Iron Homeostasis and Disorders in Dogs and Cats: A Review

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ABSTRACT

Iron is an essential element for nearly all living organisms and disruption of iron homeostasis can lead to a number of clinical manifestations. Iron is used in the formation of both hemoglobin and myoglobin, as well as numerous enzyme systems of the body. Disorders of iron in the body include iron deficiency anemia, anemia of inflammatory disease, and iron overload. This article reviews normal iron metabolism, disease syndromes of iron imbalance, diagnostic testing, and treatment of either iron deficiency or excess. Recent advances in diagnosing iron deficiency using reticulocyte indices are reviewed. (J Am Anim Hosp Assoc 2011; 47:151–160. DOI 10.5326/JAAHA-MS-5553)

Introduction

Iron is an essential element for almost all living organisms. A number of physiologic mechanisms have evolved to help animals regulate iron levels and iron distribution within the body. Disturbances in iron intake, loss, or regulation can lead to a number of clinical abnormalities, some of which may be severe or even life-threatening. Iron homeostasis is a complex process about which there is still much to be learned; however, several discoveries over the past decade have greatly improved veterinarians’ understanding of iron regulation in the body. Advances have also been made in the ability to detect situations of iron imbalance. The purpose of this article is to review the metabolism of iron, clinical manifestations of iron imbalance, diagnostic tests available to assess iron status, and treatment of either iron deficiency or excess.

Iron Metabolism

Physiologic Role of Iron

Iron is an integral component of a diverse array of biochemical pathways, including hemoglobin and myoglobin formation, neurotransmitter and myelin production, collagen formation, immune system function, energy metabolism, DNA and RNA synthesis, and many enzyme systems (e.g., catalases, cytochromes).1,2 As an example of the importance of iron in the cellular processes of the body, almost half of the enzymes of the tricarboxylic acid cycle contain iron or require iron as a cofactor.3 A small amount of iron is needed by all cells, but the vast majority of iron is used by erythroid precursors in the production of hemoglobin. Therefore, abnormalities in iron homeostasis resulting in decreased iron typically have anemia as a prominent feature.

Iron Distribution

Animals have approximately 9–22 mg of iron per pound body weight, with about 1 mg of iron contained in 2 mL of blood.4,5 In humans with normal iron balance, a total of about 1–2 mg of iron enters and leaves the body each day through the gastrointestinal tract.6 It is useful to conceptualize iron in the body as being divided into three pools: transport; functional; and storage. In circulation, iron is bound to the plasma protein transferrin. This transport pool represents ~1% of total body iron. The functional pool consists of iron in hemoglobin, myoglobin, and enzymes that require iron as a cofactor. The storage pool consists of iron that is stored in the liver, spleen, and bone marrow.7

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More than two-thirds of the body’s iron exists in hemoglobin in mature red blood cells (RBCs) and erythroid precursors. An additional 10–15% is found in either myoglobin in muscle or enzymes and cytochromes in other tissues. The remaining iron in the body is stored primarily in hepatocytes and macrophages of the mononuclear phagocyte system.

Iron Chemistry
Much of the biologic importance of iron lies in its chemistry. Iron exists in both ferrous (Fe²⁺) and ferric (Fe³⁺) oxidation states that can either donate or accept electrons, respectively. As such, iron can participate in oxidation-reduction reactions, which are essential for its biologic functions. These redox reactions can generate reactive oxygen species (ROS), such as hydroxyl and superoxide radicals, which have the potential to cause significant damage to cellular components. ROS can: cause DNA damage; impair the synthesis of proteins, lipids, and carbohydrates; induce proteases; and alter cell proliferation. Free iron, either ferric or ferrous, can also react directly with unsaturated fatty acids. This can induce lipid peroxidation, leading to impaired cellular integrity and cell death. Due to these potential toxic effects, regulation of cellular and systemic iron must be tightly controlled.

Iron Absorption
In a healthy animal, iron enters the body exclusively through the diet. There is no physiologic pathway for excretion of excess iron; therefore, regulation of the amount of iron absorbed from the diet plays a key role in maintenance of iron balance. Foods considered to be high in iron (i.e., >50 parts per million [ppm]) include organ meats (such as heart and liver), brewer’s yeast, wheat germ, egg yolks, oysters, and dried beans and fruits. Foods considered to be intermediate in iron content (10–50 ppm) include muscle meats, fish and fowl, most green vegetables, and most cereals. Foods considered to be low in iron (<10 ppm) include milk, milk products, and nongreen vegetables. In addition to the iron present in animal source ingredients, most commercial pet foods are supplemented with iron. Some commonly used sources are steamed bone meal (2.8% iron), dicalcium phosphate (1.4% iron), and ferrous sulfate heptahydrate (21.8% iron). There is very little information on the iron requirements of adult dogs, which can be quite variable due to source of iron, bioavailability, etc. The recommended allowance has been suggested as 0.36–0.5 mg/kg/day, depending on bioavailability. There are no data available on iron requirements or recommended allowances in adult cats. As such, a diet containing sufficient iron for growth is considered adequate (i.e., 1.25 mg iron/kg/day).

