engl J Med. 2004; 350:2383–2397. [PubMed: 15175440]
7. Loreal O, Gosriwatana I, Guyader D, Porter J, Brissot P, Hider RC. Determination of non-transferrin-bound iron in genetic hemochromatosis using a new HPLC-based method. J Hepatol. 2000; 32:727–733. [PubMed: 10845658]
8. Chua AC, Olynyk JK, Leedman PJ, Trinder D. Nontransferrin-bound iron uptake by hepatocytes is increased in the Hfe knockout mouse model of hereditary hemochromatosis. Blood. 2004; 104:1519–1525. [PubMed: 15155457]
9. Brissot P, Wright TL, Ma WL, Weisiger RA. Efficient clearance of non-transferrin-bound iron by rat liver. Implications for hepatic iron loading in iron overload states. J Clin Invest. 1985; 76:1463–1470. [PubMed: 4056038]
10. Gunshin H, Fujiwara Y, Custodio AO, DiRenzo C, Robine S, Andrews NC. Slc11a2 is required for intestinal iron absorption and erythropoiesis but dispensable in placenta and liver. J Clin Invest. 2005; 115:1258–1266. [PubMed: 15849611]
11. Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem. 1993; 268:10425–10432. [PubMed: 7683678]
12. Kaplan J. Mechanisms of cellular iron acquisition: another iron in the fire. Cell. 2002; 111:603–606. [PubMed: 12464171]
13. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell. 2002; 10:1033–1043. [PubMed: 12453412]
14. Yang J, Goetz D, Li JY, Wang W, Mori K, Setlik D, Du T, et al. An iron delivery pathway mediated by a lipocalin. Mol Cell. 2002; 10:1045–1056. [PubMed: 12453413]
15. Mori K, Nakao K. Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. Kidney Int. 2007; 71:967–970. [PubMed: 17342180]
16. Devarajan P. Neutrophil gelatinase-associated lipocalin: new paths for an old shuttle. Cancer Ther. 2007; 5:463–470. [PubMed: 18449360]
17. Hanai J, Mammoto T, Seth P, Mori K, Karumanchi SA, Barasch J, et al. Lipocalin 2 diminishes invasiveness and metastasis of Ras-transformed cells. J Biol Chem. 2005; 280:13641–13647. [PubMed: 15691834]
18. Mishra J, Mori K, Ma Q, Kelly C, Yang J, Mitsnefes M, et al. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol. 2004; 15:3073–3082. [PubMed: 15579510]
19. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest. 2005; 115:610–621. [PubMed: 15711640]

