

revised January 11, 2001



Overview

Despite the fact that iron is the second most abundant metal in the earth's crust, iron deficiency is the world's most common cause of anemia. When it comes to life, iron is more precious than gold. The body hoards the element so effectively that over millions of years of evolution, humans have developed no physiological means of iron excretion. Iron absorption is the sole mechanism by which iron stores are physiologically manipulated.

The average adult stores about 1 to 3 grams of iron in his or her body. An exquisite balance between dietary uptake and loss maintains this balance. About 1 mg of iron is lost each day through sloughing of cells from skin and mucosal surfaces, including the lining of the gastrointestinal tract (Cook et al., 1986). Menstruation increases the average daily iron loss to about 2 mg per day in premenopausal female adults (Bothwell and Charlton, 1982). No physiologic mechanism of iron excretion exists. Consequently, absorption alone regulates body iron stores (McCance and Widdowson, 1938). The augmentation of body mass during neonatal and childhood growth spurts transiently boosts iron requirements (Gibson et al., 1988).

Iron Absorption

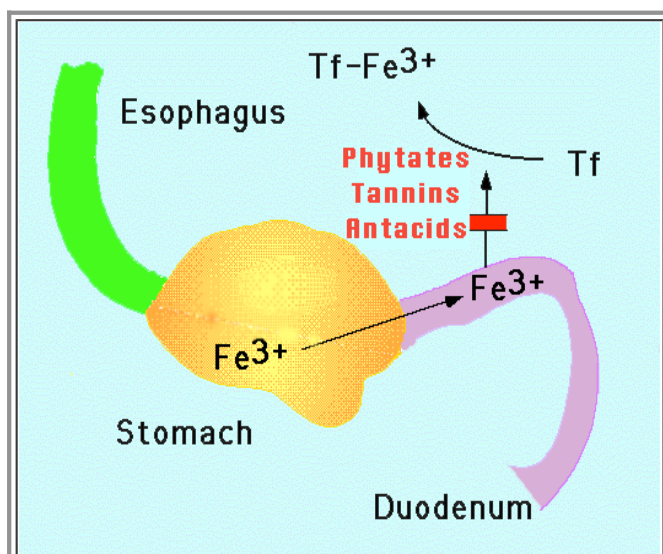


Figure 1. Iron absorption. Iron enters the stomach from the esophagus. Iron is oxidized to the Fe^{3+} state no matter its original form when taken in orally. Gastric acidity as well as solubilizing agents such as ascorbate prevent precipitation of the normally insoluble Fe^{3+} . Intestinal mucosal cells in the duodenum and upper jejunum absorb the iron.

Iron absorption occurs predominantly in the duodenum and upper jejunum (Muir and Hopfer, 1985) (Figure 1). The mechanism of iron transport from the gut into the blood stream remains a mystery despite intensive investigation and a few tantalizing hits (see below). A feedback mechanism exists that enhances iron absorption in people who are iron deficient. In contrast, people with iron overload dampen iron absorption.

The physical state of iron entering the duodenum greatly influences its absorption however. At physiological pH, ferrous iron (Fe^{2+}) is rapidly oxidized to the insoluble ferric iron (Fe^{3+}) form. Gastric acid lowers the pH in the proximal duodenum, enhancing the solubility and uptake of ferric iron (Table 1). When gastric acid production is impaired (for instance by acid pump inhibitors such as the drug, prilosec), iron absorption is reduced substantially.

Heme is absorbed by machinery completely different to that of inorganic iron. The process is more efficient and is independent of duodenal pH. Consequently meats are excellent nutrient sources of iron. In fact, blockade of heme catabolism in the intestine by a heme oxygenase inhibitor

The iron is coupled to transferrin (Tf) in the circulation which delivers it to the cells of the body. Phytates, tannins and antacids block iron absorption.

can produce iron deficiency (Kappas et al., 1993). The paucity of meats in the diets of many of the people in the world adds to the burden of iron deficiency.

A number of dietary factors influence iron absorption. Ascorbate and citrate increase iron uptake in part by acting as weak chelators to help to solubilize the metal in the duodenum (Table 1) (Conrad and Umbreit, 1993). Iron is readily transferred from these compounds into the mucosal lining cells. Conversely, iron absorption is inhibited by plant phytates and tannins. These compounds also chelate iron, but prevent its uptake by the absorption machinery (see below). Phytates are prominent in wheat and some other cereals, while tannins are prevalent in (non-herbal) teas.

