Ferroportin mediates the intestinal absorption of iron from a nanoparticulate ferritin core mimetic in mice

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ABSTRACT The ferritin core is composed of fine nanoparticulate Fe³⁺ oxohydroxide, and we have developed a synthetic mimetic, nanoparticulate Fe³⁺ polyoxohydroxide (nanoFe³⁺). The aim of this study was to determine how dietary iron derived in this fashion is absorbed in the duodenum. Following a 4 wk run-in on an Fe-deficient diet, mice with intestinal-specific disruption of the Fpn-I gene (Fpn-KO), or littermate wild-type (WT) controls, were supplemented with Fe²⁺ sulfate (FeSO₄), nanoFe³⁺, or no added Fe for a further 4 wk. A control group was Fe sufficient throughout. Direct intestinal absorption of nanoFe³⁺ was investigated using isolated duodenal loops. Our data show that FeSO₄ and nanoFe³⁺ are equally bioavailable in WT mice, and at wk 8 the mean ± SEM hemoglobin increase was 18 ± 7 g/L in the FeSO₄ group and 30 ± 5 g/L in the nanoFe³⁺ group. Oral iron failed to be utilized by Fpn-KO mice and was retained in enterocytes, irrespective of the iron source. In summary, although nanoFe³⁺ is taken up directly by the duodenum its homeostasis is under the normal regulatory control of dietary iron absorption, namely via ferroportin-dependent efflux from enterocytes, and thus offers potential as a novel oral iron supplement.—Aslam, M. F., Frazer, D. M., Faria, N., Bruggraber, S. F. A., Wilkins, S. J., Mirciov, C., Powell, J. J., Anderson, G. J., Pereira, D. I. A. Ferroportin mediates the intestinal absorption of iron from a nanoparticulate ferritin core mimetic in mice. FASEB J. 28, 3671–3678 (2014). www.fasebj.org

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Iron deficiency anemia (IDA) persists as the major nutritional deficiency disorder in the world, affecting >1 billion people (1). This places IDA in the World Health Organization’s top 10 list of target diseases for cure and prevention (2–4). Current therapy for IDA involves supplementation with an oral iron preparation. First-generation oral iron agents are simple Fe²⁺ salts, which are cheap and well absorbed, but are associated with significant upper and lower gastrointestinal side effects, such as nausea, constipation, and abdominal discomfort (5, 6). Second-generation oral iron agents are soluble chelated forms of Fe²⁺ or Fe³⁺ and, while they reduce upper gastrointestinal side effects, they are expensive to manufacture and, chronically, they appear to retain their distal gastrointestinal adverse effects (7–9). Indeed, recent studies have consistently shown that soluble oral iron negatively affects the colonic flora, promoting the presence of potentially pathogenic bacteria at the expense of beneficial bacteria (10–13). Other studies have also raised serious concerns over “available” iron in the colon as a risk factor for inflammatory signaling and colorectal carcinogenesis (8, 12).

To help address these issues, we have developed a nanodispersed, ligand-modified material, nanoparticulate Fe³⁺ polyoxohydroxide (nanoFe³⁺). Analysis by high-resolution electron microscopy showed that nanoFe³⁺ has a very similar structure to the Fe³⁺ oxohydroxide core of ferritin (14); that is, it is destabilized 2-line ferrihydrite (15, 16). It also resembles the digestion product of dietary Fe³⁺ in the small bowel (17). NanoFe³⁺ has potential advantages as a novel oral iron supplement. It is absorbed whole into duodenal enterocytes and then readily broken down intracellularly (17). Soluble iron that is released from the nanostructure in this fashion can be utilized systemi-
MATERIALS AND METHODS

Iron materials

Ferrous sulfate heptahydrate (FeSO₄) was purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Ferric citrate monohydrate was purchased from Sigma-Aldrich (Sydney, Australia). Ferric nitrilotriacetate chelate (FeNTA₂) was produced by mixing an acidified solution of FeCl₃ (10 mM) with an NTA solution to achieve a molar ratio of Fe:NTA of 1:2. The pH of the final solution was adjusted to 7.4 with NaOH.

NanoFe₃⁺ was prepared according to the protocol by Powell et al. (14). Briefly, an acidic concentrated stock solution of FeCl₃ was added to a solution containing tartaric acid and adipic acid in 0.9% (w/v) of electrolyte (KCl) to achieve a molar ratio of Fe:tartaric acid:adipic acid in the final suspension of 2:1:1 and [Fe] = 40 mM. The initial pH of the mixture was always below 2.0 and the Fe was fully soluble. The pH was then slowly increased by dropwise addition of a concentrated solution of NaOH until ca. pH 7.4. The entire mixture was then oven-dried at 45°C for a minimum of 24 h.