Dietary iron, present as both heme and nonheme iron, is noncompetitively absorbed at the apical surface of duodenal enterocytes. Heme iron enters duodenal enterocytes through a membrane protein called heme carrier protein 1. Once inside the cell, heme is degraded by intracellular heme oxygenase to release Fe²⁺, which enters the nonheme iron pool.

Nonheme iron is present in the diet primarily in the Fe³⁺ form. Fe³⁺ is insoluble at pH levels >3 whereas Fe²⁺ remains soluble even at a pH of 7 and can thus be efficiently absorbed by duodenal enterocytes. Reduction of Fe³⁺ to Fe²⁺ is accomplished by the action of cytochrome reductase b, which is highly expressed in the brush border of duodenal enterocytes. Fe²⁺ is transported into the cell through divalent metal transporter 1 (DMT1). Intestinal DMT1 requires protons for cotransport, which are provided by gastric acid entering the proximal duodenum. DMT1 is not specific for iron transport and also serves as a significant transport mechanism for other divalent metal cations, including manganese, copper, cobalt, zinc, cadmium, and lead.

Fe²⁺ in the duodenal enterocyte can either be stored as ferritin (a water soluble protein for iron storage which can hold up to 4,500 iron atoms) or transported out of the cell to reach the plasma. As the duodenal enterocyte completes its lifecycle, iron stored as ferritin is lost when the senescent cell is sloughed. This is an important mechanism of passive iron loss that helps in regulating the amount of iron that enters systemic circulation. Iron destined for exportation is transported across the basolateral surface of the duodenal enterocytes via the transporter ferroportin. Results of studies in mice have shown that ferroportin requires the accessory protein hephaestin, a multicopper iron oxidase to export iron from the duodenal enterocytes. Hephastin oxidizes Fe²⁺ released by ferroportin after it is exported from the duodenal enterocyte to the Fe³⁺ form, allowing iron to be taken up by transferrin in the circulation.

Iron Transport
Transferrin binds Fe³⁺ in circulation, keeping it soluble in an aqueous environment and delivering it to tissues. Transferrin exists in one of three forms: iron-free (apotransferrin); bound to one iron molecule (monoferric transferrin); or bound to two iron molecules (diferric transferrin). Under normal circumstances, the transferrin in circulation is 20–60% saturated with iron (i.e., in mono- or diferric forms). Mammalian apotransferrin is an 80 kDa glycoprotein with two similar iron-binding lobes that is produced and secreted primarily by hepatocytes and, to a lesser extent, by Sertoli cells and several cell types in the brain.

Iron Uptake
Transferrin provides a way to keep iron nonreactive in the circulation and extravascular fluid while delivering it to tissues that...
express transferrin receptors. Transferrin receptor 1 (TFR1) is expressed on all cells, but differs markedly in the level of expression.\textsuperscript{13,14} TFR1 is expressed in highest amounts on erythroid precursors, rapidly dividing cells, activated lymphocytes, and hepatocytes.\textsuperscript{7} In contrast, expression of TFR1 is low on non-proliferating cells. TFR1 selectively binds to circulating diferric transferrin, which initiates a pathway of receptor-mediated endocytosis. The specialized endosomal membrane formed by endocytosis contains DMT1 and a proton pump. Hydrogen ions (H\textsuperscript{+}) are pumped into the endosome, decreasing pH inside the endosome. This change in pH induces a conformational change in both the membrane-bound transferrin and TFR1, resulting in the release of iron from transferrin. Released Fe\textsuperscript{3+} is reduced to Fe\textsuperscript{2+} in the endosome by a ferrireductase. Fe\textsuperscript{2+} is subsequently transported out of the endosome into the cytoplasm by DMT1. Apotransferrin and TFR1 are recycled back to the cell surface to be reused in subsequent cycles of iron uptake.

Under normal circumstances, transferrin-TFR1 binding is the predominant mechanism for iron uptake by cells. Iron needed for metabolic processes moves into mitochondria through a poorly understood process for incorporation into heme and iron-sulfur clusters, which act as enzyme cofactors.