Lead is a particularly pernicious element to iron metabolism (Goya, 1993). Lead is taken up by the iron absorption machinery, and secondarily blocks iron through competitive inhibition. Further, lead interferes with a number of important iron-dependent metabolic steps such as heme biosynthesis. This multifaceted attack has particularly dire consequences in children, where lead not only produces anemia, but can impair cognitive development. Lead exists naturally at high levels in ground water and soil in some regions, and can clandestinely attack children's health. For this reason, most pediatricians in the U.S. routinely test for lead at an early age through a simple blood test.

Immaturity of the gastrointestinal tract can exacerbate iron deficiency in newborns. The gastrointestinal tract does not achieve competency for iron absorption for several weeks after birth. The problem is even more severe for premature infants, who tend to be anemic for a variety of reasons. A substantial portion of iron stores in newborns are transferred from the mother late in pregnancy. Prematurity shortcircuits this process. Parenteral iron replacement is possible, but not often used because of the often delicate health of premature infants. Transfusion becomes the default option in this circumstance.

Table 1. Factors That Influence Iron Absorption

Physical State (bioavailability)	heme > Fe ²⁺ > Fe ³⁺
Inhibitors	phytates, tannins, soil clay, laundry starch, iron overload, antacids
Competitors	lead, cobalt, strontium, manganese, zinc
Facilitators	ascorbate, citrate, amino acids, iron deficiency

mechanism by which iron enters the mucosal cells lining the upper gastrointestinal tract is unknown. Most cells in the rest of the body are believed to acquire iron from plasma transferrin (an iron-protein chelate), via specific transferrin receptors and receptor-mediated endocytosis (Klausner, et al, 1983). The hypothesis that apotransferrin (or an equivalent molecule) secreted by intestinal cells or present in bile chelates intestinal iron and facilitates its absorption (Huebers et al., 1983) is unsubstantiated. The transferrin gene is not expressed in intestinal cells. Later work indicated that transferrin found in the intestinal lumen is derived from plasma (Idzerda et al., 1986). Plasma transferrin entering bile is fully saturated with iron, obviating any intraluminal chelating function (Schumann et al., 1986). Furthermore, hypoxia, which greatly increases iron absorption, has no effect on intestinal transferrin levels (Simpson et al., 1986). Exogenous transferrin cannot donate iron to intestinal mucosal cells (Bezвода et al., 1986), and the brush boarder membrane lacks transferrin receptors (Parmley et al., 1985) (although they are present on the basolateral surface of intestinal epithelial cells (Levin et al., 1984); (Banerjee et al., 1986). Lastly and perhaps most compellingly, humans and mice with hypotransferrinemia paradoxically absorb more dietary iron than normal. Although the erythron is iron deficient, these individuals develop hepatic iron overload (Heilmeyer et al., 1961); (Craven et al., 1987).

Y

Mechanism of Iron Absorption

In searching for molecules involved in intestinal iron transport, Conrad and co-workers took the approach of characterizing proteins that bind iron [summarized in (Conrad and Umbreit, 1993)]. Their hypothesis of iron transport is based on identification of iron binding proteins at several key sites. They propose that mucins bind iron in the acid environment of the stomach, thereby maintaining it in solution for later uptake in the alkaline duodenum. According to their model, mucin-bound iron subsequently crosses the mucosal cell membrane in association with integrins. Once inside the cell, a cytoplasmic iron-binding protein, dubbed "mobilferrin", accepts the element, and shuttles it to the basolateral surface of the cell, where it is delivered to plasma. In this model mobilferrin could serve as a rheostat sensitive to plasma iron concentrations. Fully occupied mobilferrin would dampen mucosal iron uptake, and while the process would be enhanced by unsaturated mobilferrin (Conrad and Umbreit, 1993). This model has not gained universal acceptance however.