Rodent diets

All diets were prepared by Specialty Feeds (Glenn Forest, WA, Australia) and supplied in a powdered form. Other than varying the amount and form of the iron added, the diets were equivalent and conformed to AIN-93G purified rodent diet (Supplemental Table S1 and ref. 37). The iron materials used to supplement the rodent diet were FeSO₄, Fe³⁺ citrate, and nanoFe₃⁺ as defined above. The total iron content of the test diets was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo, Horiba-Johnin, Stanmore, UK) at 259.94 nm following digestion with concentrated nitric acid at 37°C for 4 h, followed by 16 h at 70°C, and then diluted (1:5) with UHP water. The ICP-OES calibration was with sample-based standards (the sample matrix used was that of the Fe-deficient diet similarly digested and diluted) which were spiked with iron ranging from 0 to 18 μM, in a similar fashion to previous work (38).

Animals

Mice carrying the floxed Fpn (Fpnflkox/flkox) allele were bred with vil-Cre-ER² mice, which carry a tamoxifen-inducible, intestine-specific Cre recombinase gene (30). Mice with intestine-specific deletion of Fpn (here referred to as Fpn KO) were produced by injecting the resulting vil-Cre-ER²/Fpnflkox/flkox mice subcutaneously with tamoxifen (0.075 mg/g body weight) once daily for 3 d starting at 28 d of age. Littermate wild-type (WT) control mice were also injected with tamoxifen 1×/d for 3 d starting at 28 d of age. Lack of Fpn expression in intestinal enterocytes taken from tamoxifen-injected mice was confirmed by Western blotting (Supplemental Fig. S1). This study was carried out in strict accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All animal procedures were approved by the Queensland Institute of Medical Research Berghofer Medical Research Institute Animal Ethics Committee (registration number A0192-609M). All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

Feeding study design and tissue collection

WT and Fpn-KO mice were allocated to the different diet groups (n=4–8 mice/set) as outlined in Supplemental Fig. S2. All animals were housed individually and had unlimited access to food and deionized water throughout the study. Three-week-old mice were fed an iron-deficient diet for 4 wk (i.e., 0–4 wk=iron-depletion period) to induce iron deficiency. Administration of the test diets was to the two study groups and comprised the iron-deficient diet supplemented with ca. 20 mg iron/kgdiet as FeSO₄ or nanoFe₃⁺. This began at the end of week 4 and lasted a further 4 wk (i.e., 4–8 wk; Supplemental Fig. S2). Two further sets of mice (one Fpn KO and the other WT) remained on the iron deficient diet throughout the study (here named Fe-deficient group). Similarly, two control sets of animals (one Fpn KO and the other...
WT) were fed an iron sufficient diet, with ca. 35 mg iron/kg feed as FeCl3·6H2O, or Na2FeEDTA (iron phase distribution in this solution previously described by Rebouche et al. (41)). Briefly, liver and spleen samples were wrapped in foil and dried overnight in an oven at 110°C. Two parts of the dried tissues were then mixed with a solution containing 3 M hydrochloric acid and 0.6 M trichloroacetic acid. Calibration standards were prepared in the range 2–100 μg Fe/mL. Tissues and standards were incubated at 65°C for 20 h, then vortexed and centrifuged (10,000 g, 5 min). Two parts of the supernatant solution (0.508 mM ferrozine, 1.5 M sodium acetate, and 1.5% v/v thioglycolic acid in H2O, pH 7.0) were mixed with 1 part standard/tissue supernatant, and the absorbance was read at 595 nm following 30 min incubation at room temperature.

Analysis of gene expression

RNA was extracted from snap-frozen liver and duodenal tissue samples using TRIzol reagent (Invitrogen, Melbourne, VIC, Australia) as per the manufacturer’s instructions. The RNA (500 ng) was then used for cDNA synthesis using M-MLV reverse transcriptase (Invitrogen, San Diego, CA, USA) following the manufacturer’s instructions. Gene expression was determined by quantitative real-time polymerase chain reaction (rtPCR) as described previously (42).