Iron Storage
Cellular iron in excess of immediate metabolic need is stored primarily in the cytoplasm of hepatocytes as an iron oxide in the central cavity of ferritin.\textsuperscript{11} Ferritin is a ubiquitous, water soluble protein for iron storage of a retrievable source.\textsuperscript{7,11} It is a cage-like heteropolymer of 24 subunits of H- (heavy or heart) and L- (light or liver) types.\textsuperscript{7} These subunits form a protein shell that can hold up to 4,500 atoms of iron.\textsuperscript{7} H-ferritin has ferrooxidase activity, which is important for movement of iron into the solid-state core of the protein.\textsuperscript{7} Iron enters as ferrous ion, is oxidized to the ferric form, and then hydrolyzed and polymerized to a ferric oxyhydroxide polymer.\textsuperscript{7} Iron is stored in the ferric form to keep it nonreactive and prevent formation of ROS. Ferritin is also the precursor to hemosiderin, an insoluble, nonretrievable aggregate of nonreactive iron in the cytoplasm. Under normal circumstances, 95\% of stored iron in the liver is found in hepatocytes as ferritin. The remaining 5\% of iron is found predominately in Kupffer cells as hemosiderin.\textsuperscript{11}

Systemic Iron Regulation
Iron homeostasis involves tight regulation of intestinal absorption, utilization of iron for erythropoiesis, recycling of iron from senescent erythrocytes, and storage of iron by hepatocytes and macrophages. Several regulators for controlling iron absorption and mobilization from stores are described in the ensuing paragraph.

Intestinal iron absorption is influenced by the amount of iron consumed in the diet. This mechanism is referred to as the “dietary regulator.”\textsuperscript{1,6} For instance, one study reports that duodenal enterocytes are resistant to absorbing iron for several days after a dietary iron bolus was administered to growing dogs.\textsuperscript{6} The “stores regulator” senses iron levels in circulation as a reflection of total body iron. This stores regulator probably acts at the level of the intestinal crypt cells, programming enterocytes for future iron absorption. The “erythropoietic regulator” adjusts iron absorption in response to erythropoietic demands. The erythropoietic regulator has a greater ability to increase iron absorption than the stores regulator, which is logical since the majority of iron in the body is used for erythropoiesis.\textsuperscript{6} A “humoral hypoxia regulator” induces an increase in iron absorption in the face of acute hypoxia. It is uncertain whether this regulatory pathway is distinct from the erythropoietic regulator or not. Lastly, an “inflammatory regulator” results in cellular iron retention in the setting of infection and/or inflammation. It is possible that this mechanism withholds iron from invading organisms.\textsuperscript{1,6}

The discovery of hepcidin was a major breakthrough in the search for specific effectors of iron homeostasis. Hepcidin, a 25 amino acid protein that is highly conserved across vertebrate species, is thought to be the primary regulator of iron homeostasis. Specifically, hepcidin is thought to: act as the major repressor of intestinal iron absorption and iron release from ferritin stores; and as a mediator for all other known regulators of iron.\textsuperscript{1} Hepcidin is produced by cleaving a larger precursor molecule. A canine hepcidin precursor has been identified with high homology to the human precursor.\textsuperscript{13,14} Hepcidin is primarily produced by the liver. A small amount of hepcidin is also produced by inflammatory monocytes and macrophages.\textsuperscript{1} Some research suggests that hepcidin may also be synthesized in the renal tubules.\textsuperscript{17} Because of its small size (roughly 2.8 kDa), hepcidin is probably freely filtered by the kidneys. There is no known pathway for reabsorption.\textsuperscript{18} This rapid excretion implies that regulation of serum hepcidin levels occurs predominately at the level of production.\textsuperscript{7}

Hepcidin binds to cell-surface ferroportin, triggering the internalization of ferroportin and subsequent degradation in lysosomes.\textsuperscript{7} Ferroportin is the only known cellular iron exporter in vertebrates and is found in all tissues where major iron flows are regulated (i.e., duodenal enterocytes, macrophages, and hepatocytes).\textsuperscript{15} By inducing the loss of ferroportin from cellular membranes, hepcidin inhibits the export of iron from these cells. This inhibition results in retention of absorbed dietary iron in enterocytes, iron scavenged from senescent RBCs in macrophages, and iron in hepatic stores. In addition to its effects on ferroportin, hepcidin also inhibits DMT1 expression. Inhibition of DMT1
prevents the uptake of iron into duodenal enterocytes at the apical membrane, as well as the release of iron from the endosomes formed by receptor-mediated endocytosis of the transferrin-transferrin receptor (Tfr) complex after transferrin-TFR1 mediated cellular uptake.19

