Iron Metabolism

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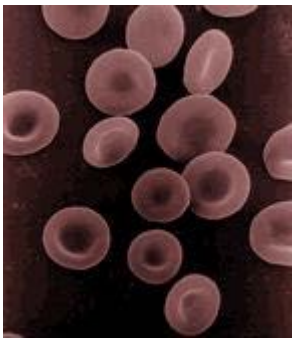
Introduction of Human Iron Metabolism

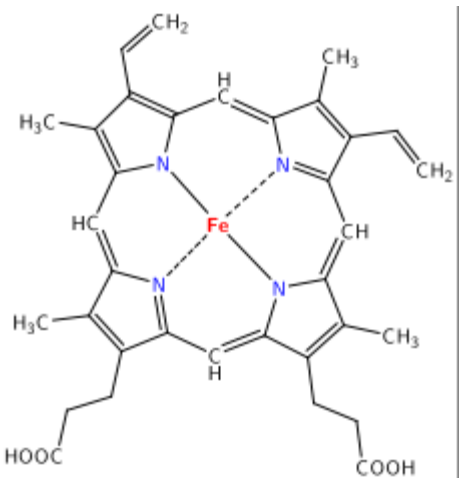


Human beings use 20 mg of iron each day for the production of new red blood cells, much of which is recycled from old red blood cells.

Human iron metabolism is the set of chemical reactions maintaining human homeostasis of iron. Iron is an essential element for most life on Earth, including human beings. The control of this necessary but potentially toxic substance is an important part of many aspects of human health and disease. Hematologists have been especially interested in the system of iron metabolism because iron is essential to red blood cells. Most of the human body's iron is contained in red blood cells. Hemoglobin and iron deficiency anemia are the most common types of anemia.

Understanding this system is also important for understanding diseases of iron overload, like hemochromatosis.

Importance of iron regulation



Structure of Heme b; "Fe" is the chemical symbol of iron.

Iron is an absolute requirement for most forms of life, including humans and most bacterial species, because plants and animals all use iron; hence, iron can be found in a wide variety of food sources.

Iron is essential to life because of its unusual flexibility to serve as both an electron donor and acceptor.

Iron can also be potentially toxic. Its ability to donate and accept electrons means that if iron is free within the cell, it can catalyze the conversion of hydrogen peroxide into free radicals. Free radicals can cause damage to a wide variety of cellular structures, and ultimately kill the cell. To prevent that kind of damage, all life forms that use iron bind the iron atoms to proteins. That allows the cells to use the benefits of iron, but also limit its ability to do harm.

The most important group of iron-binding proteins contain the heme molecules, all of which contain iron at their centers. Humans and most bacteria use variants of heme to carry out redox reactions and electron transport processes. These reactions and processes are required for oxidative phosphorylation. That process is the principal source of energy for human cells; without it, our cells would die.

The iron-sulfur proteins are another important group of iron-containing proteins. Some of these proteins are also essential parts of oxidative phosphorylation.

Humans also use iron in the hemoglobin of red blood cells, in order to transport oxygen from the lungs to the tissues and to export carbon dioxide back to the lungs. Iron is also an essential component of myoglobin to store and diffuse oxygen in muscle cells.

The human body needs iron for oxygen transport. That oxygen is required for the production and survival of all cells in our bodies. Human bodies tightly regulate iron absorption and recycling. Iron is such an essential element of human life, in fact, that humans have no physiologic regulatory mechanism for excreting iron. Most humans

prevent iron overload solely by regulating iron absorption. Those who cannot regulate absorption well enough get disorders of iron overload. In these diseases, the toxicity of iron starts overwhelming the body's ability to bind and store it.

Bacterial protection

A proper iron metabolism protects against bacterial infection. If bacteria are to survive, then they must get iron from the environment. Disease-causing bacteria do this in many ways, including releasing iron-binding molecules called siderophores and then reabsorbing them to recover iron, or scavenging iron from hemoglobin and transferrin. The harder they have to work to get iron, the greater a metabolic price they must pay. That means that iron-deprived bacteria reproduce more slowly. So our control of iron levels appears to be an important defense against bacterial infection. People with increased amounts of iron, like people with hemochromatosis, are more susceptible to bacterial infection.

Although this mechanism is an elegant response to short-term bacterial infection, it can cause problems when inflammation goes on for longer. Since the liver produces hepcidin in response to inflammatory cytokines, hepcidin levels can increase as the result of non-bacterial sources of inflammation, like viral infection, cancer, auto-immune diseases or other chronic diseases. When this occurs, the sequestration of iron appears to be the major cause of the syndrome of anemia of chronic disease, in which not enough iron is available to produce enough hemoglobin-containing red blood cells.

Body iron stores

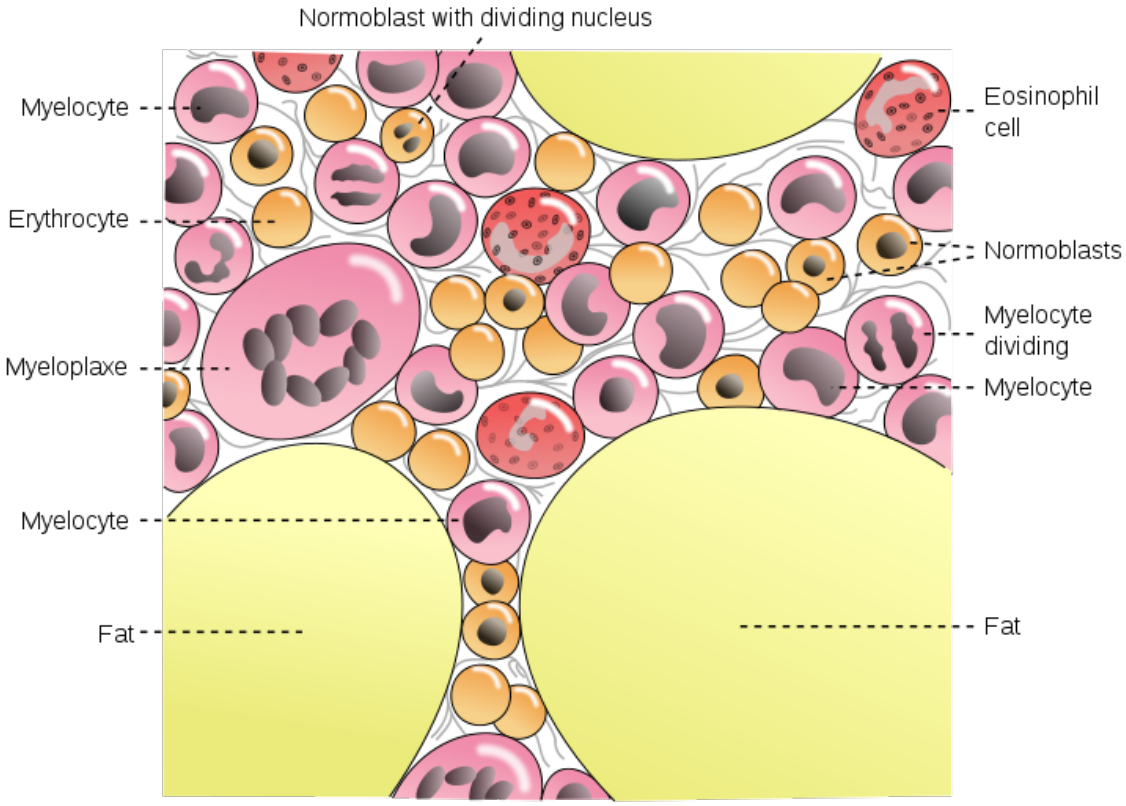


Illustration of blood cell production in the bone marrow. In iron deficiency, the bone marrow produces fewer blood cells, and as the deficiency gets worse, the cells become smaller.

Most well-nourished people in industrialized countries have 4-5 grams of iron in their bodies. Of this, about 2.5 g is contained in the hemoglobin needed to carry oxygen through the blood. Another 400 mg is devoted to cellular proteins that use iron for important cellular processes like storing oxygen (myoglobin), or performing energy-producing redox reactions (cytochromes). 3-4 mg circulates through the plasma, bound to transferrin.

Since so much iron is required for hemoglobin, iron deficiency anemia is the first and primary clinical manifestation of iron deficiency. Oxygen transport is so important to human life that severe anemia harms or kills people by depriving their organs of enough oxygen. Iron-deficient people will suffer or die from organ damage well before cells run out of the iron needed for intracellular processes like electron transport.

Some iron in the body is stored. Physiologically, most stored iron is bound by ferritin molecules; the largest amount of ferritin-bound iron is found in cells of the liver hepatocytes, the bone marrow and the spleen. The liver's stores of ferritin are the primary physiologic source of reserve iron in the body.

Macrophages of the reticuloendothelial system store iron as part of the process of breaking down and processing hemoglobin from engulfed red blood cells.

Iron is also stored as a pigment called hemosiderin in an apparently pathologic process. This molecule appears to be mainly the result of cell damage. It is often found engulfed by macrophages that are scavenging regions of damage. It can also be found among people with iron overload due to frequent blood cell destruction and transfusions.

Men tend to have more stored iron than women, particularly women who must use their stores to compensate for iron lost through menstruation, pregnancy or lactation.

How the body gets its iron

Most of the iron in the body is hoarded and recycled by the reticuloendothelial system, which breaks down aged red blood cells. However, people lose a small but steady amount by sweating and by shedding cells of the skin and the mucosal lining of the gastrointestinal tract. The total amount of loss for healthy people in the developed world amounts to an estimated average of 1 mg a day for men, and 1.5–2 mg a day for women with regular menstrual periods. People with gastrointestinal parasitic infections, more commonly found in developing countries, often lose more.

This steady loss means that people must continue to absorb iron. They do so via a tightly regulated process that under normal circumstances protects against iron overload.

Absorbing iron from the diet

The absorption of dietary iron is a variable and dynamic process. The amount of iron absorbed compared to the amount ingested is typically low. The efficiency with which iron is absorbed varies largely depending on the source. Generally the best absorbed forms of iron come from animal products. Like most mineral nutrients, the majority of the iron absorbed from digested food or supplements is absorbed in the duodenum by enterocytes of the duodenal lining. These cells have special molecules that allow them to move iron into the body.

To be absorbed, dietary iron can be absorbed as part of a protein such as heme protein or must be in its ferrous Fe^{2+} form. A ferric reductase enzyme on the enterocytes' brush border, Dcytb, reduces ferric Fe^{3+} to Fe^{2+}. A protein called divalent metal transporter 1 DMT1, which transports all kinds of divalent metals into the body, then transports the iron across the enterocyte's cell membrane and into the cell.

These intestinal lining cells can then either store the iron as ferritin (in which case the iron will leave the body when the cell dies and is sloughed off into feces) or the cell can move it into the body, using a protein called ferroportin. The body regulates iron levels by regulating each of these steps. For instance, cells produce more Dcytb, DMT1 and ferroportin in response to iron deficiency anemia.

Our bodies' rates of iron absorption appear to respond to a variety of interdependent factors, including total iron stores, the extent to which the bone marrow is producing new red blood cells, the concentration of hemoglobin in the blood, and the oxygen content of the blood. We also absorb less iron during times of inflammation. Recent discoveries demonstrate that hepcidin regulation of ferroportin (see below) is responsible for the syndrome of anemia of chronic disease.

While Dcytb and DMT1 are unique to iron transport across the duodenum, ferroportin is distributed throughout the body on all cells which store iron. Thus, regulation of ferroportin is the body's main way of regulating the amount of iron in circulation.

Hephaestin, a ferroxidase that which can oxidize Fe^{2+} to Fe^{3+} and is found mainly in the small intestine, helps ferroportin transfer iron across the basolateral end of the intestine cells.

Reasons for iron deficiency

Iron is an important topic in prenatal care because women can sometimes become iron-deficient from the increased iron demands of pregnancy.

Functional or actual iron deficiency can result from a variety of causes, explained in more detail further. These causes can be grouped into several categories:

- Increased demand for iron, which the diet cannot accommodate.
- Increased loss of iron (usually through loss of blood).
- Nutritional deficiency. This can result due to a lack of dietary iron or consumption of foods that inhibit iron absorption, including calcium, phytates and tannins. Contrary to popular belief, persons following vegetarian and vegan diets have similar iron status and the same rates of iron deficiency as non-vegetarians.
- Inability to absorb iron because of damage to the intestinal lining. Examples of causes of this kind of damage include surgery involving the duodenum, or

diseases like Crohn's or celiac sprue which severely reduce the surface area available for absorption.
- Inflammation leading to hepcidin-induced restriction on iron release from enterocytes (see below).

Iron overload

The body is able to substantially reduce the amount of iron it absorbs across the mucosa. It does not seem to be able to entirely shut down the iron transport process. Also, in situations where excess iron damages the intestinal lining itself (for instance, when children eat a large quantity of iron tablets produced for adult consumption), even more iron can enter the bloodstream and cause a potentially deadly syndrome of iron intoxication. Large amounts of free iron in the circulation will cause damage to critical cells in the liver, the heart and other metabolically active organs.

Iron toxicity results when the amount of circulating iron exceeds the amount of transferrin available to bind it, but the body is able to vigorously regulate its iron uptake. Thus, iron toxicity from ingestion is usually the result of extraordinary circumstances like iron tablet overdose rather than variations in diet. Iron toxicity is usually the result of more chronic iron overload syndromes associated with genetic diseases, repeated transfusions or other causes. Classic examples of genetic iron overload includes Hereditary Hemochromatosis (HH) and the more severe disease Juvenile Hemochromatosis (JH) caused by mutations in either the gene RGMc gene, a member of a three gene repulsive guidance molecule family , (also called hemojuvelin (HJV), and HFE2), Hemojuvelin, or the HAMP gene that encodes for hepcidin (an iron regulatory peptide).