The primers used were as follows: Hamp1, forward CCTGAGCAGCCACCACTATCTC, reverse TGCAAGACATGACACACTGGG; SOD1, forward CTGAGTGCAGCCAGC, reverse GGTGCTGGCTTTGGTACCTAC; HPRT, forward ATGATCATGATTACGCTGG, reverse TCACTATGGATGCTGTACCTAC; Cathepsin B, forward GTGAGCATAGCCAGAAGTTG, reverse CATGAGCAGCCACCACTATCTC.

Western blotting

Protein was extracted from isolated enterocytes, and expression of Fpn was assessed by Western blotting as described previously (43).

Statistical analysis

Unless otherwise stated, all values are expressed as means ± sd. Statistical differences between the Hb values for each group of mice at the various time points on a specific diet were determined with repeated measures 2-way analysis of variance (ANOVA) with the Bonferroni correction to account for multiple comparisons. Statistical differences between hepatic and splenic tissue iron levels and gene expression levels between groups of mice on the various diets were determined with 1-way ANOVA with the Bonferroni correction. Statistical differences between the serum iron levels from the duodenal loop study were determined by unpaired t test. The level of significance was set to P < 0.05. Statistical analysis was performed with GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA).

RESULTS

Dietary nanoFe3+ is absorbed via a Fpn-dependent pathway

Hb levels in the Fpn-KO mice maintained on the iron-sufficient diet (Fe-sufficient group) were lower
than the Hb levels of the WT mice maintained on the same diet, being significant from wk 5 onward (P<0.03; Fig. 1A). Similarly, Fpn-KO mice maintained on the iron-deficient diet (Fe-deficient group) had lower Hb compared to WT mice on the same diet, and this was statistically significant from wk 4 onward (P<0.003; Fig. 1B). Fpn-KO mice on both iron-supplemented test diets (i.e., FeSO₄ and nanoFe₃⁺ groups) also had similar and significantly lower Hb levels throughout the Fe repletion period compared to the WT mice (P<0.0002 from wk 5 for FeSO₄, and P<0.007 from wk 4 for nanoFe₃⁺; Fig. 1C, D).

The body weights of the animals for each dietary group are presented in Supplemental Table S2 alongside the baseline and final Hb values of Fig. 1. There were no significant differences in the body weight of the animals in any diet group at the end of the study. There were no statistically significant differences between final Hb levels of the WT mice supplemented with nanoFe₃⁺ in comparison to WT mice supplemented with FeSO₄ or WT mice in the control Fe-sufficient group. However, Hb levels at the end of the study in the nanoFe₃⁺-supplemented WT mice were significantly higher than for WT mice of the Fe-deficient group (P<0.005; Supplemental Table S2). For the Fpn-KO mice, Hb levels at the end of the study in the nanoFe₃⁺ group were similar to the Fe-deficient group and the FeSO₄ group, but were lower for these 3 groups than the levels in the Fe-sufficient group (P=0.06 for nanoFe₃⁺, P=0.01 for FeSO₄, and P=0.001 for Fe-deficient; Supplemental Table S2).

Throughout, Hb levels of Fpn-KO mice were significantly lower than for WT counterparts (all P<0.0001 by wk 8; Fig. 1).

### Tissue iron levels

The levels of iron in the duodenum, spleen, and liver of animals maintained on diets containing different forms of iron were assessed qualitatively by Perl's Prussian blue staining and quantitatively (only for the spleen and liver) using a colorimetric assay. There was no stainable iron in the small intestinal enterocytes of WT or Fpn-KO mice fed the iron-deficient diet throughout the study (Fig. 2A). Similarly, there was no accumulation of iron in the spleen (Fig. 2A) or the liver in these mice (Supplemental Fig. S4). In the three other groups (i.e., both iron-supplemented groups and the non-iron-depleted control group), no stainable iron was seen in the small intestinal enterocytes of WT mice, but distinct iron accumulation was observed in enterocytes of Fpn-KO mice (Fig. 2B–D). Conversely, iron staining was detected in the spleens of WT mice but not in the spleen of Fpn-KO mice (Fig. 2B–D). No detectable iron staining was observed in the liver of either WT or Fpn-KO animals from any group (Supplemental Fig. S3).