The role of hepcidin in iron metabolism was first established in mouse models. Mice lacking the hepcidin gene (knockout mice) developed massive iron overload and iron deposition in the liver and pancreas.20,21 In contrast, mice that overexpressed hepcidin under the control of a liver-specific promoter developed severe iron deficiency anemia. Most of these mice died at birth.22 Further, injection of a single dose a synthetic hepcidin induced a profound hypoferremia in mice within just 1 hr, the effects of which lasted for up to 72 hr.22

Hepcidin expression is influenced by a number of factors. Those relevant to dogs and cats include iron overload, inflammation, hypoxia, and anemia. For example, hepcidin expression is increased in response to iron overload and inflammation. This response minimizes availability of iron, limiting iron burden and inhibiting the growth of pathogens. In contrast, hepcidin expression is decreased in response to hypoxia and anemia, making more iron available for erythropoiesis. Neither the factor that induces hepcidin in response to iron loading nor the factor that inhibits hepcidin in response to anemia or hypoxia have been identified to date.11,15

Clinical Manifestations of Iron Imbalance
Iron Deficiency Anemia
Iron deficiency can result from either inadequate intake, inadequate absorption of iron, or chronic external blood loss. Iron deficiency related to inadequate intake is rare in veterinary medicine. Occasionally, young, growing animals may become deficient if the quantity of iron needed for growth exceeds the supply available from diet and body stores. Nursing animals are most susceptible to this problem because milk is low in iron. It is very unlikely that iron deficiency would occur in postweaning animals fed balanced, commercial growth diets. Intestinal malabsorptive diseases, such as inflammatory bowel disease, reportedly can cause iron deficiency in dogs.23

In small animals, the major cause of iron deficiency is chronic external blood loss.12 The gastrointestinal tract is a common source of chronic blood loss. Potential causes include bleeding neoplasms (such as leiomyomas, leiomyosarcomas, or carcinomas), ulcerogenic drugs (e.g., glucocorticoids, nonsteroidal anti-inflammatory drugs, and salicylates), inflammatory bowel disease, and parasites. The quantity of blood lost secondary to gastrointestinal parasites, such as hookworms and whipworms, can be as high as 100 mL/day, depending on the actual worm burden.12 Ectoparasitism can also contribute to the development of an iron deficient state. Severe flea infestation can cause substantial blood loss, especially in puppies and kittens. For example, 100 fleas can consume approximately 1 mL of blood daily.24

In humans, iron deficiency anemia continues to be a major public health problem worldwide, with an estimated three billion people affected. Iron deficiency is defined as the reduction in total body iron to an extent that iron stores are fully exhausted and some degree of tissue iron deficiency is present.25 Iron-deficient erythropoiesis occurs when the serum iron concentration is low, with only mild anemia as a consequence. Clinically significant anemia due to iron deficiency occurs at a very late stage. Anemia due to iron deficiency is characterized by absent iron stores, low serum iron concentration, low transferrin saturation, and low hemoglobin and hematocrit values.12 Clinical manifestations of iron deficiency related to anemia include pallor, weakness, lethargy, poor exercise tolerance, tachypnea, and tachycardia. Iron deficiency usually develops slowly over weeks to months; therefore, patients often have time to adapt to their anemia and may have minimal clinical signs at rest. Because iron is preferentially used for heme synthesis, anemia is usually strongly regenerative until iron is severely deficient.

Although rarely seen in modern clinical practice, iron deficiency can also cause clinical manifestations unrelated to anemia. These include: secondary to impaired proliferation, growth, and function of nonerythroid tissues depleted of iron; reduced muscle activity; abnormal behavior; and skin and nail changes.6,12 Iron deficiency can have a direct effect on the central nervous system in children with development of measurable cognitive abnormalities.26 Pica, the craving to eat, chew, or lick non-nutritive objects such as rocks, dirt, and metal, is a classic manifestation of iron deficiency.

Anemia of Inflammatory Disease
Anemia of inflammatory disease, also called anemia of chronic disease, is the second most common type of anemia (in humans) after iron deficiency.27 Anemia of inflammatory disease is typically mild to moderate and can be found in association with infection, malignancy, organ failure, trauma, or other causes of inflammation.27 Several mechanisms underlie the development of anemia in these conditions, including changes in iron homeostasis, altered proliferation of erythroid progenitor cells and production of erythropoietin, and decreased RBC life span.27 Immune stimulation from infectious agents, neoplastic cells, or other sources results in activation of T cells and monocytes. These cells produce cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor-α...
(TNF-α), interleukin (IL)-1, IL-6, and IL-10 that are responsible for a variety of changes in the body's handling of iron.

