Iron Oversupplementation Causes Hippocampal Iron Overloading and Impairs Social Novelty Recognition in Nursing Piglets

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ABSTRACT

Background: Iron oversupplementation in healthy term infants may adversely affect growth and cognitive development.

Objective: We hypothesized that early-life iron excess causes systemic and central nervous system iron overload, and compromises social behavior.

Methods: The nursing pig was used as a translational model in a completely randomized study. On postnatal day (PD) 1, 24 pigs (1.57 ± 0.28 kg mean ± standard deviation body wt) were assigned to the following treatment groups: 1) nonsupplemented iron-deficient group (NON); 2) control group (CON), intramuscularly injected with iron dextran (100 mg Fe) on PD2; 3) moderate iron group (MOD), orally administered ferrous sulfate at 10 mg Fe · kg body wt−1 · d−1; and 4) high iron group (HIG), orally administered ferrous sulfate at 50 mg Fe · kg−1 · d−1. Piglets were nursed by sows during the study from PD1 to PD21. Tissue iron was analyzed by atomic absorption spectrophotometry. Messenger RNA and protein expression of iron regulator and transporters were analyzed by quantitative reverse transcriptase-polymerase chain reaction and Western blot. A sociability test was performed on PD19–20.

Results: Both MOD and HIG treatments (5.51 and 9.85 µmol/g tissue), but not CON (0.54 µmol/g), increased hepatic iron as compared with NON (0.25 µmol/g, P < 0.05). Similarly, the hippocampal iron concentrations in the MOD and HIG groups were 14.9% and 31.8% higher than that of NON, respectively (P < 0.05). In comparison with NON, MOD and HIG treatment repressed DMT1 in duodenal mucosa by 4- and 46-fold, respectively (P < 0.05); HIG drastically induced HAMP in liver by 540-fold (P < 0.05); iron-supplemented groups reduced TFRC in the hippocampus by <1-fold (P < 0.05). However, duodenal expression of ferroportin, the predominant transporter in basal membrane, was not affected by treatment. Despite normal sociability, the MOD and HIG pigs displayed deficits in social novelty recognition (P = 0.004).

Conclusions: Duodenal ferroportin was hyporesponsive to iron excess (MOD and HIG), which caused hippocampal iron overload and impaired social novelty recognition in nursing pigs. J Nutr 2019;149:398–405.

Keywords: iron overload, nursing pig, hippocampus, oxidative stress, social recognition

Introduction

Routine iron supplementation or fortification is practiced widely during infancy, even in many developed countries where the incidence of iron deficiency anemia is relatively low. This is largely due to the fact that, for normal full-term, exclusively breastfed infants, signs of iron deficiency may start to appear as early as 4 mo of age and become more prevalent at 9 mo of age, suggesting prenatal iron endowment and breast milk iron were insufficient for the entire infancy period (1, 2). Iron-fortified infant formulas are readily available, but generally contain markedly higher concentrations of iron (4–12 mg Fe/L) than that of breast milk (~0.2–0.4 mg Fe/L) (3). There are growing concerns over the potential adverse effects of excessive iron fortification during infancy. In a randomized clinical trial, the impact of iron supplementation on growth and morbidity was assessed in Sweden and Honduras, representing a low (<3%) and high (29%) prevalence of iron deficiency anemia, respectively (4). Unexpectedly, iron supplementation (1 mg Fe · kg body wt−1 · d−1) from 4 to 9 mo of age significantly decreased linear growth of Swedish infants. The same effect was only observed in Honduran infants who were initially iron replete [hemoglobin (Hb) ≥ 110 g/L] at 4–6 mo of age. At both sites, there was an increased incidence of diarrhea when iron supplements were given to infants with initial Hb ≥ 110 g/L. The optimal strategy of iron supplementation/fortification during infancy is thus still a matter of debate. Moreover, it has recently been suggested that the iron regulatory mechanisms critical to maintain iron homeostasis in human adults might not be functionally active to limit excessive iron uptake during early infancy (3, 5).
In the central nervous system (CNS), iron-containing proteins are implicated in many biological processes critical to neurodevelopment, including myelination, neurotransmitter synthesis, and oxidative phosphorylation (6). However, iron loading may increase circulating low-molecular-weight iron species that induce nonselective oxidative stress. Abnormal loading may increase circulating low-molecular-weight iron synthesis, and oxidative phosphorylation (6). However, iron to neurodevelopment, including myelination, neurotransmitter proteins are implicated in many biological processes critical to neurodevelopment, including myelination, neurotransmitter proteins are implicated in many biological processes critical

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Supplemental Table 1 is available from the “Supplementary data” link in the web article.