Quantitation of iron levels in the liver of WT animals showed that both the FeSO₄⁻ and the nanoFe₃⁺-supplemented groups were able to increase hepatic Fe stores to levels similar to those observed in the non-iron-depleted control group (Fig. 3). Corresponding increases in spleen iron also occurred in both the FeSO₄⁻ and nanoFe₃⁺-supplemented groups, although the levels did not reach that of control mice. In contrast, both liver and spleen iron levels were consistently low in the Fpn-KO mice in all the diet groups and were significantly lower than the levels in the corresponding WT animals (P<0.006; Fig. 3). Spleen iron levels were an order of magnitude higher than liver iron levels for all diet groups (Fig. 3).

### Tissue expression of Fe-related genes

To investigate how iron derived from nanoFe₃⁺-influenced intestinal and systemic iron homeostasis, we examined the enterocyte mRNA expression of Slc11a2, the apical transporter of ferrous iron in enterocytes (25), and hepatic hepcidin (Hamp1), the master regulator of iron

![Figure 1](http://www.fasebj.org/content/28/Supplement1/3674/F1)

**Figure 1.** Hb levels in mice during dietary iron repletion. Hb values for WT (solid trace) and Fpn KO (dashed trace) mice in the control Fe-sufficient (i.e., non-iron-depleted) group (A), the Fe-deficient group (B), the FeSO₄-supplemented group (C), and the nanoFe₃⁺-supplemented group (D) after iron depletion (study wk 4), 1 wk into the iron repletion period (study wk 5), 2 wk into the iron repletion period (study wk 6), and at the end of the iron repletion period (study wk 8) as per study outline presented in Supplemental Fig. S2. Arrows (C, D) indicate the start of iron repletion with FeSO₄⁻ or nanoFe₃⁺-supplemented diets, respectively. Values are means ± sd. Numbers in each group are as follows: Fe-sufficient, n = 6 WT and n = 8 Fpn KO; Fe-deficient, n = 6 WT and n = 6 Fpn KO; FeSO₄⁻, n = 4 WT and n = 9 Fpn KO; nanoFe₃⁺⁻, n = 8 WT and n = 6 Fpn KO. *P ≤ 0.01, **P ≤ 0.007, ***P ≤ 0.0002, ****P < 0.0001 vs. Fpn-KO mice.
In WT mice, Slc11a2 expression in the duodenum was significantly higher \( (P < 0.0001) \) in the iron-deficient group than in the mice fed the iron-supplemented diets (FeSO\(_4\) and nanoFe\(^{3+}\) groups) or the non-iron-depleted controls (Fe-sufficient group) \( (P < 0.0001) \). All Fpn-KO mice had very little expression of either of the iron-regulating genes, irrespective of diet group.

Iron transport from nanoFe\(^{3+}\) in isolated duodenal loops in vivo

Duodenal loops were used to assess the direct transfer of iron, derived from nanoFe\(^{3+}\) of the lumen, into the blood circulation in WT and Fpn-KO mice. Infusing the duodenal loops with nanoFe\(^{3+}\) provides additional information on the uptake of Fe from the intact nanoparticulate material, that is, bypassing the stomach, unlike with feeding experiments. Serum iron levels were compared to a soluble Fe\(^{3+}\) control (FeNTA\(_2\)) and a saline control (no iron). All Fpn-KO animals had
significant lower serum iron levels than WT mice in the same diet group (P<0.0001). In WT mice, serum iron levels increased 30 min following infusion with nanoFe$^{3+}$ ($P=0.04$) and more so with FeNTA$_2$ ($P=0.007$) (Fig. 5). In contrast, there was no difference in serum iron levels of Fpn-KO mice 30 min following infusion with either iron preparation in comparison to the saline control (Fig. 5).

**DISCUSSION**

We have previously shown that nanoFe$^{3+}$ crosses the apical surface of Caco-2 cells via an endocytic pathway before being dissolved in endosomes or lysosomes inside the cell (17). However, the mechanism by which the iron derived from nanoFe$^{3+}$ crosses the basolateral membrane is unknown. Since Fpn is the only known mammalian iron export protein (29), we investigated the absorption of nanoFe$^{3+}$ in intestine-specific Fpn-KO mice and littermate controls. We found that, like other forms of iron in the intestine, nanoFe$^{3+}$ was ineffective at repleting Hb levels in Fpn-KO mice. Iron was retained in the enterocyte of Fpn-KO animals regardless of whether it was soluble iron or nanoFe$^{3+}$, consistent with Fpn being the common enterocyte exporter for iron irrespective of its luminal form. The low levels of iron in the liver and spleen of Fpn-KO mice provide further evidence that iron derived from nanoFe$^{3+}$ cannot bypass the Fpn-mediated efflux mechanism that is used by soluble luminal iron sources.