How cells get their iron from the body

As discussed above, most of the iron in the body is located on hemoglobin molecules of red blood cells. When red blood cells reach a certain age, they are degraded and engulfed by specialized scavenging macrophages. These cells internalize the iron-containing hemoglobin, degrade it, put the iron onto transferrin molecules, and then export the transferrin-iron complexes back out into the blood. Most of the iron used for blood cell production comes from this cycle of hemoglobin recycling.

All cells use some iron, and must get it from the circulating blood. Since iron is tightly bound to transferrin, cells throughout the body have receptors for transferrin-iron complexes on their surfaces. These receptors engulf and internalize both the protein and the iron attached to it. Once inside, the cell transfers the iron to ferritin, the internal iron storage molecule.

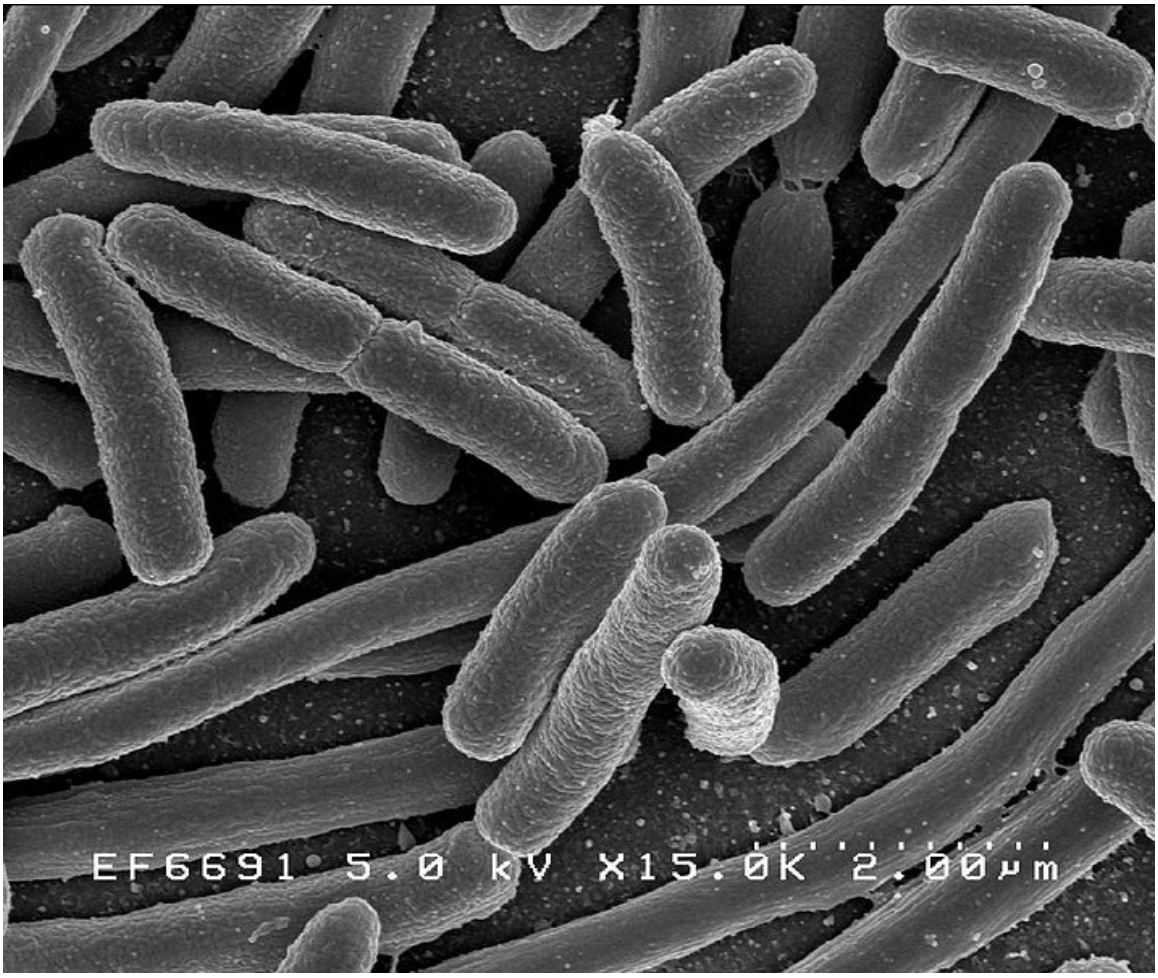
Transferrin receptor production will increase, and ferritin production will decrease.

Regulation by location

Regulation of iron levels is a task of the whole body, as well as for individual cells.

When *body* levels of iron are too low, then hepcidin in the duodenal epithelium is decreased. This causes an increase in ferroportin activity, stimulating iron uptake in the digestive system. The reverse occurs when there is an iron surplus.

In *individual cells*, an iron deficiency causes responsive element binding protein to iron responsive elements on mRNA for transferrin receptors, resulting in increased production of transferrin receptors. These receptors increase binding of transferrin to cells, and therefore stimulating iron uptake.



Electron micrograph of E. coli. All bacteria that cause human disease require iron to live and to multiply.

Diseases of iron regulation

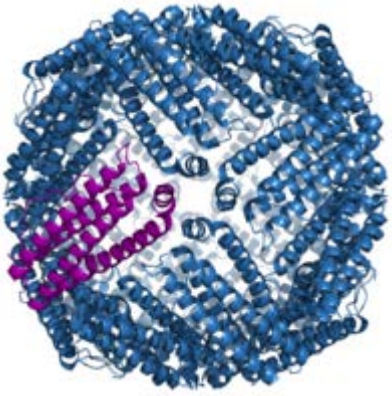
The exact mechanisms of most of the various forms of adult hemochromatosis, which make up most of the genetic iron overload disorders, remain unsolved. So while researchers have been able to identify genetic mutations causing several adult variants of

hemochromatosis, they now must turn their attention to the normal function of these mutated genes.

Chapter- 2

Ferritin

ferritin, light polypeptide



Structure of the ferritin complex.

Identifiers	
Symbol	FTL
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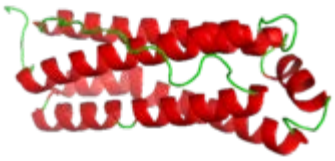
HUGO	3999
OMIM	134790
RefSeq	NM_000146
UniProt	P02792
Other data	
Locus	Chr. 19 *q13.3–13.4*

ferritin, heavy polypeptide 1

Identifiers	
Symbol	FTH1
Alt. symbols	FTHL6

Entrez	2495
HUGO	3976
OMIM	134770
RefSeq	NM_002032
UniProt	P02794
Other data	
Locus	Chr. 11 *q13*

ferritin mitochondrial



Crystallographic structure of mitochondrial ferritin.

Identifiers	
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HUGO	17345
OMIM	608847
RefSeq	NM_177478
UniProt	Q8N4E7
Other data	
Locus	Chr. 5 *q23.1*

Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including bacteria, algae and higher plants, and animals. In humans, it acts as a buffer against iron deficiency and iron overload.

Ferritin is a globular protein complex consisting of 24 protein subunits and is the primary *intracellular iron-storage protein* in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. Ferritin that is not combined with iron is called **apoferritin**.

Description

Ferritin is a 450 kDa protein consisting of 24 subunits that is present in every cell type. In vertebrates, these subunits are both the light (L) and the heavy (H) type with an apparent molecular weight of 19 kDA or 21 kDA respectively; their sequences are about 50% homologous. Amphibians have an additional ("M") type of ferritin; the single ferritin of plants and bacteria most closely resembles the vertebrate H-type. Two types have been recovered in the gastropod *Lymnaea*, the somatic ferritin being distinct from the yolk ferritin (see below). An additional subunit resembling *Lymnaea* soma ferritin is associated with shell formation in the pearl oyster. Two types are present in the parasite *Schistosoma*, one in males, the other in females. All the aforementioned ferritins are most similar, in terms of their primary sequence, with the vertebrate H-type. In *E. coli*, a 20% similarity to human H-ferritin is recovered. Inside the ferritin shell, iron ions form crystallites together with phosphate and hydroxide ions. The resulting particle is similar to the mineral ferrihydrite. Each ferritin complex can store about 4500 iron (Fe^{3+}) ions.

Some ferritin complexes in vertebrates are hetero-oligomers of two highly-related gene products with slightly different physiological properties. The ratio of the two homologous proteins in the complex depends on the relative expression levels of the two genes.

Mitochondrial ferritin was recently identified as a protein precursor. It is classified as a metal-binding protein which is located within the mitochondria. After the protein is taken up by the mitochondria it can be processed into a mature protein and assemble functional ferritin shells. Its structure was determined at 1.70 angstroms through the use of X-ray diffraction and contains 182 residues. It is 67% helical. The Ramachandran plot shows that the structure of mitochondrial ferritin is mainly alpha helical with a low prevalence of beta sheets.

Genetic structure

In human ferritin, introns are present between the 34/5th, 82/3rd, and 14/5th amino acid residues; in addition, one to two hundred untranslated bases grace either end of the combined exons. The Tyrosine residue at amino acid position 27 is thought to be associated with biomineralization.

Function

Iron storage

Ferritin serves to store iron in a non-toxic form, to deposit it in a safe form, and to transport it to areas where it is required. The function and structure of the expressed ferritin protein varies in different cell types. This is controlled primarily by how much mRNA is translated, and how stable the mRNA is. mRNA concentration is further tweaked by changes to how it is stored and how efficiently it is transcribed. The presence

of iron itself is a major trigger for the production of ferritin, with some exceptions (such as the yolk ferritin of the gastropod *Lymnaea*, which lacks an iron-responsive unit).

Free iron is toxic to cells as it acts as a catalyst in the formation of free radicals from reactive oxygen species via the Fenton Reaction. Hence vertebrates use an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored in a protein complex as ferritin or hemosiderin. Apoferritin binds to free ferrous iron and stores it in the ferric state. As ferritin accumulates within cells of the reticuloendothelial system, protein aggregates are formed as hemosiderin. Iron in ferritin or hemosiderin can be extracted for release by the RE cells although hemosiderin is less readily available. Under steady state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin FR5Rl is the most convenient laboratory test to estimate iron stores.

Because iron is an important mineral in mineralization, ferritin is employed in the shells of organisms such as molluscs to control the concentration and distribution of iron, thus sculpting shell morphology and colouration. It also plays a role in the haemolymph of the polyplacophora where it serves to rapidly transport iron to the mineralizing radula.

Immune response

Ferritin concentrations increase drastically in the presence of an infection or cancer; this is necessary to counter the infective agent's attempt to bind iron from the host's tissue. Infective agents may cause ferritin to migrate from the plasma to within cells, in order to deny iron to the infective agent.

Stress response

The concentration of ferritin has been shown to increase in response to stresses such as anoxia; this implies that it is an acute phase protein.

Mitochondria

Mitochondrial ferritin has many roles pertaining to molecular function. It participates in ferroxidase activity, binding, iron ion binding, oxidoreductase activity, ferric iron binding, metal ion binding as well as transition metal binding. Within the realm of biological processes it participates in oxidation-reduction, iron ion transport across membranes and cellular iron ion homeostasis.

Yolk

In some snails, the protein component of the egg yolk is primarily ferritin; this is a different ferritin, with a different genetic sequence, than the somatic ferritin. It is produced in the midgut glands and secreted into the haemolymph, whence it is transported to the eggs.

Human applications

Ferritin is also used in materials science as a precursor in making iron nanoparticles for carbon nanotube growth by chemical vapor deposition.

Expression

In vertebrates, ferritin is usually found within cells, although it is also present in smaller quantities in the plasma.

Diagnostic uses

Serum ferritin levels are measured in patients as part of the iron studies workup for anemia and for restless legs syndrome. The ferritin levels measured have a direct correlation with the total amount of iron stored in the body including cases of anemia of chronic disease.

A normal ferritin blood level, referred to as the "reference interval," is now determined by many testing laboratories, such as LabCorp, using the Roche enhanced chemiluminescence immunoassay (ECLIA) methodology. The Roche ECLIA reference ranges for ferritin are 30–400 ng/mL for males, and 13–150 ng/mL for females. Other tests are in usage that rely on different methods and may have different reference ranges.

Low

If the ferritin level is low, there is a risk for lack of iron, which could lead to anemia. Low ferritin levels (<50 ng/mL) have however been associated with the symptoms of restless legs syndrome, even in the absence of anemia and sickness.

In the setting of anemia, serum ferritin is the most sensitive lab test for iron deficiency anemia.

Low ferritin may also indicate hypothyroidism or vitamin C deficiency.

In a certain study in Paris, France, the level of iron in the blood (measured by ordering a ferritin serum test) has been connected to ADHD in children. Specifically, the lower the iron level, the more severe the ADHD symptoms.

Elevated

If ferritin is high there is iron in excess.

Ferritin is also used as a marker for iron overload disorders, such as hemochromatosis, hemosiderosis and porphyria in which the ferritin level may be abnormally raised.

As ferritin is also an acute-phase reactant, it is often elevated in the course of disease. A normal C-reactive protein can be used to exclude elevated ferritin caused by acute phase reactions.

Ferritin can be elevated during periods of acute malnourishment.