Methods

Animals and management

This study was approved by the Institutional Animal Care and Use Committee. The experiment used a completely randomized design.

Twenty-four crossbred piglets (birth body weight >1 kg) born in 3 litters were stratified by birth body weight, sex, and litter, and randomly assigned to 1 of 4 treatments at birth: 1) the nonsupplementation treatment group (NON) did not receive iron supplementation from postnatal day (PD) 2 to 21; 2) the positive control with iron dextran injection group (CON) piglets were injected intramuscularly with 100 mg Fe in the form of iron dextran solution on PD2 only; 3) the moderate iron treatment group (MOD), which orally received ferrous sulfate at 10 mg Fe·kg body wt−1·d−1 (PD2–21); and 4) the high iron treatment group (HIG), which orally received ferrous sulfate at 50 mg Fe·kg body wt−1·d−1 (PD2–21). A commercial ferrous sulfate solution that was designated for use in infants and toddlers (15 mg Fe/mL, Silixar) was used for iron supplementation in the MOD and HIG groups.

Piglets were raised by their sows in farrowing crates throughout the study. Body weight and rectal temperature were recorded daily, and the piglets were checked for signs of diarrhea. Blood samples were collected from the jugular vein on PD1, 7, 14, and 21 to measure hematocrit (Hct) and Hb, and to harvest plasma. On PD21, pigs were anesthetized via intramuscular injection of TIX (a solution mixture containing 50 mg/mL of telazol, ketamine, and xylazine) and killed by intracardiac injection of an overdose of pentobarbital sodium solution. Tissues and duodenal mucosa samples were collected and stored appropriately until analysis.

Hematological measurements

Hematocrit was measured by centrifugation (15,000 × g, room temperature, 5 min) of ∼50 μL of whole blood in capillary tubes (lithium heparin as anticoagulant). Hemoglobin was measured by the cyanomethemoglobin method through the use of Drabkin’s reagent (Sigma) following the manufacturer’s instruction.

Iron analysis

Tissue iron concentrations were measured as previously described (21). Briefly, ∼0.5 g of brain or liver tissues were digested in 4 mL of 16 mol/L HNO3 (trace metal grade, Fisher Scientific) for 48 h. Samples were wet-ashed on a heating plate and diluted with ultrapure water to a final volume of 5 mL. Samples were analyzed by flame atomic absorption spectrophotometry (iCE 3300 atomic absorption spectrometer, Thermo Fisher).

Gene expression analysis by qPCR

Tissue (~100 mg) was homogenized with a beadmill, and total RNA was extracted following the Trizol reagent protocol. The purity of total RNA was examined with NanoDrop (260/280 ≥ 1.9; 260/230 ≥ 2.0). Total RNA (~1 μg) was used as a template for cDNA synthesis in conjunction with a high-capacity reverse transcription kit (Applied Biosystems). qRT-PCR was performed in 20 μL reaction following the Applied Biosystems Fast SYBR Green assay protocol. Relative expression was determined by the comparative Ct method (2−ΔΔCt method) (22). Expression of target genes was normalized against a housekeeping gene [ribosomal protein L4 (RPL4)]. Sequences of the primer pairs have been previously validated (19, 23) and are listed in Supplemental Table 1.