We further investigated the absorption of nanoFe$^{3+}$ using intestinal loops in which the iron compound was administered directly into the duodenum of WT and Fpn-KO animals, thereby bypassing the stomach. Although serum iron levels increased significantly with the soluble (FeNTA$_2$) and nanoFe$^{3+}$ infusions compared to saline, the increase with nanoFe$^{3+}$ was significantly lower ($P=0.03$) than with FeNTA$_2$, despite nanoFe$^{3+}$ being as effective as ferrous sulfate at increasing Hb levels during the dietary intervention study. The difference is not related to the choice of positive controls for the two experiments, as ferrous sulfate is certainly as well absorbed as FeNTA$_2$ (44–46). Instead, the data show that either the passage of nanoFe$^{3+}$ through the stomach is required for efficient absorption, or nanoFe$^{3+}$ absorption is slower than that of soluble iron, because the direct uptake of whole nanoparticles requires their endosomal/lysosomal breakdown prior to systemic release of iron (17). Indeed, we suggest that the ca. 30 µg/dl difference in serum Fe levels between the FeNTA$_2$- and nanoFe$^{3+}$-treated WT animals is mainly due to differences in the absorption kinetics of the two materials. Studies have shown that the rate of absorption of Fe from ferritin, which contains an Fe$^{3+}$ oxohydroxide core very similar to nanoFe$^{3+}$ and is taken up by clathrin-dependent endocytosis (47–49), is slower than that of soluble Fe in the rat intestine (50). Moreover, our own observations in human volunteers comparing nanoFe$^{3+}$ to FeSO$_4$ support this difference in kinetics (unpublished results).

Despite these potential differences in the rate of iron absorption by the intestine, the overall response of the body to nanoFe$^{3+}$ appears to be almost identical to that of FeSO$_4$. The Hb repletion data in the WT mice demonstrate that iron from nanoFe$^{3+}$ is as efficient at correcting diet-induced iron deficiency anemia as FeSO$_4$. Gene expression analysis showed that Slc11a2 mRNA levels in enterocytes decreased to similar levels in response to iron repletion by either nanoFe$^{3+}$ or soluble Fe. Interestingly, the Fpn-KO animals in all the diet groups expressed very low levels of Slc11a2 even though they were iron deficient. This is likely due to the
accumulation of iron in Fpn-KO enterocytes, as seen in Fig. 2, which leads to the destabilization of Slc11a2 mRNA via the IRP/IRE system (51). Indeed, similar down-regulation of Slc11a2 levels was observed in sl mice, which develop iron-loaded enterocytes due to a deletion in the gene encoding the ferroxidase hepahestin (52). Liver Hmmp1 mRNA expression also responded similarly to iron repletion irrespective of the iron source. WT animals in the iron-supplemented groups showed significantly elevated Hmmp1 expression in the liver when compared to iron-deficient animals.

Taken together, our data clearly demonstrate the effectiveness of nanoFe3+ as a dietary supplement, consistent with iron repletion studies in rats (14). The relatively slow release of iron from nanoFe3+, with a consequent reduced rate of absorption, may be an advantage in terms of preventing the generation of nontransferrin bound Fe that can be observed following absorption of therapeutic doses of soluble iron (53–56). Notably, the iron derived from nanoFe3+ does not circumvent systemic iron regulatory mechanisms, as the intestinal efflux of iron following enterocyte uptake of nanoFe3+ is ferroportin mediated in a similar manner to that of soluble iron. This implies that iron derived from nanoFe3+ joins the common enterocyte labile Fe pool, at some point, following the direct brush border uptake of nanoFe3+.