20. Elangovan N, Lee Y-C, Tzeng W-F, Chu S-T. Delivery of ferric ion to mouse spermatozoa is mediated by lipocalin internalization. Biochem Biophys Res Commun. 2004; 319:1096–1104. [PubMed: 15194480]
21. Li J-Y, Ram G, Gast K, Chen X, Barasch K, Mori K, et al. Detection of intracellular iron by its regulatory effect. Am J Physiol Cell Physiol. 2004; 287:C1547–C1559. [PubMed: 15282194]
22. Devireddy LR, Teodoro JG, Richard FA, Green MR. Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by IL-3 deprivation. Science. 2001; 293:829–834. [PubMed: 11486081]
23. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004; 432:917–921. [PubMed: 15531878]
24. Berger T, Togawa A, Duncan GS, Elia AJ, You-Ten A, Wakeham A, et al. Lipocalin 2-deficient mice exhibit increased sensitivity to Escherichia coli infection but not to ischemia-reperfusion injury. Proc Natl Acad Sci U S A. 2006; 103:1834–1839. [PubMed: 16446425]
25. Miharada K, Hiroyama T, Sudo K, Danjo I, Nagasawa T, Nakamura Y. Lipocalin 2-mediated growth suppression is evident in human erythroid and monocyte/macrophage lineage cells. J Cell Physiol. 2008; 215:526–537. [PubMed: 18064607]
26. Miharada K, Hiroyama T, Sudo K, Nagasawa T, Nakamura Y. Lipocalin 2 functions as a negative regulator of red blood cell production in an autocrine fashion. FASEB J. 2005; 19:1881–1883. [PubMed: 16157692]
27. Hvidberg V, Jacobsen C, Strong RK, Cowland JB, Moestrup SK, Borregaard N. The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake. FEBS Lett. 2005; 579:773–777. [PubMed: 15670845]
28. Willnow TE, Goldstein JL, Orth K, Brown MS, Herz J. Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. J Biol Chem. 1992; 267:26172–26180. [PubMed: 1464627]
29. Meilinger M, Haumer M, Szakmary KA, Steinböck F, Scheiber B, Goldenberg H, et al. Removal of lactoferrin from plasma is mediated by binding to low density lipoprotein receptor-related protein/ α2-macroglobulin receptor and transport to endosomes. FEBS Lett. 1995; 360:70–74. [PubMed: 7533106]
30. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol. 2007; 18:407–413. [PubMed: 17229907]
31. Levy JE, Montross LK, Cohen DE, Fleming MD, Andrews NC. The C282Y mutation causing hereditary hemochromatosis does not produce a null allele. Blood. 1999; 94:9–11. [PubMed: 10381492]
32. Constante M, Jiang W, Wang D, Raymond V-A, Bilodeau M, Santos MM. Distinct requirements for Hfe in basal and induced hepcidin levels in iron overload and inflammation. Am J Physiol Gastrointest Liver Physiol. 2006; 291:G229–G237. [PubMed: 16565419]
33. Makui H, Soares RJ, Jiang W, Constante M, Santos MM. Contribution of Hfe expression in macrophages to the regulation of hepatic hepcidin levels and iron loading. Blood. 2005; 106:2189–2195. [PubMed: 15914561]
34. Ahmad KA, Ahmann JR, Migas MC, Waheed A, Britton RS, Bacon BR, et al. Decreased liver hepcidin expression in the hfe knockout mouse. Blood Cells Mol Dis. 2002; 29:361–366. [PubMed: 12547226]
35. Breuer W, Hershko C, Cabantchik ZI. The importance of non-transferrin bound iron in disorders of iron metabolism. Transfus Sci. 2000; 23:185–192. [PubMed: 11099894]
36. Kaplan J, Jordan I, Sturrock A. Regulation of the transferrin-independent iron transport system in cultured cells. J Biol Chem. 1991; 266:2997–3004. [PubMed: 1993673]
37. Inman RS, Wessling-Resnick M. Characterization of transferrin-independent iron transport in K562 cells. Unique properties provide evidence for multiple pathways of iron uptake. J Biol Chem. 1993; 268:8521–8528. [PubMed: 8473296]

38. Randell EW, Parkes JG, Olivieri NF, Templeton DM. Uptake of non-transferrin-bound iron by both reductive and nonreductive processes is modulated by intracellular iron. J Biol Chem. 1994; 269:16046–16053. [PubMed: 8206903]

39. Tsushima RG, Wickenden AD, Bouchard RA, Oudit GY, Liu PP, Backx PH. Modulation of iron uptake in heart by L-Type Ca2+ channel modifiers: possible implications in iron overload. Circ Res. 1999; 84:1302–1309. [PubMed: 10364568]

40. Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. Proc Natl Acad Sci U S A. 2006; 103:13612–13617. [PubMed: 16950869]

41. Gaasch J, Geldenhuys W, Lockman P, Allen D, Van der Schyf C. Voltage-gated calcium channels provide an alternate route for iron uptake in neuronal cell cultures. Neurochem Res. 2007; 32:1686–1693. [PubMed: 17404834]

42. Oudit GY, Sun H, Trivieri MG, Koch SE, Dawood F, Ackerley C, et al. L-type Ca2+ channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. Nat Med. 2003; 9:1187–1194. [PubMed: 12937413]

43. Taylor KM, Morgan HE, Johnson A, Nicholson RI. Structure-function analysis of a novel member of the LIV-1 subfamily of zinc transporters, ZIP14. FEBS Letters. 2005; 579:427–432. [PubMed: 15642354]