Protein expression analysis by Western blot

Duodenal scrapings or tissue samples (~100 mg) were homogenized in RIPA buffer containing protease inhibitor cocktail (Sigma). Total protein was harvested and analyzed for concentration with a micro BCA kit (Thermo Scientific). Total protein (30 μg) was loaded for electrophoresis and transferred to nitrocellulose membrane. Following blocking, membranes were probed overnight at 4°C with the following primary antibodies: anti-GAPDH as loading control (mouse anti-human, monoclonal IgG; Santa Cruz Biotechnology, SC-59540) and anti-ferroportin (rabbit anti-human polyclonal IgG; Novus Biologicals, NB1-21502), or anti-ferritin heavy chain (rabbit anti-pig and human polyclonal IgG; Abcam, ab231253). After washing, the membranes were incubated with mouse IgGε light-chain binding protein conjugated with CFE 790 (Santa Cruz Biotechnology, SC-516181) and goat anti-rabbit IgG (H + L) conjugated with Alexa Fluor 594 (Jackson ImmunoResearch Laboratories, 111-585-003) in the dark for 1 h at room temperature.
room temperature. The membranes were detected for the 2 fluorescent channels (Bio-Rad). Data are presented as a percentage of the mean of target protein in the CON group.

**Lipid peroxidation assay**

The lipid peroxidation end product malondialdehyde (MDA) was determined in hippocampal tissues with a commercial kit (Abcam) following the manufacturer's instruction. Briefly, ~50 mg tissue was homogenized and centrifuged (13,000 × g, 4°C, 10 min). Supernatant containing MDA was allowed to react with thiobarbituric acid. The MDA-thiobarbituric acid adduct was quantified colorimetrically in comparison with an MDA standard curve.

**Sociability test**

Pigs were tested for sociability and social novelty preference on PD19 and 20 based on a protocol used for a 3-chamber rodent sociability test (24) modified by removing chamber gates. The arena was customized with the addition of white panel board (length × width × height: 244 cm × 183 cm × 92 cm). The test constitutes 3 sequential steps: 1) acclimation; 2) test for sociability; and 3) test for social novelty preference. From PD16 to 18, each test pig was allowed a 6-min session/d to acclimate to the arena. On PD18, prior to acclimation, 2 dog training crates (Petco), intended to hold stranger pigs (S1 and S2), were installed on the lateral sides of the arena. On PD19–20, a stranger pig (S1) of similar age and body weight as the test pigs was placed in one of the crates. The test pig was allowed a 6-min period in the arena to test sociability. In order to test social novelty preference, the second stranger pig (S2) was brought to the other crate and each test pig was allowed another 6 min for socialization in the arena. The behavior was recorded by a video camera and analyzed with EthoVision (Noldus).

**Statistical analysis**

Normality and homogeneity of variance of the dataset was tested through the use of the UNIVARIATE and GLM procedures of SAS, respectively. Data were analyzed as a completely randomized design through the use of the MIXED procedure of SAS. The model includes treatment and time or exploration zone (for the sociability test only) as fixed effects, and pig nested in treatment as the random term. Serum Hb concentration and Hct on day of birth were used as covariates for the corresponding analysis. A REPEATED statement was included in the model for variables measured over time (e.g., body weight, rectal temperature, Hct, and Hb). The covariance structure (CS, AR1, and UN) that yields the smallest value of Akaike's information criterion was used in the model. Mean separation was performed for variables with significant interaction between main effects through the use of Tukey’s adjusted multiple comparison. Pearson correlation coefficients were determined with the use of the CORR procedure of SAS. Significance will be declared at P ≤ 0.05 and tendency of significance at P ≤ 0.10.

**Results**

**Body weight and body temperature**

The body weights increased with all treatments, and followed the normal growth curve of preweaning pigs. Body weight (Figure 1A) and body temperature did not differ among treatments (P > 0.10; Figure 1B).

**Hct and Hb**

There were significant interactions between treatment and time for Hb (P = 0.006; Table 1) and Hct (P < 0.001; Table 1). Hct and Hb were higher on PD14 and 21 than on PD7 for the CON, MOD, and HIG groups, whereas both hematologic indices gradually decreased in the NON group. In comparison with NON, all the other groups had higher Hb on PD14 and 21 (P < 0.05), and higher Hct on PD7, 14, and 21 (P < 0.05).