A very different scheme of iron uptake has been proposed by investigators studying iron transport in yeast. Yeast face the problem of taking in iron from the environment, a process similar to that of intestinal mucosal cells. Dancis et al. used genetic selection to isolate *Saccharomyces cerevisiae* mutants with defective iron transport (Dancis et al., 1994); (Stearman et al., 1996). They constructed an expression plasmid in which an enzyme necessary for histidine biosynthesis was under the control of an iron-repressible promoter. The plasmid was introduced into a yeast histidine auxotroph (i.e. a strain of yeast that requires histidine to survive). Mutants were selected in the absence of histidine, in the presence of high levels of iron. Among the mutants they isolated, were cells with defective iron uptake. They discovered that membrane iron transport depends absolutely upon copper transport. In this model, ferric iron in yeast culture medium is reduced to its ferrous form by an externally oriented reductase (FRE1). The element is shuttled rapidly into the cell by a ferrous transporter, which appears to be coupled to an externally oriented copper-dependent oxidase (FET3) embedded in the cell membrane (De Silva et al., 1995); (Stearman et al., 1996). FET3 is strikingly homologous to the mammalian copper oxidase ceruloplasmin. The re-oxidation of ferrous to ferric iron is apparently an obligatory step in the transport mechanism, although the coupling mechanism of oxidation and membrane transport is unclear. (De Silva et al., 1995); (Stearman et al., 1996); (Yuan et al., 1995). Although the genetic evidence for this scheme is compelling, the central component, the ferrous transporter itself, remains elusive. These investigators speculate that mammalian intestinal iron transport is analogous to the yeast iron uptake process (Harford et al., 1994). This assertion is supported by studies of copper-deficient swine, which show co-existing iron deficiency unresponsive to iron therapy (Lahey et al., 1952); (Gubler et al., 1952); (Cartwright et al., 1956).

Y

Genetic Insights into Mammalian Iron Absorption

Mouse genetics provides a different perspective on mammalian intestinal iron transport. Mouse breeders readily recognize pale animals, and have developed anemic stocks with various mutations. Intestinal mucosal iron transport is defective in two mutant strains. Microcytic (*mk*) mice and sex-linked anemia (*sla*) mice have severe iron deficiency due apparently to defects in iron uptake and release, respectively, from the intestinal cell (reviewed in [Bannerman, 1976].) Mice with the homozygous autosomal recessive *mk* mutation absorb iron poorly, have low serum iron levels, and lack stainable iron in intestinal mucosal cells. These findings are consistent with a defect in an apical iron transport molecule. Intriguingly, *mk/mk* mice

are not rescued by parenteral iron replacement. Anemia develops in normal mice transplanted with *mk* bone marrow, indicating that *mk* erythroid precursor cells also have a defect in red cell iron uptake. A common component to iron transport may therefore exist in intestinal cells and red cell precursors (Andrews, et al, 2000).

✓ Mice that are homozygous or heterozygous for the *sla* mutation (*sla/sla* or *sla/y*) also have low serum iron levels. In contrast to *mk* mice, they have abnormal iron deposits within intestinal mucosal cells, suggesting that this X-linked defect impairs intracellular iron trafficking or basolateral export of iron to the plasma. The *sla* animals differ further from the *mk* mice by correction of anemia by parenteral iron. Based on studies of these mutants, distinct apical and basolateral iron transport systems possibly exist that function coordinately to transfer iron from intestinal lumen to plasma.

✓ Whatever the mechanism of iron uptake, normally only about 10% of the elemental iron entering the duodenum is absorbed. However, this value increases markedly with iron deficiency (Finch, 1994). In contrast, iron overload reduces but does not eliminate absorption, reaffirming the fact that absorption is regulated by body iron stores. In addition, both anemia and hypoxia boost iron absorption. A portion of the iron that enters the mucosal cells is retained sequestered within ferritin. Intracellular intestinal iron is lost when epithelial cells are sloughed from the lining of the gastrointestinal tract. The remaining iron traverses the mucosal cells, to be coupled to transferrin for transport through the circulation.

Erythropoiesis and Iron Absorption

✓ Approximately 80% of total body iron is ultimately incorporated into red cell hemoglobin. An average adult produces 2×10^{11} red cells daily, for a red cell renewal rate of 0.8 percent per day. Each red cell contains more than a billion atoms of iron, and each ml of red cells contains 1 mg of iron. To meet this daily need for 2×10^{20} atoms (or 20 mg) of elemental iron, the body has developed regulatory mechanisms whereby erythropoiesis profoundly influences iron absorption. Plasma iron turnover (PIT) represents the mass turnover of transferrin-bound iron in the circulation, expressed as mg/kg/day (Huff et al., 1950). Accelerated erythropoiesis increases plasma iron turnover, which is associated with enhanced iron uptake from the gastrointestinal tract (Weintraub et al., 1965). The mechanism by which PIT alters iron absorption is unknown.