Lipopolysaccharide (LPS) and IL-6 induce hepatic production of hepcidin, causing inactivation of ferroportin and down-regulation of DMT1 expression. As discussed previously, this results in inhibition of duodenal iron absorption and decreases iron release from stores in macrophages and hepatocytes.28 IFN-γ, LPS, and TNF-α up-regulate DMT1 expression on macrophages with a resultant increased uptake of iron into these cells. That is, LPS can affect DMT1 directly (upregulation of expression on macrophages) and indirectly (stimulates hepcidin expression which causes down-regulation of DMT1). These same factors down-regulate ferroportin expression, contributing to reduced iron release from macrophages.27 Additionally, IL-10 increases transferrin receptor expression, resulting in increased uptake of iron into cells by transferrin-receptor mediated endocytosis.29 TNF-α, IL-1, IL-6, and IL-10 also up-regulate ferritin expression, promoting intracellular storage and retention of iron.27 The combined effect of all of these changes is a relative iron deficiency in both the transport and functional pools, which limits availability of iron for erythropoiesis. It is theorized that this decrease in iron availability evolved as a defense mechanism against infection in an attempt to withhold iron from microbes.

There is also evidence that hepcidin itself has moderate antimicrobial properties in vitro.30 This antimicrobial activity is only apparent at very high concentrations that may not be achieved in tissues; however, plasma and tissue hepcidin concentrations have not been determined (to the authors’ knowledge). Hepcidin’s effects on iron hemostasis are demonstrable at 100-fold lower concentrations than those needed for antimicrobial activity.15

The anemia of inflammatory disease is nonregenerative. Alterations in iron homeostasis certainly lead to decreased iron availability for heme synthesis; however, there are additional factors that contribute to the development of an anemia with lack of a regenerative response. For example, IFN-α, -β, and –γ, TNF-α, and IL-1 inhibit proliferation and differentiation of erythroid precursors (most notably erythroid burst-forming units and erythroid colony-forming units).31 Underlying mechanisms of this inhibition may involve induction of apoptosis, down-regulation of erythropoietin receptor expression on progenitor cells, and reduced expression of other prohaematopoietic factors, such as stem cell factor.31-33 RBC lifespan may also be decreased and, in most cases, there is an inadequate erythropoietin (EPO) response for the degree of anemia. Inflammatory cytokines can cause a variety of abnormalities related to EPO, including altered binding affinity of EPO-inducing transcription factors and damage to EPO-producing cells (resulting in decreased levels of circulating EPO), interference with EPO signal transduction, and down-regulation of EPO receptors.34

### Iron Overload

Iron overload disorders are defined as either hemosiderosis (an asymptomatic increase in iron deposition in tissues) or hemochromatosis (characterized by organ dysfunction secondary to iron-induced injury).35 Iatrogenic overdose or accidental ingestion of iron-containing multivitamins or other medications can result in serious toxicity. Iron administered intravenously (IV) has the greatest potential for toxicity, followed by intramuscular injections and oral administration. Oral iron has the least potential for toxicity because the amount of iron absorbed is not 100% of the ingested dose.36 Nonetheless, large oral doses of iron can overcome the normal rate-limited absorption by saturating iron-binding proteins. Free iron ions can then be absorbed in a passive, concentration-dependent manner, similar to other metals.36 Additionally, there is evidence that, in cases of toxicosis, iron may be absorbed along all parts of the intestinal tract, rather than being limited to the duodenum and upper jejunum.36

The amount of elemental iron ingested must be determined to assess the potential toxicity after oral exposure. In dogs, ingesting <20 mg/kg of elemental iron does not result in clinical signs. Mild clinical signs typically develop in dogs ingesting 20–60 mg/kg of elemental iron and serious clinical signs develop in dogs ingesting >60 mg/kg of elemental iron. Oral ingestion of between 100 and 200 mg/kg of elemental iron is potentially lethal in animals.36 Initial signs of acute iron intoxication usually present as an acute onset of gastrointestinal irritation and distress, followed by peripheral vascular collapse (characterized by depression, weak and/or rapid pulse, hypotension, cyanosis, ataxia, and possibly coma). A third phase is exhibited by pulmonary edema, vasomotor collapse, pulmonary edema, fulminant hepatic failure, as well as cyanosis, coma, and death. Animals that survive acute iron intoxication may exhibit long-term complications, including gastric fibrosis and contraction.37