**Iron concentration in liver and hippocampus**

Oral iron supplementation dose-dependently increased iron concentration in the liver (P < 0.001; Figure 2A) and hippocampus (P < 0.001; Figure 2B). Hepatic iron was higher in the HIG and MOD groups than in the NON and CON groups. The HIG group had significantly increased hippocampal iron concentration as compared with the other groups (P < 0.05). Hippocampal iron concentration was higher in MOD than in NON (P < 0.05), but it did not differ between NON and CON, or CON and MOD (P > 0.10). There was a significant positive correlation between hepatic and hippocampal iron concentrations on PD1 (P < 0.001, r = 0.69; Figure 2C).

**Gene expression**

Treatment significantly altered the expression of divalent metal transporter 1 [solute carrier family 11 member 2 (DMT1, SLC11A2)] (P = 0.014; Figure 3B) in duodenal mucosa, hepcidin antimicrobial peptide (HAMP) (P < 0.001; Figure 3D) in liver, and TFRC (P = 0.006; Figure 3E) in hippocampus. Specifically, expression of DMT1 in the MOD and HIG groups was lower than in the NON group (P < 0.05). Hepatic HAMP expression was significantly higher in HIG in comparison with NON and CON (P < 0.05). The opposite expression pattern was observed for hippocampal TFRC, which was significantly higher in NON than in all the other groups (P < 0.05). There was a tendency (P = 0.07; Figure 3C) of treatment effect on ferroportin 1 [solute carrier family 40 member 1 (FPN1; SLC40A1)] expression in duodenal mucosa which was higher in NON followed by CON, MOD and HIG. Treatment did not affect expression of cytochrome b reductase 1 (CYBRD1) in duodenal mucosa (P = 0.37; Figure 3A), or change the expression of DMT1, IREB2, and brain-derived neurotrophic factor (BDNF) in hippocampus (data not shown).
TABLE 1 Weekly measurement of hemoglobin and hematocrit in NON, CON, MOD, and HIG pigs

<table>
<thead>
<tr>
<th>PD</th>
<th>Treatment</th>
<th>Hemoglobin, g/L</th>
<th>Hematocrit, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NON</td>
<td>118 ± 6.6</td>
<td>30.2 ± 1.57</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>125 ± 6.0</td>
<td>30.6 ± 1.43</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>127 ± 6.0</td>
<td>32.6 ± 1.43</td>
</tr>
<tr>
<td></td>
<td>HIG</td>
<td>116 ± 6.0</td>
<td>30.6 ± 1.43</td>
</tr>
<tr>
<td>7</td>
<td>NON</td>
<td>99 ± 6.7</td>
<td>27.5 ± 1.55</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>107 ± 6.1</td>
<td>34.6 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>112 ± 6.1</td>
<td>35.8 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>HIG</td>
<td>112 ± 6.1</td>
<td>36.0 ± 1.41</td>
</tr>
<tr>
<td>14</td>
<td>NON</td>
<td>85 ± 6.7</td>
<td>24.4 ± 1.55</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>140 ± 6.1</td>
<td>36.1 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>150 ± 6.1</td>
<td>40.1 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>HIG</td>
<td>148 ± 6.1</td>
<td>39.5 ± 1.41</td>
</tr>
<tr>
<td>21</td>
<td>NON</td>
<td>85 ± 6.7</td>
<td>23.9 ± 1.55</td>
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<tr>
<td></td>
<td>CON</td>
<td>136 ± 6.1</td>
<td>36.1 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>156 ± 6.1</td>
<td>40.0 ± 1.41</td>
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<tr>
<td></td>
<td>HIG</td>
<td>163 ± 6.1</td>
<td>42.6 ± 1.41</td>
</tr>
</tbody>
</table>

P value

- Covariate² 0.41 0.72
- Trt <0.001 < 0.001
- Day <0.001 0.05
- Trt × day <0.001 0.006

¹Pigs were untreated (NON), received a single intramuscular injection of 100 mg Fe on PD2 (CON), or were orally administered 10 (MOD) or 50 mg Fe · kg body wt⁻¹ · d⁻¹ (HIG) from PD2 to PD21. Values are presented as least square means ± SEMs (n = 5 or 6/trt). Within a column, means without a common superscript letter differ, P < 0.05. CON, iron-injection group; HIG, high iron supplementation group; MOD, moderate iron supplementation group; NON, nonsupplemented group; PD postnatal day; trt, treatment.