Application

Cavities formed by ferritin and mini-ferritins (Dps) proteins have been successfully used as the reaction chamber for the fabrication of metal nanoparticles (NPs). Protein shells served as a template to restrain particle growth and as a coating to prevent coagulation/aggregation between NPs. Using various sizes of protein shells, various sizes of NPs can be easily synthesized for chemical, physical and bio-medical applications

Chapter- 3

Ferroportin

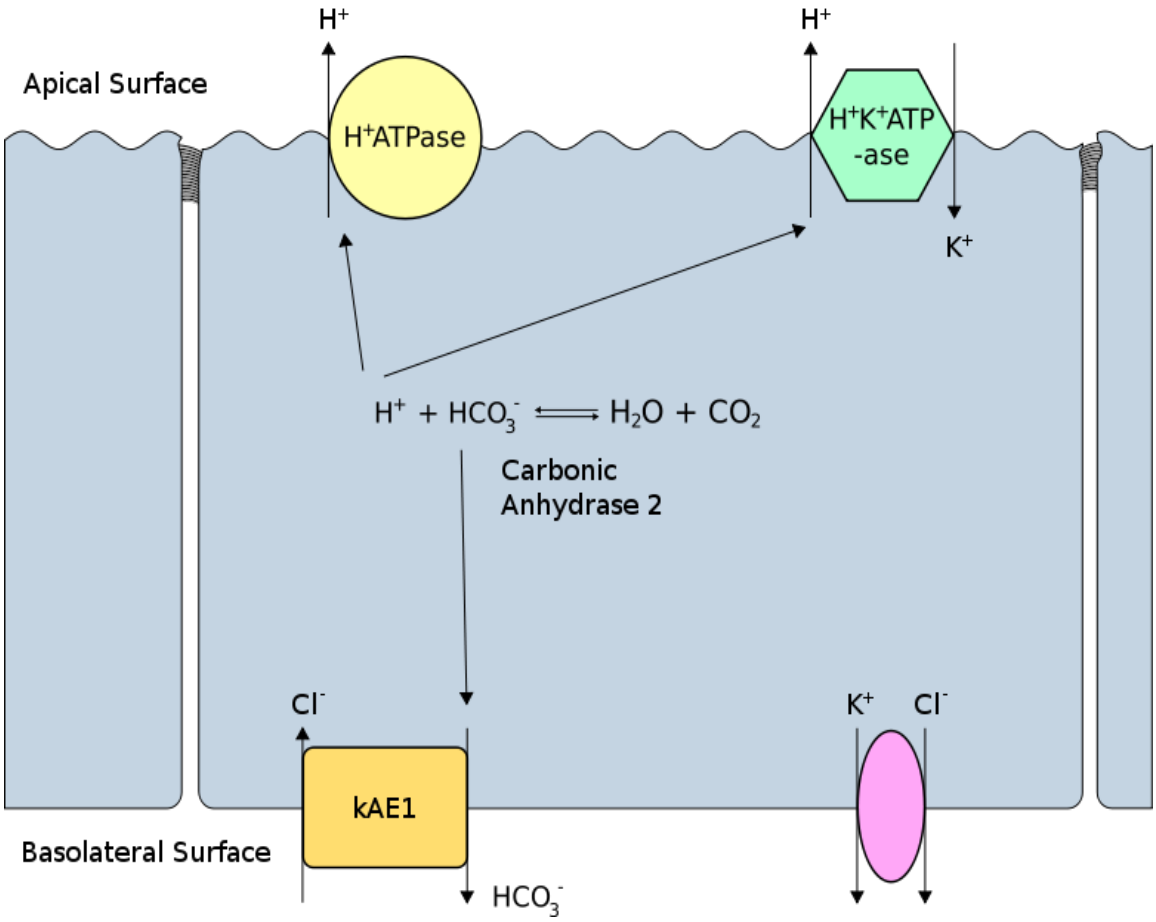
Ferroportin is a transmembrane protein that transports iron from the inside of a cell to the outside of it. It is found on the surface of cells that store or transport iron, including:

- Enterocytes in the duodenum
- Hepatocytes
- Macrophages of the reticuloendothelial system.

Enterocyte

Enterocytes, or **intestinal absorptive cells**, are simple columnar epithelial cells found in the small intestines and colon. A glycocalyx surface coat contains digestive enzymes. Microvilli on the apical surface increase surface area for the digestion and transport of molecules from the intestinal lumen. The cells also have a secretory role.

Functions



Apical membrane and Basolateral membrane

The major functions of enterocytes include:

- **Ion uptake**, including sodium, calcium, magnesium, and iron. This typically occurs through active transport.
- **Water uptake**. This follows the osmotic gradient established by Na+/K+ ATPase on the basolateral surface. This can occur transcellularly or paracellularly.
- **Sugar uptake**. Polysaccharidases and disaccharidases in the glycocalyx break down large sugar molecules, which are then absorbed. Glucose crosses the apical membrane of the enterocyte using the Na^+ dependent glucose transporter. It moves through the cytosol (cytoplasm) and exits the enterocyte via the basolateral membrane (into the blood capillary) using GLUT-2 (*SLC2A2*). Galactose uses the same transport system. Fructose, on the other hand, crosses the apical membrane of the enterocyte, using GLUT-5 (*SLC2A5*). It is thought to cross into the blood capillary using one of the other GLUT transporters.
- **Peptide and amino acid uptake**. Peptidases in the glycocalyx cleave proteins to amino acids or small peptides. Enteropeptidase is responsible for activating pancreatic trypsinogen into trypsin, which activates other pancreatic zymogens.
- **Lipid uptake**. Lipids are broken down by pancreatic lipase and bile, and then diffuse into the enterocytes. Smaller lipids are transported into intestinal

capillaries, while larger lipids are processed by the Golgi and smooth endoplasmic reticulum into lipoprotein chylomicra and exocytozed into lacteals.

- **Vitamin B12 uptake**. Receptors bind to the vitamin B_{12}-gastric intrinsic factor complex and are taken into the cell.
- **Resorption of unconjugated bile salts**. Bile that was released and not used in emulsification of lipids are reabsorbed in the ileum. Also known as the enterohepatic circulation.
- **Secretion of immunoglobulins**. IgA from plasma cells in the mucosa are absorbed through receptor mediated endocytosis on the basolateral surface and released as a receptor-IgA complex into the intestinal lumen. The receptor component confers additional stability to the molecule.

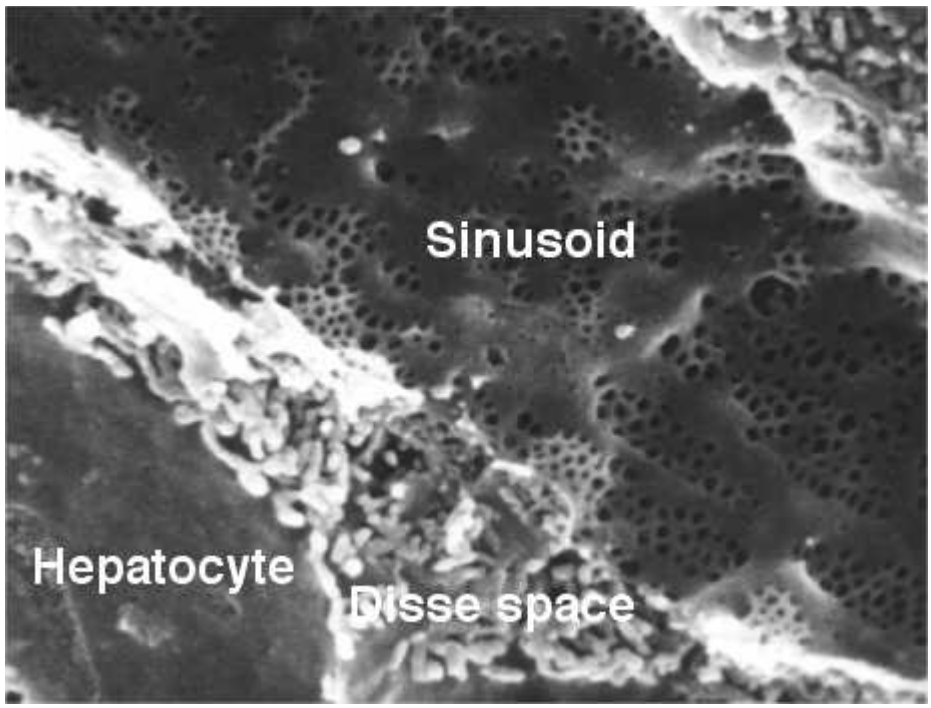
Pathology

Dietary fructose intolerance occurs when there is a deficiency in the amount of fructose carrier.

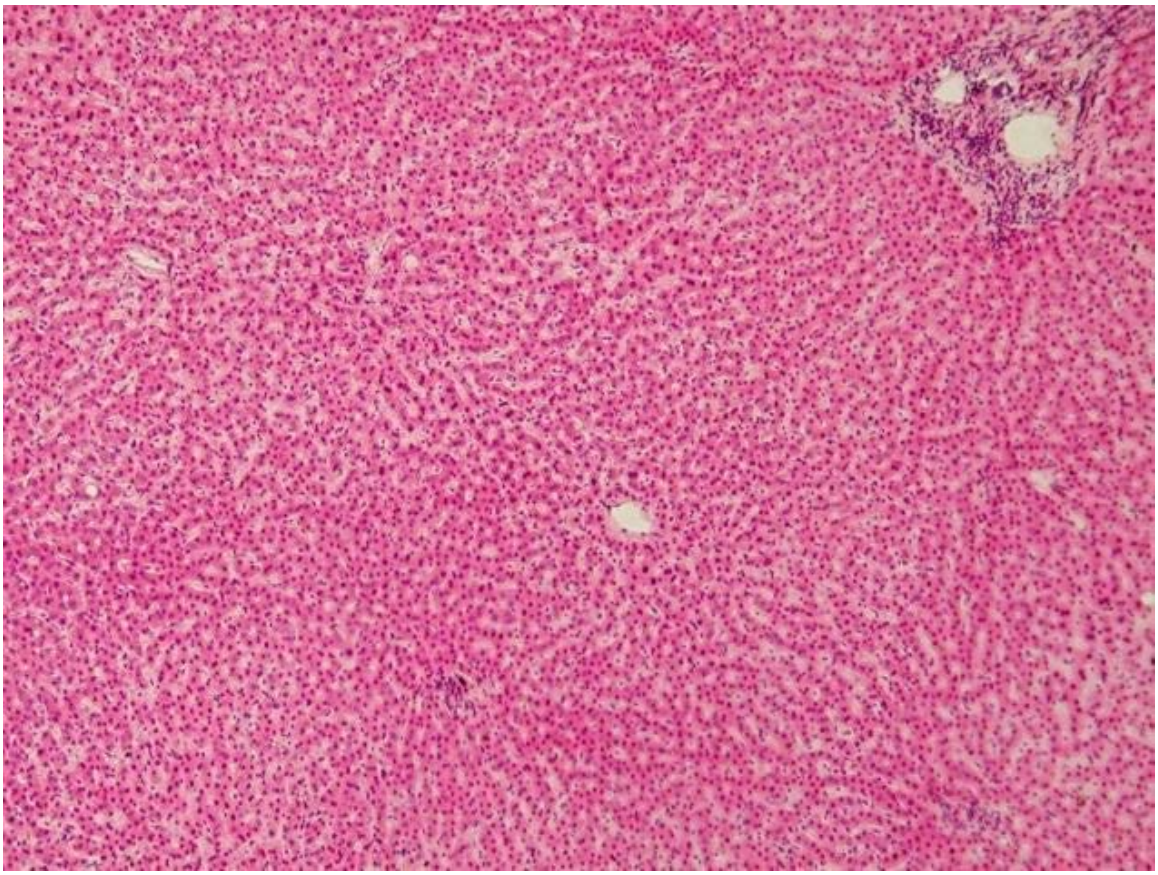
Lactose intolerance is the most common problem of carbohydrate digestion and is created by an insufficient amount of lactase (a disaccharidase) enzyme, which is used to break down the sugar. As a result of this deficiency, undigested lactose cannot be absorbed and is instead passed on to the colonic bacteria, which metabolize the lactose. The bacteria release gas and metabolic products that enhance colonic motility. Although rare, if one is to completely eliminate lactose products from the diet, within a month, the subject will develop lactose intolerance.

Problems with the gastric intrinsic factor or its receptor can result in pernicious anemia.

Hepatocyte



Sinusoid of a rat liver with fenestrated endothelial cells. Fenestration are approx 100 nm diameter, and the sinusoidal width 5 μm. Scanning electron micrograph by Robin Fraser, University of Otago.



Cross-section of the human liver.

A **hepatocyte** is a cell of the main tissue of the liver. Hepatocytes make up 70-80% of the liver's cytoplasmic mass. These cells are involved in:

- Protein synthesis
- Protein storage
- Transformation of carbohydrates
- Synthesis of cholesterol, bile salts and phospholipids
- Detoxification, modification, and excretion of exogenous and endogenous substances

The hepatocyte also initiates formation and secretion of bile.

Hepatocyte histology

Hepatocytes display an eosinophilic cytoplasm, reflecting numerous mitochondria, and basophilic stippling due to large amounts of rough endoplasmic reticulum and free ribosomes. Brown lipofuscin granules are also observed (with increasing age) together with irregular unstained areas of cytoplasm; these correspond to cytoplasmic glycogen

and lipid stores removed during histological preparation. The average life span of the hepatocyte is 5 months; they are able to regenerate.

Hepatocyte nuclei are round with dispersed chromatin and prominent nucleoli. Anisokaryosis is common and often reflects tetraploidy and other degrees of polyploidy, a normal feature of over 50% of hepatocytes. Binucleate cells are also common.

Hepatocytes are organised into plates separated by vascular channels (sinusoids), an arrangement supported by a reticulin (collagen type III) network. The hepatocyte plates are one cell thick in mammals and two cells thick in the chicken. Sinusoids display a discontinuous, fenestrated endothelial cell lining. The endothelial cells have no basement membrane and are separated from the hepatocytes by the space of Disse, which drains lymph into the portal tract lymphatics.

Kupffer cells are scattered between endothelial cells; they are part of the reticuloendothelial system and phagocytose spent erythrocytes. Stellate (Ito) cells store vitamin A and produce extracellular matrix and collagen; they are also distributed amongst endothelial cells but are difficult to visualise by light microscopy.

Hepatocytes are an important physiological example for evalutation of both biological and metabolic effects of xenobiotics. They are separated from the liver by collagenase digestion, which is a two step process. In the first step, the liver is placed in an isotonic solution, in which calcium is removed to disrupt cell-cell tight junctions by the use of a calcium chelating agent. Next, a solution containing collagenase is added to separate the hepatocytes from the liver stroma. This process creates a suspension of hepatocytes, which can be cultured and plated on 96 well plates for immediate use, or cryopreserved by freezing. They do not proliferate in culture. Hepatocytes are intensely sensitive to damage during the cycles of cryopreservation including freezing and thawing. Even after the addition of classical cryoprotectants there is still damage done while being cryopreserved.