In addition to acute intoxication, hepatic cirrhosis resembling hemochromatosis has been induced in dogs receiving chronic, large doses of parenteral iron (i.e., up to 5.8 g/kg IV iron dextran over 10 mo).38 Liver failure secondary to iron overload has been described in basenjis with pyruvate kinase deficiency.39 There is one case report of transfusional hemochromatosis in a miniature schnauzer who was given a whole RBC transfusions every 6–8 wk for 3 yr to treat pure red cell aplasia.35 The iron chelator, deferoxamine, administered as a constant rate infusion of 15 mg/kg/hr
should be considered in animals either at risk or actively displaying signs of severe iron toxicosis.\(^4^0\)

**Diagnostic Testing**

**Direct Tests**

There are several serum analytes that can be used to help assess iron status in the body. Serum iron concentrations are not only decreased in iron deficiency, but also with inflammatory reactions, hypoproteinemia, hypothyroidism, renal disease, and chronic inflammatory states.\(^4^1\) Serum iron levels can also be affected by hemolysis, iron supplementation, recent transfusions, time of day, corticosteroid administration, and consumption of meat.\(^4^2,4^3\)

Therefore, serum iron concentrations should not be used as a measure of total body stores.

Although total transferrin can be measured, it is not commonly determined. Instead, it is usually measured indirectly as the total iron binding capacity (TIBC), which is the iron content when transferrin is saturated with iron.\(^4^4\) TIBC is greater than measurable serum iron concentrations because transferrin can bind more iron than what is present in the circulation. TIBC is either at the low end of the reference range or decreased with anemia of inflammatory disease, but is typically normal in dogs with chronic iron deficiency anemia.\(^4^4\) Percent transferrin saturation, with a normal range of 20–60%, can be calculated as the ratio of serum iron to TIBC.\(^2^5\) Transferrin saturation is markedly decreased with iron deficiency, although the clinical utility of this test may be limited due to diurnal variation in serum iron levels and the impact of numerous other clinical disorders.

In health, serum ferritin concentration correlates well with tissue iron stores and is a good surrogate measurement of total body iron. Since ferritin is an acute-phase protein, ferritin concentrations can be increased in animals with coexisting inflammation. It can also be increased during liver disease, hemolytic diseases, and some neoplastic disorders.\(^4^1\)

Examination of the bone marrow for stainable iron with Prussian blue can help evaluate iron stores in dogs. This iron is present in macrophages as hemosiderin. This is a subjective and relatively insensitive and invasive method of assessing iron stores. Since hemosiderophages are most readily found in bone marrow spicules, an adequately cellular sample is essential. It should also be noted that stainable iron is not present in the bone marrow of cats.\(^4^4\)

**Indirect Tests**

Iron deficiency anemia is initially strongly regenerative. Because iron is preferentially used in heme formation, classic hematologic changes do not occur until late in iron deficiency. The anemia becomes nonregenerative only when severe iron deficiency occurs. In the severely iron-depleted state, RBCs become microcytic and hypochromic due to decreased hemoglobin synthesis, delayed cell maturation, and extra mitotic divisions.\(^4^5\) Hypochromic cells are recognized on a blood smear by their increased central pallor. The decrease in mean cell volume (MCV) precedes the decrease in mean cell hemoglobin concentration (MCHC) in iron deficiency. While a low MCHC is often present in severely affected dogs, it is rarely present in adult cats with iron deficiency anemia. Red cell distribution width (RDW) is often increased due to the presence of both microcytic and normocytic cells.\(^4^6\) In contrast, the anemia of inflammatory disease is classically characterized as nonregenerative, normochromic, and normocytic and is typically only mild to moderate in severity.\(^4^4\)

**Reticulocyte Indices**

Iron deficiency is classically diagnosed by demonstration of a microcytic, hypochromic anemia and decreased serum ferritin concentrations.\(^4^7,4^8\) Since iron is preferentially used for heme synthesis, typical hematologic and biochemical changes do not occur until late in iron deficiency, making the conventional indices of MCV and MCHC insensitive indicators of iron deficiency. Diagnosis of iron deficiency can also be difficult when complicated by concurrent disease, such as neoplasia or inflammation, due to highly variable results obtained for biochemical markers of iron status (i.e., serum iron concentration, TIBC). Recent investigations of reticulocyte parameters as indices of iron status in humans and animals have shown promise for improved ability to diagnose iron deficiency states. Flow cytometric hematology analyzers can differentiate mature erythrocytes from reticulocytes by use of a cellular RNA stain. These instruments measure traditional erythrocyte indices as well as a number of reticulocyte indices, such as mean cell volume and hemoglobin concentration.\(^4^5,4^9\) Reticulocytes circulate for 1–2 days before maturing into erythrocytes, providing a real-time evaluation of iron status and response to iron therapy.\(^5^0\)