²Hemoglobin and hematocrit values on PD1 were used as covariates for analysis.

Protein expression

There was a significant treatment effect on hepatic ferritin-heavy chain (FTH1) expression (P < 0.001; Figure 4B). Both MOD and HIG significantly increased hepatic FTH1 expression in comparison with NON and CON. Hippocampal FTH1 expression, however, did not differ among treatments (P = 0.66; Figure 4C). Treatment tended to alter FPN1 expression in duodenal mucosa (P = 0.107; Figure 4A), which was lower in NON than in the other groups.

Hippocampal lipid peroxidation

Treatment tended to affect hippocampal MDA concentration (P = 0.076; Figure 5). The concentration was higher in HIG than in the other groups.

Sociability test

Piglets from all treatment groups displayed normal social activity by spending more time (exploration zone: P < 0.001) exploring a stranger piglet than the empty crate (Figure 6A). Interestingly, there was a significant interaction between treatment and zone (P = 0.004; Figure 6B) on the exploration pattern in the subsequent test for social novelty preference. Piglets from both the NON and CON groups spent more time exploring a stranger piglet rather than the familiar conspecific, whereas piglets from the MOD and HIG groups spent equivalent time exploring both stranger and familiar conspecifics.

Discussion

Due to limited iron stores at birth (<50 mg) and low iron concentrations in sow milk (~1 mg/L), nursing piglets, like human infants, require iron supplementation to maintain a replete status. In general, retaining 7–16 mg Fe/d ensures adequate Hb and iron storage before weaning (25). In our current study, the MOD and HIG treatments, on average, provide 3–15 times the iron requirement during the first 3 wk after birth without accounting for absorption rate. It should be noted that even the high oral doses used in our study are still much lower than the toxic level, which was reported to be 600 mg/kg body wt for nursing pigs (26). In comparison with NON, both a single intramuscular injection of iron dextran (CON) and oral iron supplementation (MOD and HIG) effectively prevented iron deficiency anemia as indicated by the concentration of Hb (>110 g/L). However, Hb did not seem to be a sensitive marker for iron excess: MOD and HIG significantly increased hepatic iron load in a dose-dependent manner, whereas there was little difference in Hb among the CON, MOD, and HIG groups. It is not unexpected that increased hepatic iron load was mirrored by elevated protein expression of FTH1 for iron storage. This might be mediated through the binding of iron to iron-regulatory proteins (IRPs), and release the translation repression that IRPs imposed on FTH1 (27). Importantly, in comparison with NON and both NON and CON, there was a significant increase in iron overload in nursing piglets.
of hippocampal iron in the MOD or HIG groups, suggesting iron excess enhanced its accumulation in the developing brain. This is confirmed by the observation of significant correlation between hepatic iron and hippocampal iron. Nevertheless, the rise of hippocampal iron did not significantly increase FTH1 expression.

Iron supplementation (e.g., 1 mg Fe · kg body wt−1 · d−1 from 4 to 9 mo of age) to iron-replete, healthy term infants or young children has been shown to adversely affect linear growth, head circumference, and weight gain, which cannot be explained by the difference in morbidity rates. (4, 28). The deleterious effect of iron excess on growth was also observed in postweaning pigs (7 wk of age) (29). In our current study, both MOD and HIG treatments provided more iron than the requirement for normal growth (21 mg Fe/kg weight gain) (25). However, neither treatment compromised growth performance in postweaning pigs (29). In our current study, all piglets were devoid of diarrhea (personal observation) and fever as reflected by normal rectal temperature throughout the study. However, our study was conducted on a research farm, where high environmental hygiene was maintained.