Protein synthesis

The hepatocyte is a cell in the body that manufactures serum albumin, fibrinogen, and the prothrombin group of clotting factors(except for Factor3,4).

It is the main site for the synthesis of lipoproteins, ceruloplasmin, transferrin, complement, and glycoproteins. Hepatocytes manufacture their own structural proteins and intracellular enzymes.

Synthesis of proteins is by the rough endoplasmic reticulum (RER), and both the rough and smooth endoplasmic reticulum (SER) are involved in secretion of the proteins formed.

The endoplasmic reticulum (ER) is involved in conjugation of proteins to lipid and carbohydrate moieties synthesized by, or modified within, the hepatocytes.

Carbohydrate metabolism

The liver forms fatty acids from carbohydrates and synthesizes triglycerides from fatty acids and glycerol. Hepatocytes also synthesize apoproteins with which they then assemble and export lipoproteins (VLDL, HDL).

The liver is also the main site in the body for gluconeogenesis, the formation of carbohydrates from precursors such as alanine, glycerol, and oxaloacetate.

Lipid metabolism

The liver receives many lipids from the systemic circulation and metabolizes chylomicron remnants. It also synthesizes cholesterol from acetate and further synthesizes bile salts. The liver is the sole site of bile salts formation.

Detoxification

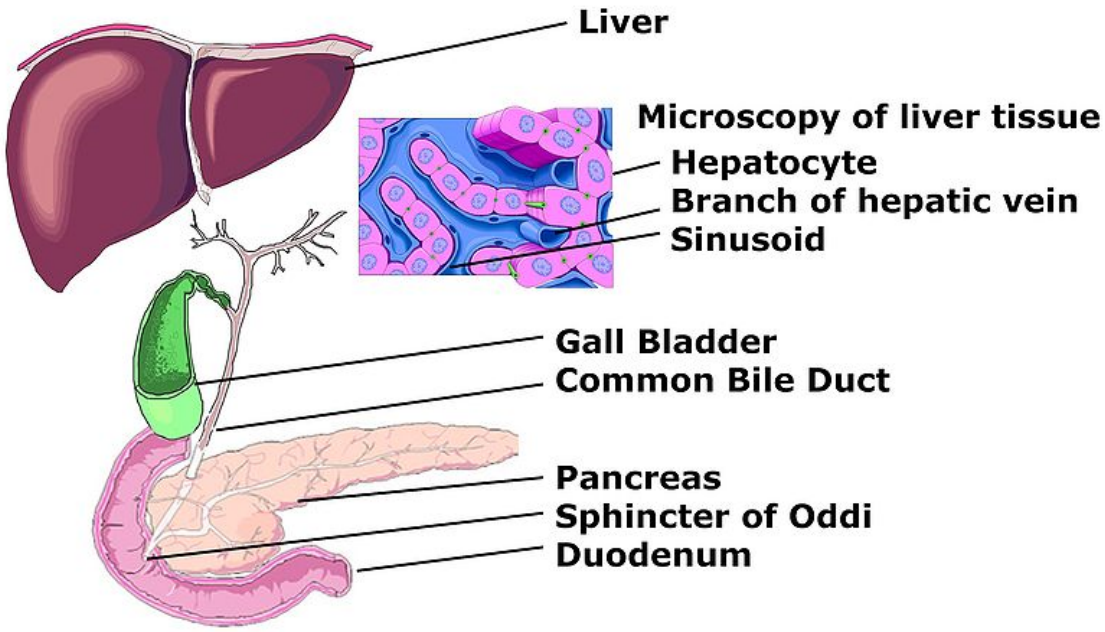
These liver cells have the ability to metabolize, detoxify, and inactivate exogenous compounds such as drugs, (drug metabolism), and insecticides, and endogenous compounds such as steroids.

The drainage of the intestinal venous blood into the liver requires efficient detoxification of miscellaneous absorbed substances to maintain homeostasis and protect the body against ingested toxins.

One of the detoxifying functions of hepatocytes is to modify ammonia into urea for excretion.

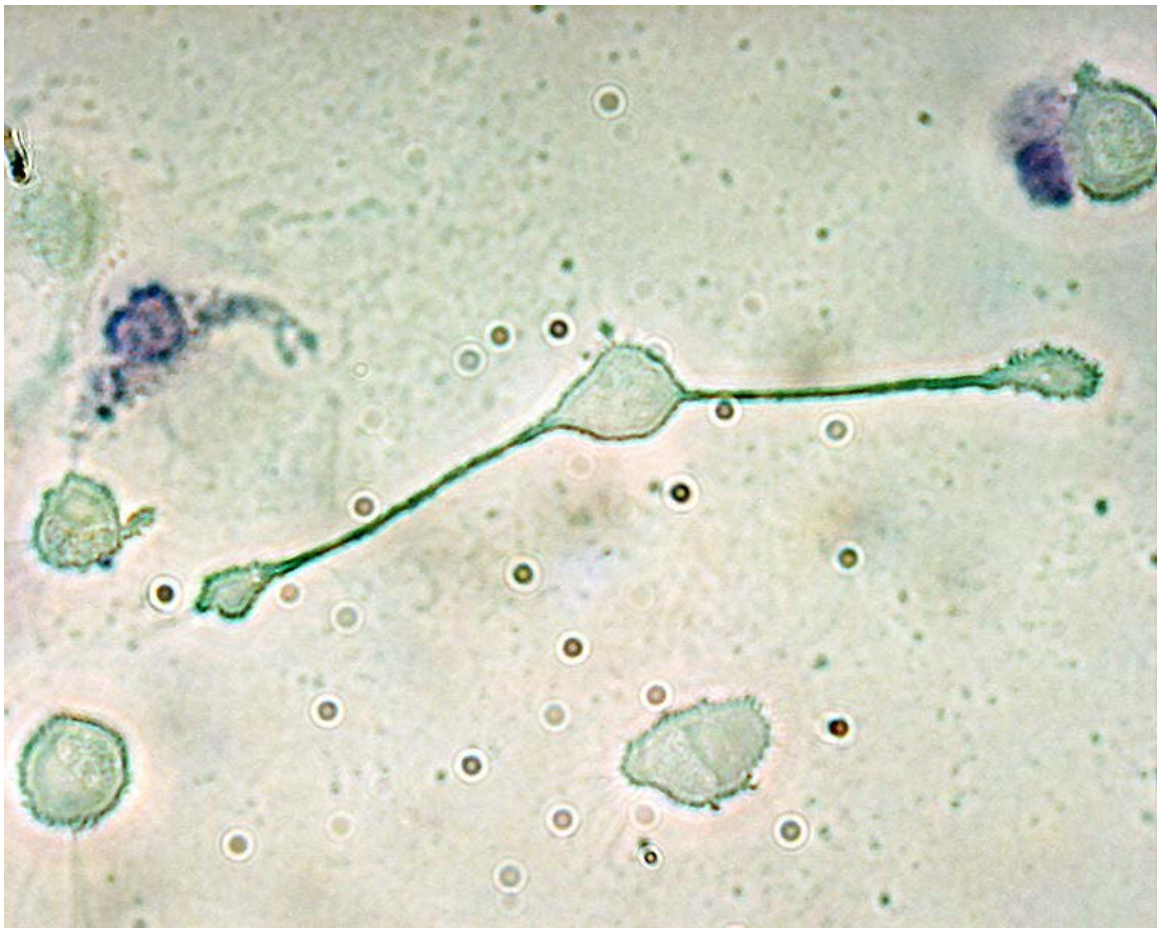
The most abundant organelle in liver cell is the smooth endoplasmic reticulum.

Additional images



Schemic diagram of Biliary system

Macrophage



A macrophage of a mouse stretching its "arms" (Pseudopodia) to engulf two particles, possibly pathogens

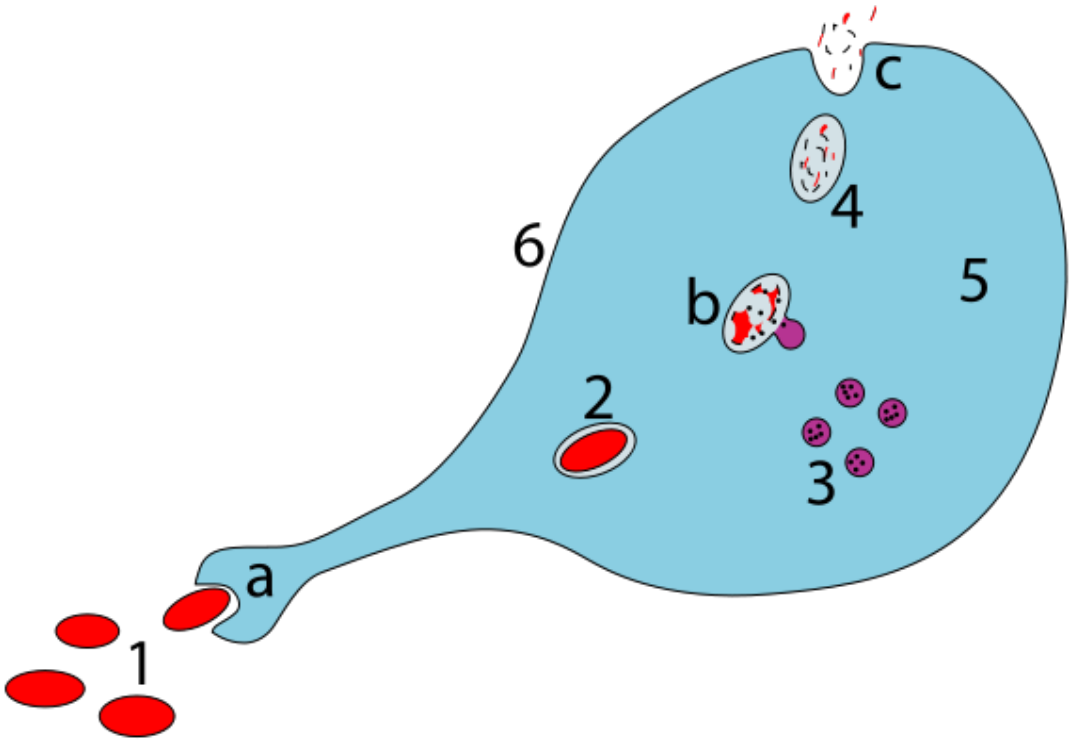
Macrophages (Greek: big eaters, from *makros* "large" + *phagein* "eat"; abbr. **MΦ**) are white blood cells within tissues, produced by the differentiation of monocytes. Human macrophages are about 21 micrometres (0.00083 in) in diameter. Monocytes and macrophages are phagocytes, acting in both non-specific defense (innate immunity) as well as to help initiate specific defense mechanisms (adaptive immunity) of vertebrate animals. Their role is to phagocytose (engulf and then digest) cellular debris and pathogens either as stationary or as mobile cells, and to stimulate lymphocytes and other immune cells to respond to the pathogen. They can be identified by specific expression of a number of proteins including CD14, CD11b, F4/80 (mice)/EMR1 (human), Lysozyme M, MAC-1/MAC-3 and CD68 by flow cytometry or immunohistochemical staining. They move by action of Amoeboid movement.

Life cycle

When a leukocyte enters damaged tissue through the endothelium of a blood vessel (a process known as the leukocyte extravasation), it undergoes a series of changes to become a macrophage. Monocytes are attracted to a damaged site by chemical substances

through chemotaxis, triggered by a range of stimuli including damaged cells, pathogens and cytokines released by macrophages already at the site. At some sites such as the testis, macrophages have been shown to populate the organ through proliferation. Unlike short-lived neutrophils, macrophages survive longer in the body up to a maximum of several months.

Function



Steps of a macrophage ingesting a pathogen:
a. Ingestion through phagocytosis, a phagosome is formed
b. The fusion of lysosomes with the phagosome creates a phagolysosome; the pathogen is broken down by enzymes
c. Waste material is expelled or assimilated (the latter not pictured)
Parts:
1. Pathogens
2. Phagosome
3. Lysosomes
4. Waste material
5. Cytoplasm
6. Cell membrane

Phagocytosis

One important role of the macrophage is the removal of necrotic cellular debris in the lungs. Removing dead cell material is important in chronic inflammation, as the early stages of inflammation are dominated by neutrophil granulocytes, which are ingested by macrophages if they come of age.

The removal of necrotic tissue is, to a greater extent, handled by *fixed macrophages*, which will stay at strategic locations such as the lungs, liver, neural tissue, bone, spleen and connective tissue, ingesting foreign materials such as pathogens, recruiting additional macrophages if needed.

When a macrophage ingests a pathogen, the pathogen becomes trapped in a phagosome, which then fuses with a lysosome. Within the phagolysosome, enzymes and toxic peroxides digest the pathogen. However, some bacteria, such as *Mycobacterium tuberculosis*, have become resistant to these methods of digestion. Macrophages can digest more than 100 bacteria before they finally die due to their own digestive compounds.

Role in adaptive immunity

Macrophages are versatile cells that play many roles. As scavengers, they rid the body of worn-out cells and other debris. They are foremost among the cells that "present" antigen, a crucial role in initiating an immune response. As secretory cells, monocytes and macrophages are vital to the regulation of immune responses and the development of inflammation; they produce a wide array of powerful chemical substances (monokines) including enzymes, complement proteins, and regulatory factors such as interleukin-1. At the same time, they carry receptors for lymphokines that allow them to be "activated" into single-minded pursuit of microbes and tumour cells.

After digesting a pathogen, a macrophage will present the antigen (a molecule, most often a protein found on the surface of the pathogen, used by the immune system for identification) of the pathogen to the corresponding helper T cell. The presentation is done by integrating it into the cell membrane and displaying it attached to an MHC class II molecule, indicating to other white blood cells that the macrophage is not a pathogen, despite having antigens on its surface.