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REFERENCES
1. World Health Organization (2008) Global Database on Anaemia, World Health Organization, Geneva, Switzerland
2. World Health Organization (2002) The World Health Report. Reducing Risks, Promoting Healthy Life, World Health Organization, Geneva, Switzerland
3. Ezzati, M., Lopez, A. D., Rodgers, A., Vander Hoorn, S., and Murray, C. J. (2002) Selected major risk factors and global and regional burden of disease. Lancet 360, 1347–1360
4. World Health Organization (2004) The Global Burden of Disease: 2000 Update pp. 1–146, World Health Organization, Geneva, Switzerland
5. Cancelo-Hidalgo, M. J., Castelo-Branco, C., Palacios, S., Haya-Palazuelos, J., Ciriá-Rencasens, M., Manasanch, J., and Pérez-Edo, L. (2013) Tolerability of different oral iron supplements: a systematic review. Curr. Med. Res. Opin. 29, 291–305
6. Peña-Rosas Juan, P., De-Regil Luz, M., Dosswell, T., and Viteri Fernandez, E. (2012) Daily oral iron supplementation during pregnancy. In Cochrane Database of Systematic Reviews, John Wiley & Sons, Ltd., Chichester, UK
7. Seril, D. N., Liao, J., Ho, K. L., Warsi, A., Yang, C. S., and Yang, G. Y. (2002) Dietary iron supplementation enhances DSS-induced colitis and associated colorectal carcinoma development in mice. Dig. Dis. Sci. 47, 1296–1278
8. Radulescu, S., Brookes, M. J., Salgueiro, P., Ridgway, R. A., McGhee, E., Anderson, K., Ford, S. J., Stones, D. H., Iqbal, T. H., Tselpis, C., and Sansom, O. J. (2012) Luminal iron levels govern intestinal tumorigenesis after aper loss in vivo. Cell Rep. 2, 270–282
9. Coplin, M., Schuette, S., Leichtmann, G., and Lashner, B. (1991) Tolerability of iron: a comparison of bis-glycino iron II and ferrous sulfate. Clin. Ther. 13, 606–612
10. Dostal, A., Chassard, C., Hilty, F. M., Zimmermann, M. B., Jaeggi, T., Rossi, S., and Lacroix, C. (2012) Iron depletion and repletion with ferrous sulfate or electrolytic iron modifies the composition and metabolic activity of the gut microbiota in rats. J. Nutr. 142, 271–277
11. Zimmermann, M. B., Chassard, C., Rohner, F., N’Goran, E., K., Nindjin, C., Dostal, A., Uztung, J., Ghattas, H., Lacroix, C., and Hurrell, R. F. (2010) The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d’Ivoire. Am. J. Clin. Nutr. 92, 1406–1415
12. Werner, T., Wagner, S. J., Martinez, I., Walter, J., Chang, J. S., Clasen, T., Kising, S., Schuemann, K., and Haller, D. (2011) Depletion of luminal iron alters the gut microbiota and prevents Crohn’s disease-like ileitis. Gut 60, 325–333
13. Kortman, G. A., Boleij, A., Swinkels, D. W., and Tjabana, H. (2012) Iron availability increases the pathogenic potential of Salmonella typhimurium and other enteric pathogens at the intestinal epithelial interface. PLoS One 7, e29722
14. Powell, J. J., Bruggaber, S. F., Faria, N., Poots, L. K., Hondow, N., Pennycook, T. J., Latunde-Dada, G. O., Simpson, R. J., Brown, A. P., and Pereira, D. I. (2014) A nano-disperse ferritin-iron core mimetic that efficiently corrects anemia without luminal iron redox activity. [Epub ahead of print] Nanomedicine doi: 10.1016/j.nano.2013.12.011
15. Pan, Y. H., Sader, K., Powell, J. J., Blech, A., Gass, M., Trinick, J., Warner, A., Li, A., Readson, R., and Brown, A. (2009) 3D morphology of the human hepatic ferritin mineral core: new evidence for a subunit structure revealed by single particle analysis of HAADF-STEM images. J. Struct. Biol. 166, 22–31
16. Toshia, T., Behera, R. K., Ng, H. L., Bhattasali, O., Alber, T., and Theil, E. C. (2012) Ferritin protein nanoparticle iron channels: gating by N-terminal extensions. J. Biol. Chem. 287, 13016–13025
17. Patzelt, D. I., Merger, B. L., Faria, N., Bruggaber, S. F., Aslam, M. F., Poots, L. K., Prassmayer, L., Lonnerdal, B., Brown, A. P., and Powell, J. J. (2013) Caco-2 cell acquisition of dietary iron(III) invokes a nanoparticulate endocytic pathway. PLoS One 8, e81250