✓ A circulating factor related to erythropoiesis that modulates iron absorption has been hypothesized, but not identified (Beutler and Bittenweiser, 1960); (Finch, 1994). Several candidate factors have been excluded, including transferrin (Aron et al., 1985) and erythropoietin (Raja et al., 1986). Clinical manifestations of this apparent communication between the marrow and the intestine includes iron overload that develops in patients with severe thalassemia in the absence of transfusion. The accelerated (but ineffective) erythropoiesis in this condition substantially boosts iron absorption. In some cases, the coupling of increased PIT and increased gastrointestinal iron absorption is beneficial. In pregnancy, placental removal of iron raises the PIT. This process enhances gastrointestinal iron absorption thereby increasing the availability of the element to meet the needs of the growing and developing fetus.

✓ Competition studies suggest that several other heavy metals share the iron intestinal absorption pathway. These include lead, manganese, cobalt and zinc (Table 1). Enhanced iron absorption induced by iron deficiency also augments the uptake of these elements. As iron deficiency often coexists with lead intoxication, this interaction can produce particularly serious medical complications in children (Piomelli et al., 1987). Interestingly, copper absorption and metabolism appear to be handled mechanisms different to

those of iron.

ÝÝÝ

References:

- Andrews NC. (2000). Intestinal iron absorption: current concepts circa 2000. *Dig Liver Dis* Jan-Feb;32(1):56-61.
- Banerjee, D., Flanagan, P. R., Cluett, J., and Valberg, L. S. (1986). Transferrin receptors in the human gastrointestinal tract. Relationship to body iron stores. *Gastroenterology* 91, 861.
- Bannerman, R. M. (1976). Genetic defects of iron transport. *Federation Proceedings* 35, 2281.
- Beutler, E., and Buttenweiser, E. (1960). The regulation of iron absorption. I. A search for humoral factors. *Journal of Laboratory and Clinical Medicine* 55, 274.
- Bezwoda, W. R., MacPhail, A. P., Bothwell, T. H., Baynes, R. D., Derman, D. P., and Torrance, J. D. (1986). Failure of transferrin to enhance iron absorption in achlorhydric human subjects. *Br. J. Haematol.* 63, 749.
- Bothwell, T. H., and Charlton, R. W. (1982). A general approach of the problems of iron deficiency and iron overload in the population at large. *Seminars in Hematology* 19, 54.
- Cartwright, G. E., Gubler, C. J., Bush, J. A., and Wintrobe, M. M. (1956). Studies on copper metabolism. XVII. Further observations on the anemia of copper deficiency in swine. *Blood* 11, 143.
- Conrad, M. E., and Umbreit, J. N. (1993). A concise review: Iron absorption - the mucin-mobilferrin-integrin pathway. A competitive pathway for metal absorption. *American Journal of Hematology* 42, 67.
- Cook, J. D., Skikne, B. S., and al., e. (1986). Estimates of iron sufficiency in the US population. *Blood* 68, 726.
- Craven, C. M., Alexander, J., Eldridge, M., Kushner, J. P., Bernstein, S., and Kaplan, J. (1987). Tissue distribution and clearance kinetics of non-transferrin-bound iron in the hypotransferrinemic mouse: a rodent model for hemochromatosis. *Proceedings of the National Academy of Sciences (USA)* 84, 3457.
- Dancis, A., Haile, D., Yuan, D.S., Klausner, R.D. (1994). The *Saccharomyces cerevisiae* copper transport protein (Ctr1p). Biochemical characterization, regulation by copper, and physiologic role in copper uptake. *J. Biol. Chem.* 269, 25660-7
- De Silva, D. M., Askwith, C. C., Eide, D., and Kaplan, J. (1995). The FET3 gene product required for high affinity iron transport in yeast is a cell surface ferroxidase. *Journal of Biological Chemistry* 270, 1098-101.
- Finch, C. (1994). Regulators of iron balance in humans. *Blood* 84, 1697.
- Gibson, R. S., MacDonald, A. C., and Smit-Vanderkooy, P. D. (1988). Serum ferritin and dietary iron parameters in a sample of Canadian preschool children. *J. Can. Dietetic Assoc.* 49, 23.
- Goyer, RA. (1993) Lead toxicity: current concerns. *Environ Health Perspect* 100: 177-187.
- Gubler, C. J., Lahey, M. E., Chase, M. S., Cartwright, G. E., and Wintrobe, M. M. (1952). Studies on copper metabolism. III. The metabolism of iron in copper deficient swine. *Blood* 7, 1075.
- Harford, J. B., Rouault, T. A., Huebers, H. A., and Klausner, R. D. (1994). Molecular mechanisms of iron metabolism. In *The Molecular Basis of Blood Diseases*, G. Stamatoyannopoulos, A. W. Nienhuis, P. W. Majerus and H. Varmus, eds. (Philadelphia: W.B. Saunders Co.), pp. 351-378.
- Heilmeyer, L., Keller, W., Vivell, O. Keiderling, W., Betke, K., Wohler, F., Schultze, H.E. (1961) Congenital transferrin deficiency in a seven-year old girl. *German Medical Monthly* 6, 385