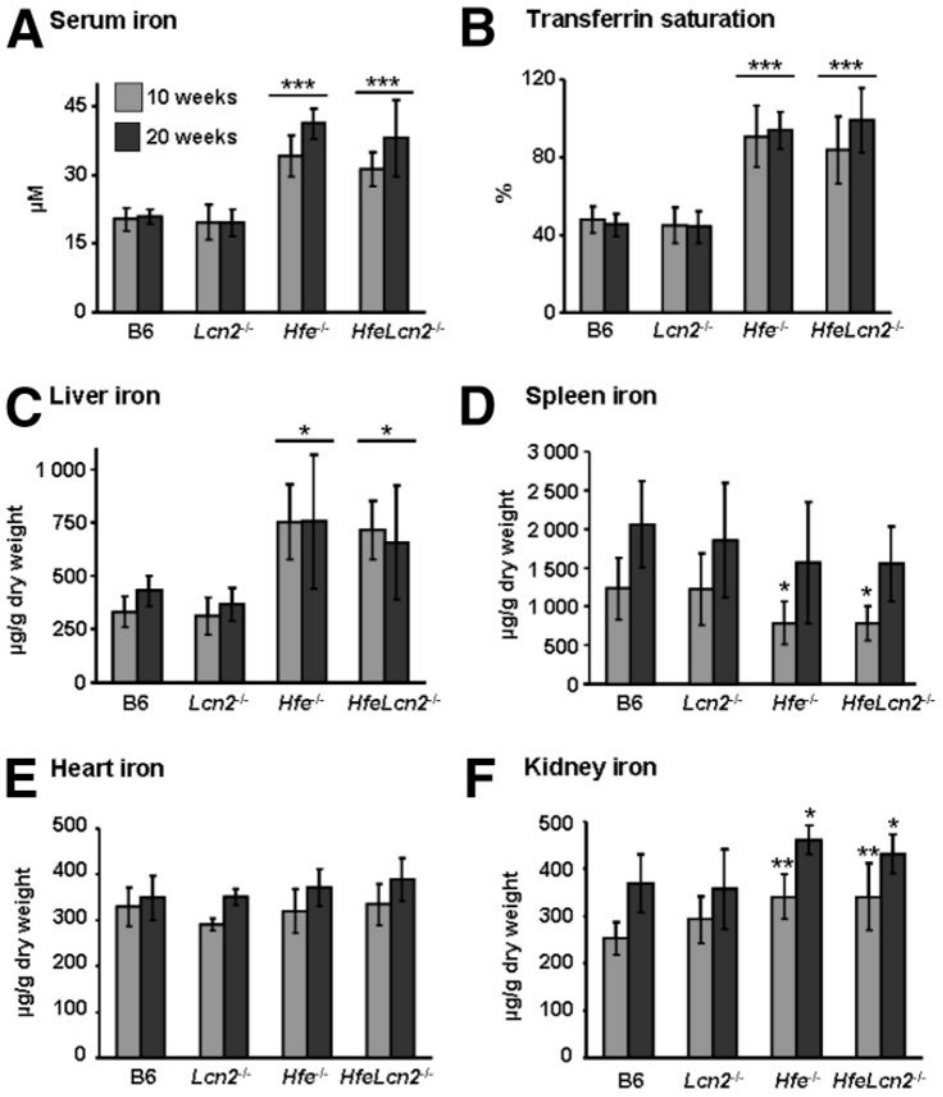

Fig. 1.

Iron parameters in 10- and 20-week-old wild-type (B6) and mutant mice. (A) Serum iron. (B) Transferrin saturation. (C–F) Iron concentration in the (C) liver, (D) spleen, (E) heart, and (F) kidney. The results are expressed as the mean ± standard deviation (n = 6 to 8 mice per group). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (mutant mice versus B6 mice of the same age).