Eventually, the antigen presentation results in the production of antibodies that attach to the antigens of pathogens, making them easier for macrophages to adhere to with their cell membrane and phagocytose. In some cases, pathogens are very resistant to adhesion by the macrophages.

The antigen presentation on the surface of infected macrophages (in the context of MHC class II) in a lymph node stimulates TH1 (type 1 helper T cells) to proliferate (mainly due to IL-12 secretion from the macrophage). When a B-cell in the lymph node recognizes the same unprocessed surface antigen on the bacterium with its surface bound antibody, the antigen is endocytosed and processed. The processed antigen is then presented in MHCII on the surface of the B-cell. TH1 receptor that has proliferated recognizes the

antigen-MHCII complex (with co-stimulatory factors- CD40 and CD40L) and causes the B-cell to produce antibodies that help opsonisation of the antigen so that the bacteria can be better cleared by phagocytes.

Macrophages provide yet another line of defense against tumor cells and somatic cells infected with fungus or parasites. Once a T cell has recognized its particular antigen on the surface of an aberrant cell, the T cell becomes an activated effector cell, chemical mediators known as lymphokines that stimulate macrophages into a more aggressive form. These activated macrophages can then engulf and digest affected cells much more readily. The macrophage does not generate a response specific for an antigen, but attacks the cells present in the local area in which it was activated.

Role in Muscle Regeneration

The first step to understanding the importance of macrophages in muscle repair, growth, and regeneration is that there are two "waves" of macrophages with the onset of damageable muscle use – subpopulations that do and do not directly have an influence on repairing muscle. The initial wave is a phagocytic population that comes along during periods of increased muscle use that are sufficient to cause muscle membrane lysis and membrane inflammation, which that can enter and degrade the contents of injured muscle fibers.,,. These early-invading, phagocytic macrophages reach their highest concentration about 24 hours following the onset of some form of muscle cell injury or reloading. Their concentration rapidly declines after 48 hours. The second group is the non-phagocytic types that are distributed near regenerative fibers. These peak between two and four days and remain elevated for several days during the hopeful muscle rebuilding. The first subpopulation has no direct benefit to repairing muscle, while the second non-phagocytic group does.

It is thought that macrophages release soluble substances that influence the proliferation, differentiation, growth, repair, and regeneration of muscle, but at this time the factor that is produced to mediate these effects is unknown. It is known that macrophages involved in promoting tissue repair is not muscle specific; they accumulate in numerous tissues during the healing process phase following injury.

A study conducted in 2006 showcased macrophage influences on muscle repair of soleus muscle on mice.

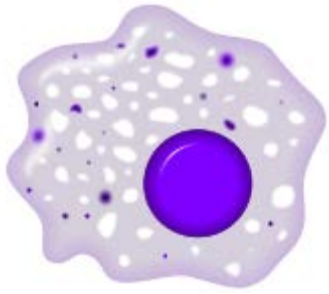
The first procedural step was to make sure macrophages are present in the muscle after onset of muscle injury, and then decrease their presence to see what effects were had on the muscle. By using anti-F4/80 to bind to macrophages and render them useless, it was seen that when the second wave of macrophages were depleted, there were many more lesions in the muscle cell membrane between the second and fourth day – showing muscle damage when repairing is suppose to occur. After testing for membrane lesions in both the total amount of muscle fibers present, it was noticed that most of the damage occurred in muscle cells that did not have the second subpopulation of macrophages present. Macrophages depletion prevents muscle membrane repair.

When examining muscle regeneration, there was a significant reduction in the amount of myonuclei. Depletion of macrophages caused, between the second and fourth day of repair, much less muscle regeneration compared to muscle with macrophage population. Macrophages promote muscle regeneration between the second and fourth day.

To determine the influence of macrophages in muscle growth, muscle cross-sectional area in macrophage-depleted muscle area was measured against two muscle sets: muscle that was injured and had macrophage presence and muscle that was not injured and had macrophage presence. The macrophage-depleted muscle showed less growth after four days, and injured muscle with macrophages nearly grew back to the level of uninjured muscle. Macrophage depletion reduces muscle growth during a growth period.

The study attempted to examine the appearances of Pax7 and MyoD, but data was not consistent with previous findings.

Fixed macrophages



Macrophage

A majority of macrophages are stationed at strategic points where microbial invasion or accumulation of dust is likely to occur. Each type of macrophage, determined by its location, has a specific name:

Name of cell	Location
Dust cells/Alveolar macrophages	pulmonary alveolus of lungs
Histiocytes	connective tissue
Kupffer cells	liver
Microglia	neural tissue
Epithelioid cells	granulomas
Osteoclasts	bone
Sinusoidal lining cells	spleen

Investigations concerning Kupffer cells are hampered because in humans Kupffer cells are only accessible for immunohistochemical analysis from biopsies or autopsies. From

rats and mice they are difficult to isolate and after purification only approximately 5 million cells can be obtained from one mouse.

Macrophages can express paracrine functions within organs that are specific to the function of that organ. In the testis for example, macrophages have been shown to be able to interact with Leydig cells by secreting 25-hydroxycholesterol, an oxysterol that can be converted to testosterone by neighbouring Leydig cells. Also, testicular macrophages may participate in creating an immune privileged environment in the testis, and in mediating infertility during inflammation of the testis.

Involvement in symptoms of diseases

Due to their role in phagocytosis, macrophages are involved in many diseases of the immune system. For example, they participate in the formation of granulomas, inflammatory lesions that may be caused by a large number of diseases.

Some disorders, mostly rare, of ineffective phagocytosis and macrophage function have been described.

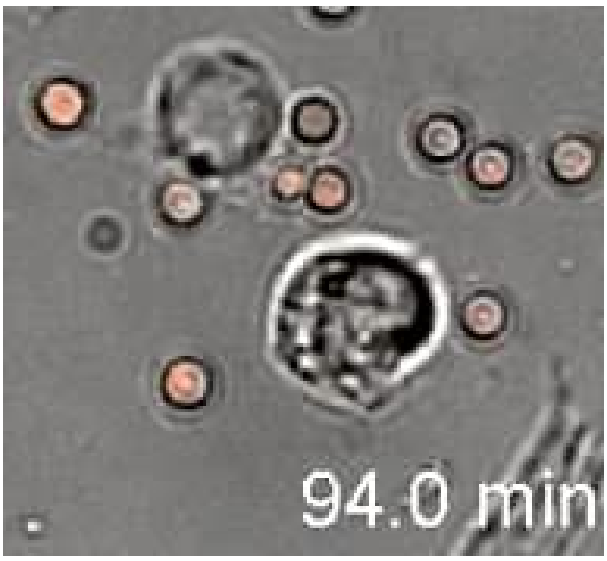
Macrophages are the predominant cells involved in creating the progressive plaque lesions of atherosclerosis.

Macrophages also play a role in Human Immunodeficiency Virus (HIV) infection. Like T cells, macrophages can be infected with HIV, and even become a reservoir of ongoing virus replication throughout the body.

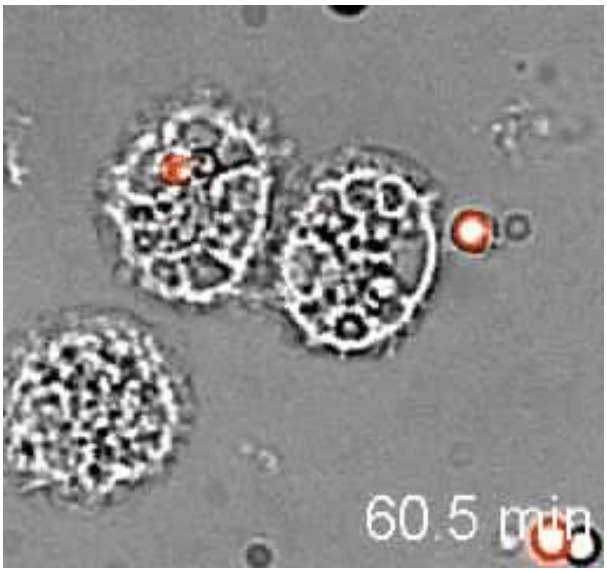
Macrophages are believed to help cancer cells proliferate as well. They are attracted to oxygen-starved (hypoxic) tumour cells and promote chronic inflammation. Inflammatory compounds such as Tumor necrosis factor (TNF) released by the macrophage activates the gene switch nuclear factor-kappa B. NF-κB then enters the nucleus of a tumour cell and turns on production of proteins that stop apoptosis and promote cell proliferation and inflammation.

Recent investigations point a link between streptococcal infection and autoimmune behaving microglia which cause OCD

Media



An active J774 macrophage is seen taking up four
conidia in a cooperative manner. The J774 cells were treated with 5 ng/ml interferon-γ one night before filming with conidia. The observation was made over a period of 2.5 h every 30 s



Two highly active alveolar macrophages can be seen ingesting conidia. Time lapse is 30 s per frame over 2.5 h

Recent research suggests that ferroportin is inhibited by hepcidin, which therefore is the "master regulator" of human iron metabolism. Hepcidin binds to ferroportin, and results in the internalisation of ferroportin within the cell, followed by degradation by the proteasome. This results in retention of iron within the cell, and a reduction in iron levels within the plasma. This is part of the mechanism that causes anaemia of chronic disease; hepcidin is released from the liver in response to inflammatory cytokines, namely

interleukin-6, which results in an increased hepcidin concentration and a consequent decrease in plasma iron levels.

Clinical significance

Mutations in the ferroportin gene are known to cause an autosomal dominant form of iron overload known as Type IV Haemochromatosis or Ferroportin Disease. The effects of the mutations are generally not severe but a spectrum of clinical outcomes are seen with different mutations. Ferroportin is also associated with African iron overload. Ferroportin and hepcidin are critical proteins for the regulation of systemic iron homeostasis. Both ferroportin and hepcidin are expressed in cultured human breast epithelial cells and hepcidin regulates ferroportin in these cells. Transfection of breast cancer cells with ferroportin significantly reduces their growth after orthotopic implantation in the mouse mammary fat pad. Ferroportin is a pivotal protein in breast biology and a strong and independent predictor of prognosis in breast cancer.

Chapter- 4

Hemojuvelin

hemochromatosis type 2 (juvenile)

Identifiers	
Symbols	HFE2; HFE2A; MGC23953; JH; RGMC; HJV
External IDs	OMIM: 608374 MGI: 1916835 HomoloGene: 17060 GeneCards: HFE2 Gene

Orthologs		
Species	**Human**	**Mouse**
Entrez	148738	69585
Ensembl	ENSG00000168509	ENSMUSG00000038403
UniProt	Q6ZVN8	Q7TQ32
RefSeq (mRNA)	NM_145277	NM_027126
RefSeq (protein)	NP_998818	NP_081402
Location (UCSC)	Chr 1: 144.12 - 144.13 Mb	Chr 3: 96.33 - 96.33 Mb

Hemojuvelin (HJV/RGMc/HFE2) is a membrane-bound and soluble protein in mammals that is responsible for the iron overload condition known as juvenile hemochromatosis in humans, a severe form of hemochromatosis. In humans, the hemojuvelin protein is encoded by the *HFE2* gene. HJV is also called RGMc, a member of a three gene family

(in vertebrates) called the repulsive guidance molecules. Both RGMa and RGMb are found in the nervous system, while RGMc is found in skeletal muscle and the liver.

Function

For many years the signal transduction pathways that regulate systemic iron homeostasis have been unknown. However it has been demonstrated that hemojuvelin interacts with bone morphogenetic protein (BMP), possibly as a co-receptor, and may signal via the SMAD pathway to regulate hepcidin expression. Associations with BMP2 and BMP4 have been described.

Mouse HJV knock-out models confirmed that HJV is the gene responsible for juvenile hemochromatosis. Hepcidin levels in the liver are dramatically depressed in these knockout animals.

A soluble form of HJV may be a molecule that suppresses hepcidin expression.

Gene structure and transcription

RGMc/HJV is a 4-exon gene in mammals that undergoes alternative RNA splicing to yield 3 mRNAs with different 5' untranslated regions (5'UTRs). Gene transcription is induced during myoblast differentiation, producing all 3 mRNAs. There are three critical promoter elements responsible for transcriptional activation in skeletal muscle (the tissue that has the highest level of RGMc expressesion per weight), comprising paired E-boxes, a putative Stat and/or Ets element, and a MEF2 site, and muscle transcription factors myogenin and MEF2C stimulate RGMc promoter function in non-muscle cells. As these elements are conserved in RGMc genes from multiple species, these results suggest that RGMc has been a muscle-enriched gene throughout its evolutionary history.

Isoforms

Two classes of GPI-anchored and glycosylated HJV molecules are targeted to the membrane and undergo distinct fates.

- Full-length HJV is released from the cell surface and accumulates in extracellular fluid, where its half-life exceeds 24 hours. There appears to be two potential soluble isoforms and two membrane-associated isoforms.
- The predominant membrane-associated isoform, a disulfide-linked two-chain form composed of N- and C-terminal fragments, is not found in the extracellular fluid, and is short-lived, as it disappears from the cell surface with a half-life of < 3 hours after interruption of protein synthesis.

RGMc appears to undergo a complex processing that generates 2 soluble, single-chain forms, and two membrane-bound forms found as a (i) single-chain, and (ii) two-chain

species which appears to be cleaved at a site within a partial von Willebrand factor domain.

Using a combination of biochemical and cell-based approaches, it has demonstrated that BMP-2 could interact in biochemical assays with the single-chain HJV species, and also could bind to cell-associated HJV. Two mouse HJV amino acid substitution mutants, D165E and G313V (corresponding to human D172E and G320V), also could bind BMP-2, but less effectively than wild-type HJV, while G92V (human G99V) could not. In contrast, the membrane-spanning protein, neogenin, a receptor for the related molecule, RGMa, preferentially bound membrane-associated heterodimeric RGMc and was able to interact on cells only with wild-type RGMc and G92V. These results show that different isoforms of RGMc/HJV may play unique physiological roles through defined interactions with distinct signaling proteins and demonstrate that, in some disease-linked HJV mutants, these interactions are defective.