Iron metabolism is unique as there is no efficient route of excretion. Therefore, a fine-tuned mechanism to regulate intestinal iron uptake is critical. In both humans and pigs, iron absorption primarily occurs in the duodenum through carrier-mediated transportation (32). Apical expression of DMT1 serves as the main entry portal for nonheme iron, which is subsequently exported to blood predominantly through FPN1 in the basolateral membrane (33). Hepcidin plays a major role in controlling dietary iron absorption by binding to FPN1 on the surface of the basolateral membrane and leading to its degradation. In the CNS, iron uptake mainly relies on endothelial expression of TFRC, which mediates transferrin-bound iron uptake through endocytosis and, to a lesser extent, transcytosis (34). In our current study, in comparison with the control NON group, iron supplementation altered mRNA expression of iron regulatory proteins (DMT1, HAMP, TFRC, and FPN1) in a direction that reduces iron uptake and trafficking in both intestine and brain. Our results are in agreement with others who also observed that increase of dietary iron reduced DMT1 and FPN1 expression in duodenum and enhanced hepatic expression of HAMP in postweaning pigs (29). It thus highlights a shared iron-dependent transcriptional modulation. Apical expression of CYBRD1, a ferric reductase, is thought to facilitate ferric iron absorption in gut epithelium through cooperation with DMT1 in rodents. The mRNA expression of CYBRD1 in the duodenum was shown to be induced by iron availability (35). However, in our current study, CYBRD1 expression was insensitive to iron supplementation, making it less likely to play an important role in regulating iron metabolism in piglets. In humans, its role in iron absorption has also been questioned by the finding that the different iron statuses observed in various pathophysiologic conditions did not alter CYBRD1 expression (36). Downregulation of TFRC might result from increased cellular iron that competitively reduced the binding of IRPs to the iron-response element in the 5′ untranslated region, which increases RNA degradation of TFRC (37). Others have also reported that TFRC expression in the prefrontal cortex of preweaning pigs is negatively associated with iron availability in the context of iron deficiency (38). Hepatic expression of HAMP was shown to be positively correlated with urine and plasma hepcidin in 4-wk-old piglets.
Protein expression of FPN1 (A) in duodenal mucosa and expression of FTH1 in liver (B) and hippocampus (C) of nursing pigs on PD21. Pigs were untreated (NON), received a single intramuscular injection of 100 mg Fe on PD2 (CON), or were orally administered 10 (MOD) or 50 mg Fe · kg body wt⁻¹ · d⁻¹ (HIG) from PD2 to PD21. Data were normalized to GAPDH for loading control and are presented as relative expression (%) to CON. Values are least square means ± SEMs, n = 5 or 6. Means without a common letter differ, P < 0.05. Representative Western blot images are shown on the left side of each panel. CON, positive control group with iron dextran injection; FPN1, ferroportin 1; FTH1, ferritin heavy chain; HIG, high iron treatment group; MOD, moderate iron treatment group; NON, nontreatment group; PD, postnatal day; Trt, treatment.

Hence, in our current study, the dramatic induction of hepatic HAMP expression by oral iron supplementation can explain the higher amounts of circulating hepcidin. Despite a well-established role of the hepcidin-ferroportin axis in controlling intestinal iron uptake (40), intriguingly, iron supplementation did not affect, let alone increase, duodenal protein expression of FPN1 in our study. Our results are consistent with a previous finding that dietary iron intake did not alter duodenal FPN1 expression in postweaning pigs (29). In addition, parenteral administration of iron dextran was found to dramatically increase circulating hepcidin and liver HAMP expression, but did not affect ferroportin expression in the duodenal mucosa of weanling pigs in comparison with a nonsupplemented group (41). The same hyporesponsiveness was also observed in studies with suckling rodents (42, 43), in which constitutive overexpression of hepcidin failed to induce ferroportin degradation in enterocytes (42). There is no direct

Hippocampal lipid peroxidation in nursing pigs on PD21. Pigs were untreated (NON), received a single intramuscular injection of 100 mg Fe on PD2 (CON), or were orally administered 10 (MOD) or 50 mg Fe · kg body wt⁻¹ · d⁻¹ (HIG) from PD2 to PD21. Values are least square means ± SEMs, n = 6. CON, positive control group with iron dextran injection; HIG, high iron treatment group; MOD, moderate iron treatment group; NON, nontreatment group; PD, postnatal day; Trt, treatment.