Several retrospective studies have evaluated various novel hematologic parameters in association with iron deficiency. In humans, reticulocyte hemoglobin content (CHr) and percentage of hypochromic erythrocytes (%Hypo) have shown to be sensitive in detecting iron deficiency.\(^5^0–5^7\) The %Hypo allows for earlier detection of iron-deficient erythropoiesis than evaluation of MCV; however, a few weeks of iron deficiency are still needed before abnormalities are detected.\(^5^6\) In contrast, the CHr decreases within a few days of iron-deficient erythropoiesis.\(^5^5,5^6\) One retrospective study established reference intervals for CHr and reticulocyte mean cell volume (rMCV) in healthy dogs. The
prevalence of low CHr and rMCV was also determined in a large group of dogs. Hematologic and biochemical parameters previously reported to be affected by, or associated with, changes in iron status were compared between the dogs with decreased values of CHr and rMCV and a group of healthy control dogs. Dogs with low CHr values had significantly lower hematocrit, MCV, percent transferrin saturation, and serum iron concentrations than the healthy control dogs. Dogs with low rMCV values had significantly lower MCV, slightly lower (but not significantly lower) hematocrit, and significantly lower percent transferring saturation, and serum iron concentrations than the control dogs. The association of low CHr and rMCV with these hematologic and biochemical abnormalities suggests that CHr and rMCV may be useful in evaluating iron-dependent erythropoiesis in dogs.

It is noteworthy that a few other disease processes alter CHr values. For example, human patients with α and β thalassemias exhibit an abnormally low CHr, but α and β thalassemias have not been reported in dogs. Further, human patients undergoing chemotherapy often have increased CHr due to transient megaloblastic/microcytic erythropoiesis with production of large reticulocytes.

One prospective study evaluated the utility of reticulocyte indices in a nutritional model of iron deficiency in dogs. Changes in reticulocyte indices were evident as iron deficiency developed and resolved. In some cases, changes in reticulocyte indices were detectable as soon as, or sooner, than changes in conventional hematologic and biochemical indices. Additionally, changes in reticulocyte indices were often much more pronounced than changes in conventional hematologic or biochemical indices. The authors of this study therefore suggested that reticulocyte indices were better indicators of iron status than erythrocyte MCV or MCHC. When iron deficiency was diagnosed based on a decrease in ferritin concentration, rMCV, CHr, percentage of macrocytic reticulocytes, or percentage of reticulocytes with high hemoglobin content or an increase in percentage of reticulocytes with low hemoglobin content, were all superior to both MCV and MCHC for detecting iron deficiency. Neither percentage of microcytic reticulocytes nor reticulocyte hemoglobin concentration was superior to conventional hematologic or biochemical indices in diagnosing iron deficiency.

**Treatment**

Iron supplementation is indicated and effective in the treatment of iron deficiency (Table 1). Iron supplementation has no other therapeutic use and no beneficial effects in other forms of anemia, with the exception of patients being treated with erythropoietic agents. Treatment of anemia of inflammatory disease relies solely on managing the underlying condition.

Oral iron supplementation is considered the safest and least expensive route of administration. Ferrous sulfate and ferrous gluconate are the recommended forms of oral iron because of their low cost and high bioavailability. Ferrous sulfate is commercially available in two forms, a “regular” and a “dried” form. Regular ferrous sulfate is freely soluble in water. It contains approximately 200 mg of elemental iron/gram. Dried ferrous sulfate, also known as exsiccated ferrous sulfate, is slowly soluble in water and contains 300 mg of elemental iron/gram. Ferrous sulfate preparations should be stored in air-tight, light-resistant containers. As mentioned previously, iron requirements for small animals have not been established; however, the following doses of ferrous sulfate have been used to treat iron deficiency: 11 mg/kg/day with a meal, 60–300 mg/day for 2 wk or more (dog), 100–300 mg/day (total dose, dog), 50–100 mg per os once daily (cat), and 30–200 mg/day for 2 wk or more (cat).