Structure

The Rosetta ab initio protein structure prediction software has been used to create a three dimensional model of the RGM family of proteins.

Mechanism of action

Furin-like proprotein convertases (PPC) are responsible for conversion of 50 kDa HJV to a 40 kDa protein with a truncated COOH-terminus, at a conserved polybasic RNRR site. This suggests a potential mechanism to generate the soluble forms of HJV/hemojuvelin (s-hemojuvelin) found in the blood of rodents and humans.

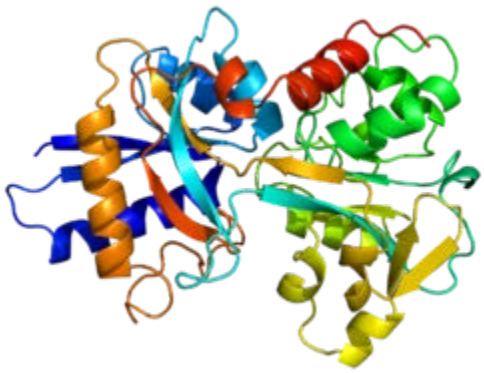
Clinical significance

Mutations in HJV are responsible for the vast majority of juvenile hemochromatosis patients. A small number of patients have mutations in the hepcidin (HAMP) gene. The gene was positionally cloned. Hemojuvelin is highly expressed in skeletal muscle and heart, and to a lesser extent in the liver. One insight into the pathogenesis of juvenile hemochromatosis is that patients have low to undetectable urinary hepcidin levels, suggesting that hemojuvelin is a positive regulator of hepcidin, the central iron regulatory hormone. As a result, low hepcidin levels would result in increased intestinal iron absorption. Thus, HJV/RGMc appears to play a critical role in iron metabolism.

Chapter- 5

Transferrin

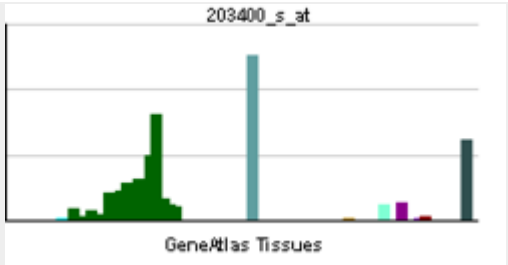
Transferrin

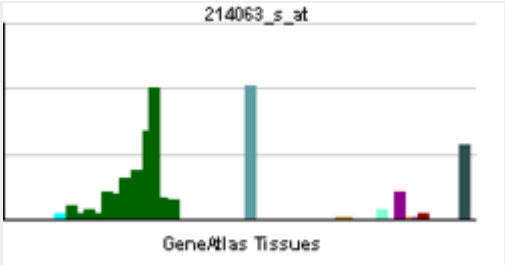


PDB rendering based on 1a8e.

Identifiers	
Symbols	TF; DKFZp781D0156; PRO1557; PRO2086
External IDs	OMIM: 190000 MGI: 98821 HomoloGene: 68153 GeneCards: TF Gene

RNA expression pattern





More reference expression data

Orthologs		
Species	**Human**	**Mouse**
Entrez	7018	22041
Ensembl	ENSG00000091513	ENSMUSG00000032554
UniProt	P02787	Q3UBW7
RefSeq (mRNA)	NM_001063	NM_133977
RefSeq (protein)	NP_001054	NP_598738
Location (UCSC)	Chr 3: 134.95 - 134.98 Mb	Chr 9: 103.07 - 103.09 Mb

Transferrin

Identifiers	
Symbol	Transferrin
Pfam	PF00405
InterPro	IPR001156
PROSITE	PDOC00182
SCOP	1lcf

Transferrins are iron-binding blood plasma glycoproteins that control the level of free iron in biological fluids. In humans, it is encoded by the *TF* gene.

Transferrin is a glycoprotein that binds iron very tightly but reversibly. Although iron bound to transferrin is less than 0.1% (4 mg) of the total body iron, it is the most important iron pool, with the highest rate of turnover (25 mg/24 h). Transferrin has a molecular weight of around 80 kDa and contains 2 specific high-affinity Fe(III) binding sites. The affinity of transferrin for Fe(III) is extremely high (10^{23} M^{-1} at pH 7.4) but decreases progressively with decreasing pH below neutrality.

When not bound to iron, it is known as "apo-transferrin".

Transport mechanism

When a transferrin protein loaded with iron encounters a transferrin receptor on the surface of a cell (e.g., to erythroid precursors in the bone marrow), it binds to it and, as a consequence, is transported into the cell in a vesicle by receptor-mediated endocytosis. The pH of the vesicle is reduced by hydrogen ion pumps (H^+ ATPases), causing transferrin to release its iron ions. The receptor (with its ligand, transferrin, bound) is then transported through the endocytic cycle back to the cell surface, ready for another round of iron uptake. Each transferrin molecule has the ability to carry two iron ions in the ferric form (Fe^{3+}).

The gene coding for transferrin in humans is located in chromosome band 3q21. Research on kingsnakes by Dessauer and Zwiefel in 1981 revealed that the inheritance of transferrin is a codominant trait.

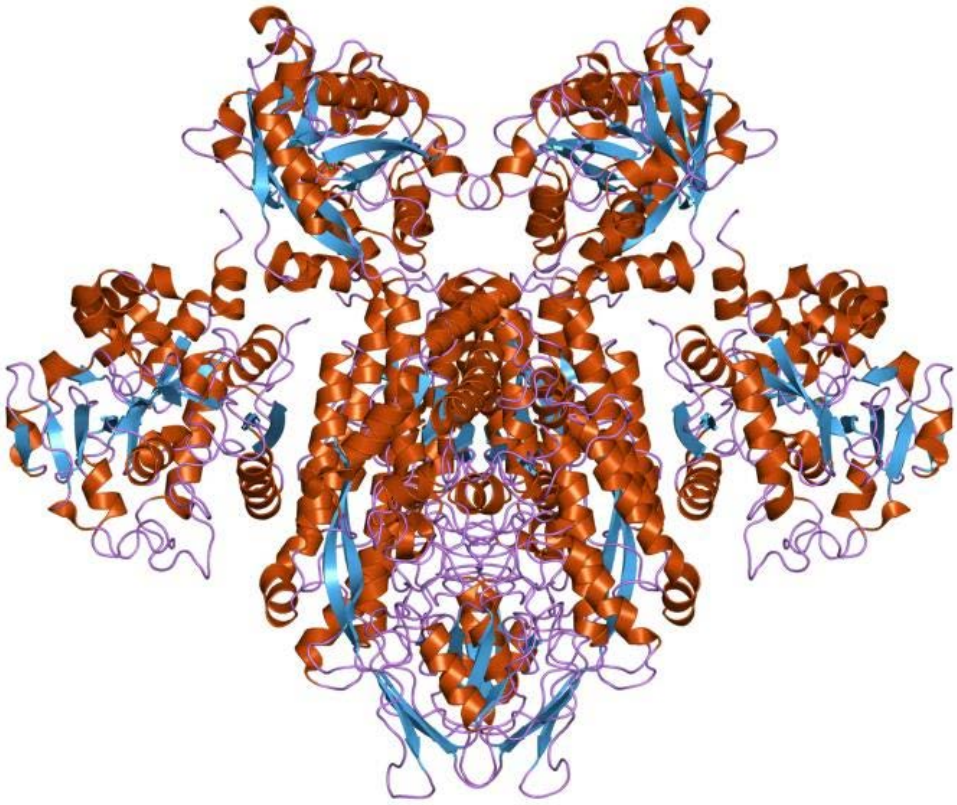
Medical professionals may check serum transferrin level in iron deficiency, hemochromatosis, and other iron overload disorders.

Structure

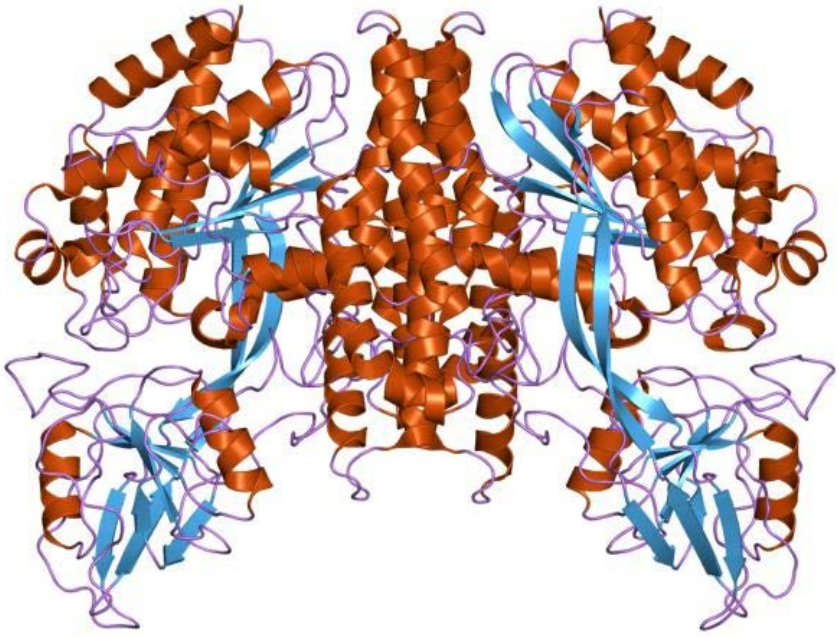
In humans, transferrin consists of a polypeptide chain containing 679 amino acids. It is a complex composed of alpha helices and beta sheets to form two domains (the first situated in the N-terminus and the second in the C-terminus). The N- and C- terminal sequences are represented by globular lobes and between the two lobes is an iron-binding site.

The amino acids which bind the iron ion to the transferrin are identical for both lobes; two tyrosines, one histidine, and one aspartic acid. In order for the iron ion to bind an anion is required, preferably carbonate (CO_3^{2-}).

Transferrin also has a transferrin iron-bound receptor; it is a disulfide-linked homodimer. In humans, each monomer consists of 760 amino acids. It enables ligand bonding to the transferrin, as each monomer can bind to one or two molecules of iron. Each monomer consists of three domains: the protease domain, the helical domain, and apical domain. The shape of transferrin receptor resembles a butterfly-like complex, due to the three clearly shaped domains.



Transferrin bound to its receptor.



Transferrin receptor complex.

Tissue distribution

The liver is the main source of manufacturing transferrin, but other sources such as the brain also produce this molecule . The main role of transferrin is to deliver iron from absorption centres in the duodenum and red blood cell macrophages to all tissues. Predominantly, transferrin plays a key role where erythropoiesis and active cell division occur. In order for iron ion to be introduced into the cell a carrier protein is used, known as a transferrin receptor. The receptor helps maintain iron homeostasis in the cells by controlling iron concentrations.

Immune system

Transferrin is also associated with the innate immune system. Transferrin is found in the mucosa and binds iron, thus creating an environment low in free iron that impedes bacteria survival. The levels of transferrin decreases in inflammation, seeming contradictory to its function.

Transferrin imbalance can have serious health effects for those with low or high serum transferrin levels. A patient with an increased serum transferrin level often suffers from

iron deficiency anemia. A patient with decreased plasma transferrin can suffer from iron overload diseases and protein malnutrition. An absence of transferrin in the body creates a rare genetic disorder known as atransferrinemia; a condition characterized by anemia and hemosiderosis in the heart and liver that leads to many complications including heart failure. Most recently, transferrin and its receptor have been tested to diminish tumour cells by using the receptor to attract antibodies.

Other effects

The metal binding properties of transferrin have a great influence on the biochemistry of plutonium in humans. Transferrin has a bacteriocidal effect on bacteria, in that it makes Fe^{3+} unavailable to the bacteria.

Carbohydrate deficient transferrin increases in the blood with heavy ethanol consumption and provides a laboratory test to help detect it.

Pathology

A deficiency is associated with atransferrinemia.

Reference ranges

Normal reference ranges for transferrin are 204–360 mg/dL.

Reference ranges for blood tests, comparing blood content of transferrin and other iron-related compounds (shown in brown and orange) with other constituents.

A high transferrin level may indicate iron deficiency anemia. Levels of serum iron and total iron binding capacity (TIBC) are used in conjunction with transferrin to specify any abnormality.

Interactions

Transferrin has been shown to interact with Insulin-like growth factor 2 and IGFBP3. Transcriptional regulation of transferrin is upregulated by Retinoic Acid

Related proteins

Members of the family include blood serotransferrin (siderophilin); milk lactotransferrin (lactoferrin); egg white ovotransferrin (conalbumin); and membrane-associated melanotransferrin.