18. Doorey, C., Cooper, C., Dickson, D. P., Gibson, J. F., Simpson, R. J., and Peters, T. J. (1995) Iron speziatiol in media containing ascorbate and oxygen. Br. J. Nutr. 70, 157–169
19. Simpson, R. J., and Peters, T. J. (1990) Forms of soluble iron in mouse stomach and duodenal lumen: significance for mucosal uptake. Br. J. Nutr. 63, 79–89
20. Miret, S., Simpson, R. J., and McKie, A. T. (2003) Physiology and molecular biology of dietary iron absorption. Annu. Rev. Nutr. 23, 283–301
21. McKie, A. T., Marciani, P., Rolfs, A., Brennan, K., Wehr, K., Barrow, D., Miret, S., Bomford, A., Peters, T. J., and Farzaneh, A. (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol. Cell 5, 299–309
22. McKie, A. T. (2008) The role of Dbyer in iron metabolism: an update. Biochem. Soc. Trans. 36, 1239–1241
23. Ohgami, R. S., Campagna, D. R., McDonald, A., and Fleming, M. D. (2006) The Steap proteins are metalloreductases. Blood 108, 1388–1394
24. Gunshin, H., Fujisawa, Y., Custudio, A. O., Direnzio, C., Robine, S., and Andrews, N. C. (2005) Slc11a2 is required for intestinal iron absorption and erythropoesis but dispensable in placenta and liver. J. Clin. Invest. 115, 1258–1266
25. Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., Boron, W. F., Nussberger, S., Gollan, J. L., and Hediger, M. F.
26. Theil, E. C. (2004) Iron, ferritin, and nutrition. Annu. Rev. Nutr. 24, 327–343
27. Donovan, A., Brownlie, A., Zhou, Y., Shepard, J., Pratt, S. J., Moynihan, J., Paw, B. H., Dryer, A., Barut, B., Zapata, A., Law, T. C., Brugnara, C., Kingsley, P. D., Palis, J., Fleming, M. D., Andrews, N. C., and Zon, L. I. (2000) Positional cloning of zebrafish ferroporin1 identifies a conserved vertebrate iron exporter. Nature 403, 776–781
28. Abboud, S., and Haile, D. J. (2000) A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J. Biol. Chem. 275, 19906–19912
29. Nemeth, E., and Ganz, T. (2006) Regulation of iron metabolism by hepcidin. Annu. Rev. Nutr. 26, 323–342
30. Donovan, A., Lima, C. A., Pinkus, J. L., Pinkus, G. S., Zon, L. I., Robine, S., and Andrews, N. C. (2005) The iron exporter ferroporin/Sic40a1 is essential for iron homeostasis. Cell Metab. 1, 191–200
31. Ganz, T. (2003) Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 102, 783–788
32. Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, B. M., Donovan, A., Ward, D. M., Ganz, T., and Kaplan, J. (2004) Hepcidin regulates cellular iron efflux by binding to ferroporin and inducing its internalization. Science 306, 2090–2093
33. Ganz, T., and Nemeth, E. (2012) Hepcidin and iron homeostasis. Biochim. Biophys. Acta 1825, 1434–1443
34. Drakesmith, H., and Prentice, A. M. (2012) Hepcidin and the iron-infection axis. Science 338, 768–772
35. Bregman, D. B., Morris, D., Koch, T. A., He, A., and Goodnough, L. T. (2013) Hepcidin levels predict nonresponsiveness to oral iron therapy in patients with iron deficiency anemia. Am. J. Hematol. 88, 97–101
36. Prentice, A. M., Doherty, G. P., Abrams, S. A., Cox, S. E., Atkinson, S. H., Verhoef, H., Armitage, A. E., and Drakesmith, H. (2012) Hepcidin is the major predictor of erythrocyte iron incorporation in anemic African children. Blood 119, 1922–1928
37. Reeves, P. G., Nielsen, F. H., and Fahey, G. C., Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123, 1939–1951
38. Powell, J. J., McNaughton, S. A., Jugdaohsingh, R., Anderson, S. H., Dear, J., Khot, F., Mowatt, L., Gleason, K. L., Sykes, M., Thompson, R. P., Bolton-Smith, C., and Hodson, M. J. (2005) A provisional database for the silicon content of foods in the United Kingdom. Br. J. Nutr. 94, 804–812
39. Frazer, D., Wilkins, S., Becker, E., Murphy, T., Vulpe, C., McKie, A., and Anderson, G. (2005) A rapid decrease in the expression of DMT1 and DcytB but not Ireg1 or hephaestin explains the mucosal block phenomenon of iron absorption. Gut 52, 340–346
40. Bunting, H. (1949) The histochemical detection of iron in tissues. Biotech. Histochem. 24, 109–115