Sociability (A) and social novelty preference (B) of nursing pigs. Pigs were untreated (NON), received a single intramuscular injection of 100 mg Fe on PD2 (CON), or were orally administered 10 (MOD) or 50 mg Fe · kg body wt⁻¹ · d⁻¹ (HIG) from PD2 to PD21. Data are presented as duration of socialization (s) within each zone area that contains either an empty crate or stranger pigs (S1 and S2). Values are least square means ± SEMs, n = 6. CON, positive control group with iron dextran injection; HIG, high iron treatment group; MOD, moderate iron treatment group; NON, nontreatment group; PD, postnatal day; S, stranger pig; Trt, treatment; TxZ, treatment × zone interaction.
evidence showing the hyporesponsiveness of FPN1 to the action of hepcidin in human infants. However, in a study with 6-month-old breastfed infants, the iron absorption rate was found to be refractory to daily supplementation with ferrous sulfate (7.5 mg Fe), which would otherwise decrease the absorption rate in adults (44). Collectively, the available evidence challenges the functional maturity of the hepcidin-ferroportin axis in neonates.

Iron excess to an extent that exceeds the binding capacity of transferrin is likely to increase circulating nontransferrin-bound iron, a known pro-oxidant that generates hydroxyl radicals and induces oxidative damage to protein and DNA. Our hypothesis that excessive iron provision early in life results in CNS oxidative stress is partially supported by the findings that lipid peroxidation biomarkers tended to increase (particularly in the HIG group) in response to iron supplementation as compared with the NON group. Oxidative stress is involved in the pathogenesis of developmental neuropsychiatric disorders (e.g., autism spectrum disorders) (45–48). Social deficits are a major component of clinical manifestations. Few studies have evaluated the impact of iron loading on sociability and social recognition memory. Pigs are a social species. Maternal viral infection in sows during late gestation was found to moderately impair both sociability and social novelty preference of offspring (49). Our finding is novel in that iron excess (in the MOD and HIG groups) diminished social novelty preference without affecting overall sociability in nursing piglets. This cannot be entirely attributed to iron-induced hippocampal oxidative stress: both MDA and iron concentrations in the hippocampus were similar in the CON and MOD groups. The CA2 region of the hippocampus has been increasingly recognized as playing a critical function in social recognition. High expression of social neuropeptide receptors (e.g., oxytocin and vasopressin 1b receptors) in CA2 pyramidal neurons is considered to contribute to the formation and retrieval of social memory in mice (50, 51). Global deletion of vasopressin 1b receptor reduced aggression and moderately impaired social recognition in a manner independent of olfactory cues (50, 52). More convincing evidence comes from a study that selectively silenced output of CA2 pyramidal neurons in mice, leading to specific loss of social recognition memory, which was characterized as indiscrimination between familiar and novel conspecifics in a 3-chamber social novelty test. However, overall sociability and other hippocampal-dependent cognitive functions were unaffected (16). The phenotypes of social behavior are the same as those observed for the MOD and HIG groups in the current study. The adverse effect of iron overloading on social recognition has not been reported in human infants. Nevertheless, in a follow-up study that evaluated the impact of iron fortification during infancy on cognitive development at 10 y of age, provision of iron-fortified formula (12.7 mg Fe/L) to iron-replete infants compromised their neurodevelopmental measures including spatial memory and visual–motor integration (9).

In conclusion, our results suggested that iron excess led to iron overloading in both the periphery and developing brain in nursing pigs. This might be due to the fact that FPN1 is refractory to regulation of hepcidin in early life. The finding that iron overloading impaired social recognition is novel, and the underlying mechanism for this effect remains unclear.

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