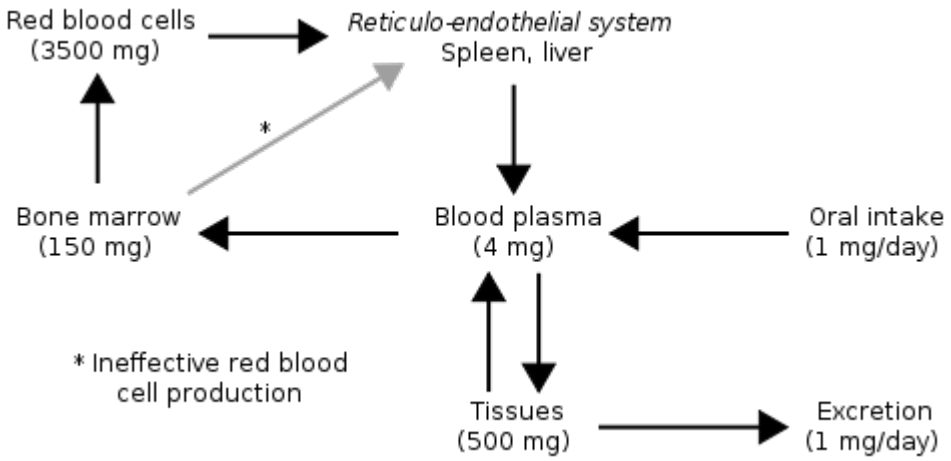
Chapter- 6

HFE Hereditary Hemochromatosis

Hemochromatosis type 1	
ICD-10	E83.1
ICD-9	275.0
OMIM	235200
DiseasesDB	5490
eMedicine	med/975 derm/878
MeSH	D006432

Hemochromatosis type 1 (or **HFE hereditary hemochromatosis**, or **HFE-related hereditary haemochromatosis**) is a hereditary disease characterized by excessive absorption of dietary iron resulting in a pathological increase in total body iron stores. Humans, like most animals, have no means to excrete excess iron. Excess iron accumulates in tissues and organs disrupting their normal function. The most susceptible organs include the liver, adrenal glands, heart, skin, gonads, joints, and the pancreas; patients can present with cirrhosis, polyarthropathy, adrenal insufficiency, heart failure or diabetes. The hereditary form of the disease is most common among those of Northern European ancestry, in particular those of Celtic descent.

Pathophysiology



The normal distribution of body iron stores

Since the regulation of iron metabolism is still poorly understood, a clear model of how haemochromatosis operates is still not available. A working model describes the defect in the HFE gene, where a mutation puts the intestinal absorption of iron into overdrive. Normally, HFE facilitates the binding of transferrin, which is iron's carrier protein in the blood. Transferrin levels are typically elevated at times of iron depletion (low ferritin stimulates the release of transferrin from the liver). When transferrin is high, HFE works to increase the intestinal release of iron into the blood. When HFE is mutated, the intestines perpetually interpret a strong transferrin signal as if the body were deficient in iron. This leads to maximal iron absorption from ingested foods and iron overload in the tissues. However, HFE is only part of the story, since many patients with mutated HFE do not manifest clinical iron overload, and some patients with iron overload have a normal HFE genotype. A possible explanation is the fact that HFE normally plays a role in the production of hepcidin in the liver, a function that is impaired in HFE mutations.

People with abnormal iron regulatory genes do not reduce their absorption of iron in response to increased iron levels in the body. Thus the iron stores of the body increase. As they increase, the iron which is initially stored as ferritin is deposited in organs as haemosiderin and this is toxic to tissue, probably at least partially by inducing oxidative stress. Iron is a pro-oxidant. Thus, haemochromatosis shares common symptomology (e.g., cirrhosis and dyskinetic symptoms) with other "pro-oxidant" diseases such as Wilson's disease, chronic manganese poisoning, and hyperuricaemic syndrome in Dalmatian dogs. The latter also experience "bronzing".

Terminology

The term "hemochromatosis" is used by many different sources in many different ways.

It is often used to imply an association with the HFE gene. For many years, HFE was the only known gene associated with hemochromatosis, and the term "hereditary

hemochromatosis" was used to describe hemochromatosis type 1. However, it is now known that there are many different genetic associations with this condition. The older the text, or the more general the audience, the more likely that HFE is implied.

The term "hemochromatosis" has also been in contexts where there had not been a known genetic association for the iron accumulation. However, it should be noted that in some cases, the understanding of a condition that was considered due to behavior can be refined to accommodate new known genetic associations, as in African iron overload.

History

The disease was first described in 1865 by Armand Trousseau in a report on diabetes in patients presenting with a bronze pigmentation of their skin. Trousseau did not associate diabetes with iron accumulation; the recognition that infiltration of the pancreas with iron might disrupt endocrine function resulting in diabetes was made by Friedrich Daniel von Recklinghausen in 1890.

Signs and symptoms

Haemochromatosis is protean in its manifestations, *i.e.*, often presenting with signs or symptoms suggestive of other diagnoses that affect specific organ systems. Many of the signs and symptoms below are uncommon and most patients with the hereditary form of haemochromatosis do not show any overt signs of disease nor do they suffer premature morbidity.

The classic triad of cirrhosis, bronze skin and diabetes is not as common anymore because of earlier diagnosis.

The more common clinical manifestations include:

- Fatigue
- Malaise
- Liver cirrhosis (with an increased risk of hepatocellular carcinoma) Liver disease is always preceded by evidence of liver dysfunction including elevated serum enzymes specific to the liver. Presence of Cirrhosis can also be discovered by the victim suffering from jaundice (yellowing of the skin).
- Insulin resistance (often patients have already been diagnosed with diabetes mellitus type 2) due to pancreatic damage from iron deposition
- Erectile dysfunction and hypogonadism, resulting in decreased libido
- Congestive heart failure, arrhythmias or pericarditis
- Arthritis of the hands (especially the second and third MCP joints), but also the knee and shoulder joints
- Damage to the adrenal gland, leading to adrenal insufficiency

Less common findings including:

- Deafness
- Dyskinesias, including Parkinsonian symptoms
- Dysfunction of certain endocrine organs:
    - Parathyroid gland (leading to hypocalcaemia)
    - Pituitary gland
- More commonly a slate-gray or less commonly darkish colour to the skin
- An increased susceptibility to certain infectious diseases caused by siderophilic microorganisms:
    - *Vibrio vulnificus* infections from eating seafood or wound infection
    - *Listeria monocytogenes*
    - *Yersinia enterocolica*
    - *Salmonella enterica* (serotype Typhymurium)
    - *Klebsiella pneumoniae*
    - *Escherichia coli*
    - *Rhizopus arrhizus*
    - *Mucor* species

Males are usually diagnosed after their forties and fifties, and women several decades later, owing to regular iron loss through menstruation (which ceases in menopause). The severity of clinical disease in the hereditary form varies considerably. There is evidence suggesting that hereditary haemochromatosis patients affected with other liver ailments such as hepatitis or alcoholic liver disease suffer worse liver disease than those with either condition alone. There are also juvenile forms of hereditary haemochromatosis that present in childhood with the same consequences of iron overload.

Diagnosis

The diagnosis of haemochromatosis is often made following the incidental finding on routine blood screening of elevated serum liver enzymes or elevation of the transferrin saturation. Arthropathy with stiff joints, diabetes, or fatigue, may be the presenting complaint.

Blood tests

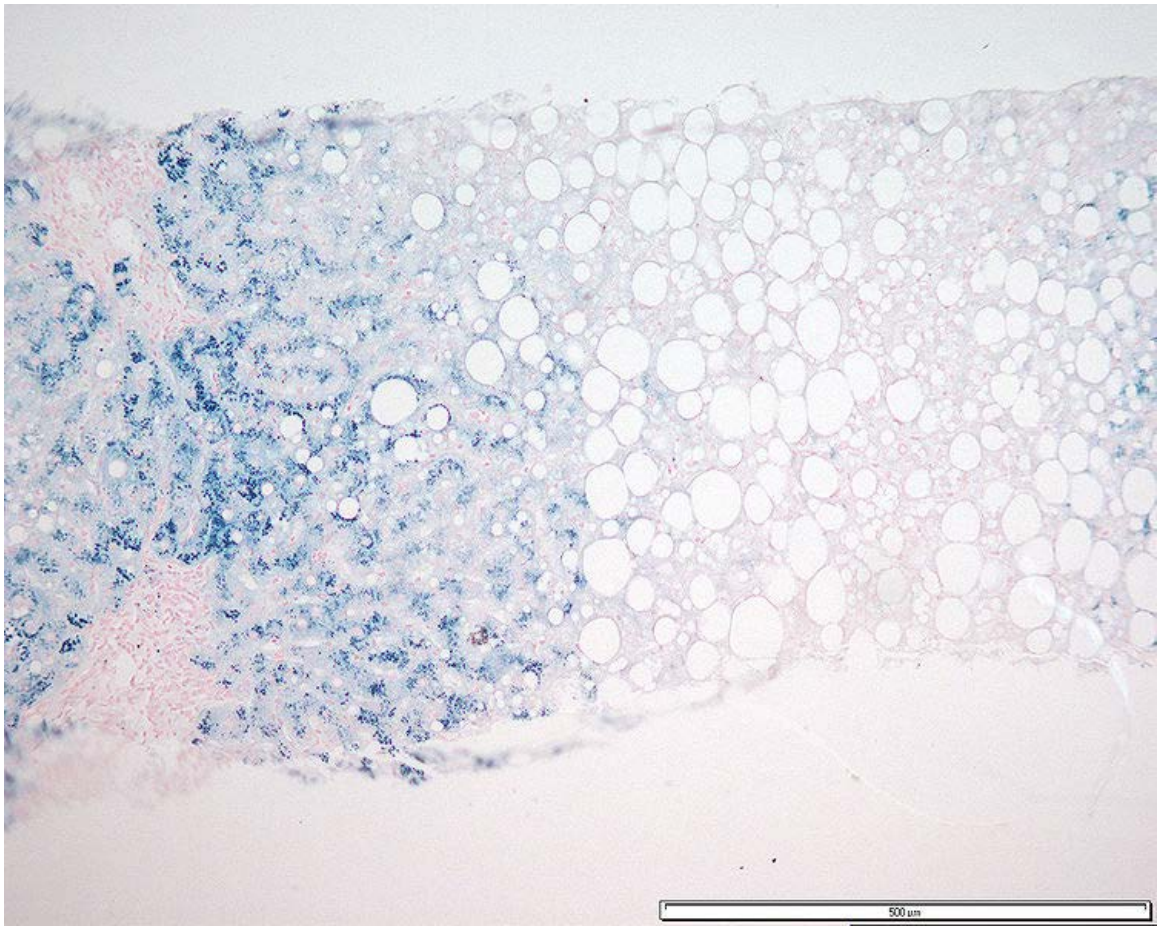
Serum transferrin and transferrin saturation are commonly used as screening for haemochromatosis. Transferrin binds iron and is responsible for iron transport in the blood. Measuring transferrin provides a crude measure of iron stores in the body. Fasting transferrin saturation values in excess of 45% (or 35% in premenopausal women) are recognized as a threshold for further evaluation of haemochromatosis. Transferrin saturation greater than 62% is suggestive of homozygosity for mutations in the HFE gene.

Serum Ferritin: Ferritin, a protein synthesized by the liver is the primary form of iron storage within cells and tissues. Measuring ferritin provides another crude estimate of whole body iron stores though many conditions notably inflammation can elevate serum ferritin. Normal values for males are 12–300 ng/ml (nanograms per milliliter) and for

female, 12–150 ng/ml. Serum ferritin in excess of 1000 nanograms per millilitre of blood is almost always attributable to haemochromatosis.

Other blood tests routinely performed: blood count, renal function, liver enzymes, electrolytes, glucose (and/or an oral glucose tolerance test (OGTT)).

Liver biopsy



Iron accumulation demonstrated by Prussian blue staining in a patient with homozygous genetic hemochromatosis (microscopy, 10x magnified). Parts of normal pink tissue are scarcely present.

Liver biopsies involve taking a sample of tissue from the liver, using a thin needle. The amount of iron in the sample is then quantified and compared to normal, and evidence of liver damage, especially cirrhosis, measured microscopically. Formerly, this was the only way to confirm a diagnosis of haemochromatosis but measures of transferrin and ferritin along with a history are considered adequate in determining the presence of the malady. Risks of biopsy include bruising, bleeding and infection. Now, when a history and measures of transferrin or ferritin point to haemochromatosis, it is debatable whether a liver biopsy is still necessary to quantify the amount of accumulated iron.

MRI

FerriScan is a MRI-based test to non-invasively and accurately measure liver iron concentrations. It is safer and generally cheaper to perform than liver biopsy; does not suffer from problems with sampling variability; and can be used more frequently than performing liver biopsies. FerriScan has regulatory approval in the USA, Canada, Europe and Australasia. A FerriScan takes about 10 minutes to perform and doesn't require any contrast agents. It can be quickly and easily established on most 1.5 T MRI scanners.

Other Imaging

Clinically the disease may be silent, but characteristic radiological features may point to the diagnosis. The increased iron stores in the organs involved, especially in the liver and pancreas, result in characteristic findings on unenhanced CT and a decreased signal intensity in MRI scans. Haemochromatosis arthropathy includes degenerative osteoarthritis and chondrocalcinosis. The distribution of the arthropathy is distinctive, but not unique, frequently affecting the second and third metacarpophalangeal joints of the hand. The arthropathy can therefore be an early clue as to the diagnosis of haem-ochromatosis.

Functional testing

Based on the history, the doctor might consider specific tests to monitor organ dysfunction, such as an echocardiogram for heart failure, or blood glucose monitoring for patients with haemochromatosis diabetes.

Differential diagnosis

There exist other causes of excess iron accumulation, which have to be considered before haemochromatosis is diagnosed.

- **African iron overload**, formerly known as Bantu siderosis, was first observed among people of African descent in Southern Africa. Originally, this was blamed on ungalvanised barrels used to store home-made beer, which led to increased oxidation and increased iron levels in the beer. Further investigation has shown that only some people drinking this sort of beer get an iron overload syndrome, and that a similar syndrome occurred in people of African descent who have had no contact with this kind of beer (*e.g.,* African Americans). This led investigators to the discovery of a gene polymorphism in the gene for ferroportin which predisposes some people of African descent to iron overload.
- **Transfusion hemosiderosis** is the accumulation of iron, mainly in the liver, in patients who receive frequent blood transfusions (such as those with thalassemia).
- **Dyserythropoeisis**, also known as myelodysplastic syndrome is a disorder in the production of red blood cells. This leads to increased iron recycling from the bone marrow and accumulation in the liver.