- Huebers, H. A., Huebers, E., and al., e. (1983). The significance of transferrin for intestinal iron absorption. *Blood* 61, 283.
- Huff, R. L., Hennessey, T. G., Austin, R. E., Garcia, J. F., Roberts, B. M., and Lawrence, J. H. (1950). Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders. *Journal of Clinical Investigation* 29, 1041.
- Idzerda, R. L., Huebers, H., and al., e. (1986). Rat transferrin gene expression: tissue-specific regulation by iron deficiency. *Proceedings of the National Academy of Sciences (USA)* 83, 3723.
- Kappas, A., Drummond, G. S., and Galbraith, R. A. (1993). Prolonged clinical use of a heme oxygenase inhibitor: hematological evidence for an inducible but reversible iron-deficiency state. *Pediatrics* 91, 537-539.
- Klausner R.D., van Renswoude J., Ashwell G., Kempf, C, Schechter, AN, Dean, A, Bridges, KR. (1983) Receptor-mediated endocytosis of transferrin in K562 cells. *J. Biol. Chem.* 258: 4715 - 4724.
- Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E., and Wintrobe, M. M. (1952). Studies on copper metabolism. II. Hematologic manifestations of copper deficiency in swine. *Blood* 7, 1075.
- Levin, M. J., Tuil, D., and al., e. (1984). Expression of transferrin gene during development of non-hepatic tissues: High level of transferrin mRNA in fetal muscle and adult brain. *Biochem. Biophys. Res. Commun.* 122, 212.
- McCance, R. A., and Widdowson, E. M. (1938). The absorption and excretion of iron following oral and intravenous administration. *J. Phys.* 94, 148.
- Muir, A., and Hopfer, U. (1985). Regional specificity of iron uptake by small intestinal brush-boarder membranes from normal and iron deficient mice. *Gastrointestinal and Liver Pathology* 11, 6376-6379.
- Parmley, R. T., Barton, J. C., and Conrad, M. E. (1985). Ultrastructural localization of transferrin, transferrin receptor and iron-binding sites on human placental and duodenal microvilli. *Br. J. Haematol.* 60, 81.
- Piomelli, S., Seaman, C., and Kapoor, S. (1987). Lead-induced abnormalities of porphyrin metabolism. The relationship with iron deficiency. *Ann. NY Acad. Sci.* 514, 278.
- Raja, K. N., Pippard, M. J., Simpson, R. J., and Peters, T. J. (1986). Relationship between erythropoiesis and the enhanced intestinal uptake of ferric iron in hypoxia in the mouse. *British Journal of Haematology* 64, 587.
- Schumann, K., Schafer, S. G., and Forth, W. (1986). Iron absorption and biliary excretion of transferrin in rats. *Res. Exp. Med.* 186, 215.
- Simpson, R. J., Osterloh, K. R. S., Raja, K. B., Snape, S. D., and Peters, T. J. (1986). Studies on the role of transferrin and endocytosis on the uptake of Fe³⁺ from Fe-nitriloacetate by mouse duodenum. *Biochim. Biophys. Acta* 884, 166.
- Stearman, R., Yuan, D. S., Yamaguchi-Iwai, Y., Klausner, R. D., and Dancis, A. (1996). A permease-oxidase complex involved in high-affinity iron uptake in yeast. *Science* 271, 1552-1557.
- Weintraub, L. R., Conrad, M. E., and Crosby, W. H. (1965). Regulation of the intestinal absorption of iron by the rate of erythropoiesis. *British Journal of Hematology* 2, 432.
- Yuan, D. S., Stearman, R., Dancis, A., Dunn, T., Beeler, T., and Klausner, R. D. (1995). The Menkes/Wilson disease gene homologue in yeast provides copper to a ceruloplasmin-like oxidase required for iron uptake. *Proceedings of the National Academy of Sciences (USA)* 92, 2632-2636.