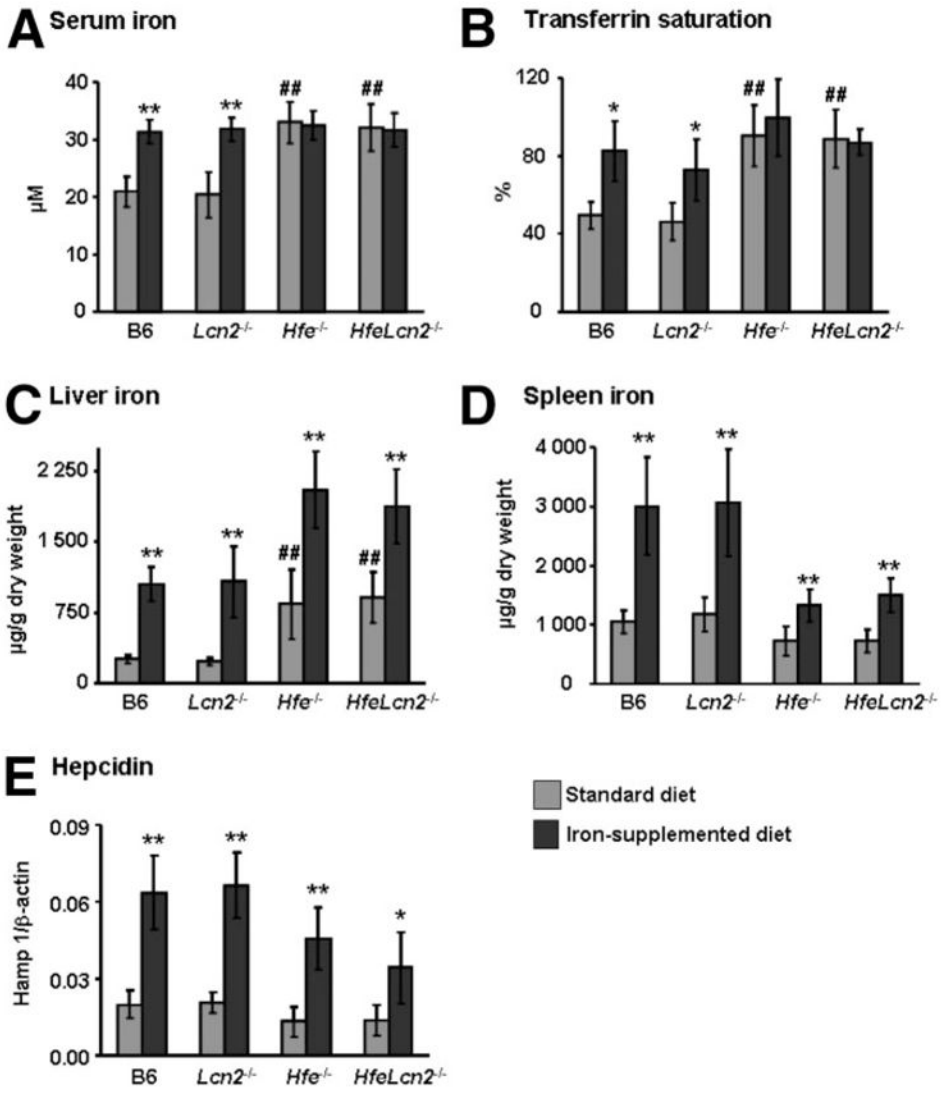

Fig. 2.

Response to iron-loading in wild-type (B6) and mutant mice. (A) Serum iron. (B) Transferrin saturation. (C) Iron concentration in the liver. (D) Iron concentration in the spleen. (E) Hepatic hepcidin 1 messenger RNA expression (Hamp 1) in mice on a standard diet (gray bars) and mice on a diet supplemented with 2.5% carbonyl iron for 2 weeks (black bars). The results are expressed as the mean ± standard deviation (n = 6 to 8 mice per group). $^{\#\#}P < 0.001$ (mutant mice versus B6 mice). $^{*}P < 0.01$, $^{**}P < 0.001$ (iron-supplemented versus standard diet).