Antacids, eggs, and milk can decrease the bioavailability of iron and should be administered as far apart from iron supplementation as possible. Due to limitations on iron absorption from the gastrointestinal tract, treatment with oral iron preparations must be continued for weeks to months and the body’s iron stores may

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**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Dose</th>
<th>Indication</th>
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<tr>
<td>Ferrous sulfate</td>
<td>PO</td>
<td>11 mg/kg/day; 60–300 mg/day (dog); 100–300 mg/day (dog); 50–100 mg/day (cat); 30–200 mg/day (cat)</td>
<td>Iron deficiency</td>
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<tr>
<td>Iron dextran</td>
<td>IM</td>
<td>100 mg total dose (dog); 10–20 mg/kg (dog); 50 mg total dose (cat)</td>
<td>Iron deficiency</td>
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<td>Iron gluconate</td>
<td>IV</td>
<td>No veterinary studies</td>
<td>Iron deficiency</td>
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<tr>
<td>Iron sucrose</td>
<td>IV</td>
<td>No veterinary studies</td>
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<td>Iron deficiency</td>
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<tr>
<td>Deferoxamine</td>
<td>IV</td>
<td>15 mg/kg/hr</td>
<td>Iron overload</td>
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</table>

IM, intramuscular; IV, intravascular; PO, per os
never be fully replenished. Oral iron supplementation can cause black coloration of feces and false-positives with the guaiac test for fecal occult blood. Iron does not usually affect the benzidine test for occult blood. Side effects of ferrous sulfate are typically limited to mild gastrointestinal upset, which may be reduced with division of the daily dosage. Many over-the-counter vitamin supplements containing iron are available. For example, Pet-Tabs Iron-Plus for Dogs & Cats (formerly Pet-Tinic) contains 2.5 mg iron/mL. Fairly large amounts are required to achieve an effective dose (e.g., it would take 20 mL of this product to provide a 50 mg dose).

Parenteral iron may be appropriate in patients who cannot tolerate or absorb oral formulations or in patients with profound iron deficits, such as blood loss that exceeds the absorptive capacity for iron. The most commonly used parenteral formulation is iron dextran, which is a dark brown, slightly viscous liquid complex of ferric oxide and low-molecular-weight, partially hydrolyzed, dextran derivative. Iron dextran is slowly absorbed, primarily via the lymphatic system after IM injection, with approximately 60% of the drug absorbed within 3 days and up to 90% absorbed after 1–3 wk. Iron dextran administration can result in a brown discoloration of serum, with resultant falsely elevated bilirubin measurement and falsely decreased serum calcium measurement. IM administration of iron dextran is recommended in veterinary medicine. Anaphylactic and anaphylactoid reactions are possible. These reactions appear to be due to the dextran moiety; therefore, a test dose of iron dextran is recommended. IV administration of iron dextran has been advocated for humans by some authors, although IM administration is still recommended by the manufacturer. IM injections in humans frequently lead to complications and, even though there are rare cases of anaphylaxis, the IV route is both safe and effective. Up to 10% of humans report an adverse reaction within 48–72 hr after IV administration, including urticaria, pruritis, nausea, vomiting, fever, malaise, arthralgia, and myalgia. Reported veterinary dosages are a maximal dose of 100 mg IM daily, 10–20 mg/kg once, followed by oral therapy with ferrous sulfate, and 50 mg IM q 3–4 wk (cat).3,12,61

Iron gluconate and iron sucrose are parenteral iron formulations that have been widely used by physicians in Europe, but have only been approved for use in the United States in the last decade. Iron gluconate is a stable complex of sodium ferric gluconate in sucrose containing 12.5 mg elemental iron/mL. Approximately 80% of administered iron is delivered to transferrin within 24 hr after IV administration. Iron gluconate has not been associated with serious anaphylactic reactions and a test dose is not required. Iron sucrose is supplied as a solution containing 20 mg iron/mL. As with iron gluconate, serious anaphylactic reactions have not been reported. To the authors’ knowledge, no veterinary studies have investigated the clinical use of either iron gluconate or iron sucrose in dogs or cats.

Blood transfusions also provide a good source of iron (assuming the blood comes from a patient that is not iron deficient); however, a blood transfusion would not be enough to solely treat an iron deficient animal. Two milliliters of blood contains 1 mg of iron, but transfusions should be restricted to severely anemic patients.12

Conclusion

Although iron physiology and pathophysiology are intricate, new advances in recent years have provided a better understanding of these complex processes. Iron deficiency most commonly manifests as anemia; however, anemia may be underdiagnosed due to a delay in onset of anemia until iron deficiency is advanced. There are several tests available for assessing iron status, but evaluation of reticulocyte indices may allow for an earlier recognition of iron deficiency, improved ability to diagnose iron disorders in the face of confounding factors or concurrent disease, and improved treatment monitoring. Treatment of iron deficiency is indicated once recognized.

FOOTNOTES

a Pet-Tabs Iron-Plus for Dogs & Cats; Virbac Animal Health, Fort Worth, TX

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