41. Rebouché, C. J., Wilcox, C. L., and Widness, J. A. (2004) Microanalysis of non-ferrous iron in animal tissues. J. Biochem. Biophys. Meth. 58, 293–295
42. Darshan, D., Wilkins, S. J., Frazer, D. M., and Anderson, G. J. (2011) Reduced expression of ferroporin-1 mediates hyporesponsiveness of suckling rats to stimuli that reduce iron absorption. Gastroenterology 141, 300–309
43. Frazer, D. M., Wilkins, S. J., Becker, E. M., Vulpe, C. D., McKie, A. T., Trinder, D., and Anderson, G. J. (2002) Hepcidin expression inversely correlates with the expression of duodenal iron transporters and iron absorption in rats. Gastroenterology 123, 835–844
44. Barrand, M. A., Hider, R. C., and Callingham, B. A. (1990) The importance of reductive mechanisms for intestinal uptake of iron from ferric molal and ferric nitrolitriacetic acid (NTA). J. Pharm. Pharmacol. 42, 279–282
45. Zimmermann, M. B., Biebinger, R., Egli, I., Zeder, C., and Hurrell, R. F. (2011) Iron deficiency up-regulates iron absorption from ferrous sulphate but not ferric pyrophosphate and consequently food fortification with ferrous sulphate has relatively greater efficacy in iron-deficient individuals. Br. J. Nutr. 105, 1245–1250
46. Wollenberg, P., and Rummel, W. (1987) Dependence of intestinal iron absorption on the valency state of iron. Naunyn Schmiedeberg’s Arch. Pharmacol. 336, 578–582
47. Antileo, E., Garri, C., Tapia, V., Munoz, J. P., Chiong, M., Nualart, F., Lavandero, S., Fernandez, J., and Nunez, M. T. (2013) Endocytic pathway of exogenous iron-loaded ferritin in intestinal epithelial (Caco-2) cells. Am. J. Physiol. Gastrointest. Liver. Physiol. 304, G655–G661
48. Kalganovar, S., and Lonnerdal, B. (2009) Receptor-mediated uptake of ferritin-bound iron by human intestinal Caco-2 cells. J. Nutr. Biochem. 20, 304–311
49. San Martin, C. D., Garri, C., Pizarro, F., Walter, T., Theil, E. C., and Nunez, M. T. (2008) Caco-2 intestinal epithelial cells absorb soybean ferritin by mu2 (AP2)-dependent endocytosis. J. Nutr. 138, 659–666
50. Theil, E. C., Chen, H., Miranda, C., Janser, H., Eslenhans, B., Núñez, M. T., Pizarro, F., and Schümann, K. (2012) Absorption of iron from ferritin is independent of heme iron and ferrous salts in women and rat intestinal segments. J. Nutr. 142, 478–483
51. LaVaque, T., Smith, S., Cooperman, S., Iwai, K., Land, W., Meyron-Holtz, E., Drake, S. K., Miller, G., Abu-Asab, M., and Tsokos, M. (2001) Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. Nat. Genet. 27, 209–214
52. Chen, H., Su, T., Attieh, Z. K., Fox, T. C., McKie, A. T., Anderson, G. J., and Vulpe, C. D. (2003) Systemic regulation of Hephaestin and Ireg1 revealed in studies of genetic and nutritional iron deficiency. Blood 102, 1893–1899
53. Dresow, B., Petersen, D., Fischer, R., and Nielsen, P. (2008) Non-transferrin-bound iron in plasma following administration of oral iron drugs. Biometals 21, 273–276
54. Hurrell, R. F. (2011) Safety and efficacy of iron supplements in malaria-endemic areas. Ann. Nutr. Metab. 59, 64–66
55. Lomer, M. C., Cook, W. B., Jan-Mohamed, H. J., Hutchinson, C., Liu, D. Y., Hider, R. C., and Powell, J. J. (2012) Iron requirements based upon iron absorption tests are poorly predicted by haematological indices in patients with inactive inflammatory bowel disease. Br. J. Nutr. 107, 1806–1811
56. Schumann, K., Solomons, N. W., Romero-Abal, M. E., Orozco, M., Weiss, G., and Marx, J. (2012) Oral administration of ferrous sulfate, but not of iron polynaleate or sodium iron ethylenediaminetetraacetic acid (NaFeEDTA), results in a substantial increase of non-transferrin-bound iron in healthy iron-deficient men. Food. Nutr. Bull. 33, 128–136
57. Pollegioni, L., Bruggaber, S., Faria, N., and Pereira, D., inventors (2008, August 14) Ligand modified poly oxo-hydroxy metal ion materials, their uses and processes for their preparation. UK patent WO/2008/096130

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