End-organ damage

Iron is stored in the liver, the pancreas and the heart. Long term effects of haemochromatosis on these organs can be very serious, even fatal when untreated. For example, similar to alcoholism, haemochromatosis can cause cirrhosis of the liver. The liver is a primary storage area for iron and will naturally accumulate excess iron. Over time the liver is likely to be damaged by iron overload. Cirrhosis itself may lead to additional and more serious complications, including bleeding from dilated veins in the oesophagus and stomach (varices) and severe fluid retention in the abdomen (ascites). Toxins may accumulate in the blood and eventually affect mental functioning. This can lead to confusion or even coma (hepatic encephalopathy).

Liver cancer: Cirrhosis and haemochromatosis together will increase the risk of liver cancer. (Nearly one-third of people with haemochromatosis and cirrhosis eventually develop liver cancer.)

Diabetes: The pancreas which also stores iron is very important in the body's mechanisms for sugar metabolism. Diabetes affects the way the body uses blood sugar (glucose). Diabetes is in turn the leading cause of new blindness in adults and may be involved in kidney failure and cardiovascular disease.

Congestive heart failure: If excess iron in the heart interferes with the its ability to circulate enough blood, a number of problems can occur including death. The condition may be reversible when haemochromatosis is treated and excess iron stores reduced.

Heart arrhythmias: Arrhythmia or abnormal heart rhythms can cause heart palpitations, chest pain and light-headedness and are occasionally life threatening. This condition can often be reversed with treatment for haemochromatosis.

Pigment changes: Bronze or gray coloration of the skin is caused primarily by increased melanin deposition, with iron deposition playing a lesser role.

Treatment

Phlebotomy

Early diagnosis is important because the late effects of iron accumulation can be wholly prevented by periodic phlebotomies (by venesection) comparable in volume to blood donations. Treatment is initiated when ferritin levels reach 300 milligrams per litre (or 200 in nonpregnant premenopausal women).

Every bag of blood (450–500 ml) contains 200–250 milligrams of iron. Phlebotomy (or bloodletting) is usually done at a weekly interval until ferritin levels are less than 20 milligrams per litre. After that, 1–6 donations per year are usually needed to maintain iron balance.

Desferrioxamine mesilate

Where venesection is not possible, long-term administration of desferrioxamine mesilate is useful. Desferrioxamine is an iron-chelating compound, and excretion induced by desferrioxamine is enhanced by administration of Vitamin C. It cannot be used during pregnancy or breast-feeding due to risk of defects in the child.

Treatment of organ damage

- Treatment of organ damage (heart failure with diuretics and ACE inhibitor therapy).

Diet

- Limiting intake of alcoholic beverages, vitamin C (increases iron absorption in the gut), red meat (high in iron) and potential causes of food poisoning (shellfish, seafood).
- Increasing intake of substances that inhibit iron absorption, such as high-tannin tea, calcium, and foods containing oxalic and phytic acids (such as collard greens, which must be consumed at the same time as the iron-containing foods in order to be effective).

Screening

Standard diagnostic measures for haemochromatosis, transferrin saturation and ferritin tests, are not a part of routine medical testing. Screening for haemochromatosis is recommended if the patient has a parent, child or sibling with the disease.

Routine screening of the general population for hereditary haemochromatosis is generally not done. Mass genetic screening has been evaluated by the U.S. Preventive Services Task Force (USPSTF), among other groups. The USPSTF recommended against genetic screening of the general population for hereditary haemochromatosis because the likelihood of discovering an undiagnosed patient with clinically relevant iron overload is less than 1 in 1,000. Although there is strong evidence that treatment of iron overload can save lives in patients with transfusional iron overload, no clinical study has shown that for asymptomatic carriers of hereditary haemochromatosis treatment with venesection (phlebotomy) provides any clinical benefit. Recently, it has been suggested that patients be screened for iron overload using serum ferritin as a marker: If serum ferritin exceeds 1000 ng/mL, iron overload is very likely the cause.

Epidemiology

Haemochromatosis is one of the most common heritable genetic conditions in people of northern European extraction with a prevalence of 1 in 200. The disease has a variable penetration and about 1 in 10 people of this demographic carry a mutation in one of the

genes regulating iron metabolism, the most common allele being the C282Y allele in the *HFE* gene. The prevalence of mutations in iron metabolism genes varies in different populations. A study of 3,011 unrelated white Australians found that 14% were heterozygous carriers of an HFE mutation, 0.5% were homozygous for an *HFE* mutation, and only 0.25% of the study population had clinically relevant iron overload. Most patients who are homozygous for HFE mutations will not manifest clinically relevant haemochromatosis. Other populations have a lower prevalence of both the genetic mutation and the clinical disease.

Genetic studies suggest the original haemochromatosis mutation arose in a single person, possibly of Celtic ethnicity, who lived 60–70 generations ago. At that time when dietary iron may have been scarcer than today, the presence of the mutant allele may have provided a natural selection reproductive advantage by maintaining higher iron levels in the blood.

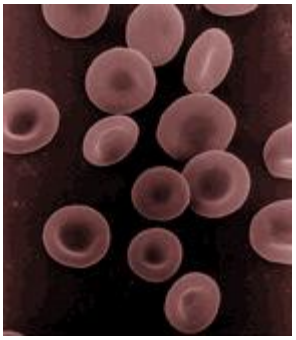
Genetics

The regulation of dietary iron absorption is complex and our understanding is incomplete. One of the better characterized genes responsible for hereditary hemochromatosis is HFE on chromosome 6 which codes for a protein that participates in the regulation of iron absorption. The HFE gene has two common alleles, C282Y and H63D. The C282Y allele is a transition point mutation from guanine to adenine at nucleotide 845 in the HFE gene, resulting in a missense mutation that replaces the cysteine residue at position 282 with a tyrosine amino acid. Heterozygotes for either allele do not manifest clinical iron overload but may display an increased iron uptake. Mutations of the *HFE* gene account for 90% of the cases of non-transfusional iron overload. This gene is closely linked to the HLA-A3 locus. Homozygosity for the C282Y mutation is the most common genotype responsible for clinical iron accumulation, though heterozygosity for C282Y/H63D mutations, so-called compound heterozygotes, results in clinically evident iron overload. There is considerable debate regarding the penetrance—the probability of clinical expression of the trait given the genotype—is for clinical disease in HHC homozygotes. Most, if not all, males homozygous for HFE C282Y will show manifestations of liver dysfunction such as elevated liver-specific enzymes such as serum gamma glutamyltransferase (GGT) by late middle age. Homozygous females can delay the onset of iron accumulation because of iron loss through menstruation. Each patient with the susceptible genotype accumulates iron at different rates depending on iron intake, the exact nature of the mutation and the presence of other insults to the liver such as alcohol and viral disease. As such the degree to which the liver and other organs is affected, expressivity, is highly variable and is dependent on such these other factors and co-morbidities as well as age at which they are studied for manifestations of disease. Penetrance differs between different populations.

Chapter- 7

Iron Deficiency Anemia

Iron deficiency anemia



Red blood cells

ICD-10	D50.
ICD-9	280
DiseasesDB	6947
eMedicine	med/1188
MeSH	D018798

Iron deficiency anemia (or **iron deficiency anaemia**) is a common anemia that occurs when iron loss (often from intestinal bleeding or menses) occurs, and/or the dietary intake or absorption of iron is insufficient. In such a state, haemoglobin, which contains iron, cannot be formed.

Iron deficiency is the most common single cause of anemia worldwide, accounting about about half of anemia. Estimates of iron deficiency world wide range very widely, but the the number almost certainly exceeds one billion persons globally.. Worldwide, the most important cause of iron deficiency anemia is parasitic infection caused by hookworms, whipworms, and roundworms, in which intestinal bleeding caused by the worms can lead

to undetected blood loss in the stool. These are especially important problems in growing children. Chronic inflammation caused by hookworms and malaria infections contributes to anemia during pregnancy in most developing countries. In adults of post-menopausal age (over 50 years old) the most common cause of iron-deficiency anemia is chronic gastrointestinal bleeding from nonparasitic causes, such as from gastric ulcer, duodenal ulcer or a gastrointestinal cancer.

In the developed world, where intestinal worm parasite burden is less than in many undeveloped countries, about 20% of all women of childbearing age have iron deficiency anemia, compared with only 3% of adult men. The principal cause of iron deficiency anemia in premenopausal women is blood lost during menses.

Iron deficiency *anemia* is an advanced stage of iron deficiency. When the body has sufficient iron to meet its needs (functional iron), the remainder is stored for later use in the bone marrow, liver, and spleen as part of a finely tuned system of human iron metabolism. Iron deficiency ranges from iron depletion, which yields little physiological damage, to iron deficiency anemia, which can affect the function of numerous organ systems. Iron depletion causes the amount of stored iron to be reduced, but has no effect on the functional iron. However, a person with no stored iron has no reserves to use if the body enters a state in which it requires more iron than is being absorbed from the diet.

Symptoms and Signs

Iron deficiency anemia is characterized by pallor (reduced amount of oxyhemoglobin in skin or mucous membrane), fatigue and weakness. Because it tends to develop slowly, adaptation occurs and the disease often goes unrecognized for some time. In severe cases, dyspnea (trouble breathing) can occur. Unusual obsessive food cravings, known as pica, may develop. Pagophagia or pica for ice is a very specific symptom and may disappear with correction of iron deficiency anemia. Hair loss and lightheadedness can also be associated with iron deficiency anemia.

Other symptoms and signs of iron deficiency anemia include:

- Constipation
- Sleepiness
- Tinnitus
- Palpitations
- Hair loss
- Fainting or feeling faint
- Depression
- Breathlessness on exertion.
- Twitching muscles
- Tingling, numbness, or burning sensations
- Missed menstrual cycle
- Heavy menstrual period
- Slow social development

- Glossitis (inflammation or infection of the tongue)
- Angular cheilitis (inflammatory lesions at the mouth's corners)
- Koilonychia (spoon-shaped nails) or nails that are weak or brittle
- Poor appetite
- Pruritus (Itchiness)
- Dysphagia due to formation of esophageal webs (Plummer-vinson syndrome).
- Angular stomatitis
- RLS (Restless Leg Syndrome)

Infant development

Iron deficiency anemia for infants in their earlier stages of development may have significantly greater consequences than it does for adults. An animal made severely iron deficient during its earlier life cannot recover to normal iron levels even with iron therapy. In contrast, iron deficiency during later stages of development can be compensated with sufficient iron supplements. Iron deficiency anemia affects neurological development by decreasing learning ability, altering motor functions, and permanently reducing the number of dopamine receptors and serotonin levels. Iron deficiency during development can lead to reduced myelination of the spinal cord, as well as a change in myelin composition. Additionally, iron deficiency anemia has a negative effect on physical growth. Growth hormone secretion is related to serum transferrin levels, suggesting a positive correlation between iron-transferrin levels and an increase in height and weight.

Cause

The diagnosis of iron deficiency anemia requires further investigation as to its cause. Iron deficiency can be caused by increased iron demand or decreased iron intake, and can occur in both children and adults.

The most important cause of iron deficiency world-wide is infestation with parasitic worms (hookworms, whipworms, roundworms). Esimates of infection in the world population vary from a minimum of a billion humans to as many as 5 million out of a total of 6 billion.. In addition to parasitiosis, dietary insufficiency, malabsorption, chronic blood loss, diversion of iron to fetal erythropoiesis during pregnancy, intravascular hemolysis and hemoglobinuria or other forms of chronic blood loss should all be considered, according to the patient's sex, age, and history. Other common causes include gastrointestinal blood loss due to drug therapy (often in the case of NSAIDs or aspirin), and hypochlorhydria/achlorhydria (often due to long-term proton pump inhibitor therapy). In babies and adolescents, rapid growth may outpace dietary intake of iron, and result in deficiency without disease or grossly abnormal diet.

Especially in adults over the age of 50, iron deficiency is often a sign of other disease in the gastrointestinal tract, such as chronic bleeding from any cause (for example, a colon cancer) that causes loss of blood in the stool. Such loss is often undetectable, except with special testing. In adults, 60% of patients with iron deficiency anemia have underlying

gastrointestinal disorders leading to chronic blood loss, and this percentage increases with patient age. Particularly, iron deficiency in adult men from purely dietary causes is quite rare, and in such cases, other causes of iron loss must be vigorously sought until found.

Diagnosis

Anemia may be diagnosed from symptoms and signs, but when anemia is mild it may not be diagnosed from mild non-specific symptoms. Pica, an abnormal craving for dirt, ice, or other "odd" foods occurs variably in iron and zinc deficiency, but is neither sensitive or specific to the problem so is of little diagnostic help.

Anemia is often first shown by routine blood tests, which generally include a complete blood count (CBC) which is performed by an instrument which gives an output as a series of index numbers. A sufficiently low hemoglobin (HGB) by definition makes the diagnosis of anemia, and a low hematocrit (HCT) value is also characteristic of anemia. Further studies will be undertaken to determine the anemia's cause. If the anemia is due to iron deficiency, one of the first abnormal values to be noted on a CBC, as the body's iron stores begin to be depleted, will be a high red blood cell distribution width (RDW), reflecting a varied size distribution of red blood cells (RBCs). In the course of slowly depleted iron status, an increasing RDW normally appears even before anemia appears.

A low mean corpuscular volume (abbreviated MCV) often appears next during the course of body iron depletion. It is the result of many red blood cells which are abnormally small. A low MCV, a low mean corpuscular hemoglobin (MCH) and/or Mean corpuscular hemoglobin concentration (MCHC), and the appearance of the RBCs on visual examination of a peripheral blood smear narrows the problem to a microcytic anemia (literally, a "small red blood cell" anemia). The numerical values for red blood count, blood hemoglobin, MCV, MCH, MCHC are all calculated by modern laboratory equipment.

The blood smear of a patient with iron deficiency shows many hypochromic (pale and relatively colorless) and rather small RBCs, and may also show poikilocytosis (variation in shape) and anisocytosis (variation in size). With more severe iron deficiency anemia the peripheral blood smear may show target cells, hypochromic pencil-shaped cells, and occasionally small numbers of nucleated red blood cells. Very commonly, the platelet count is slightly over normal limits in iron deficiency anemia (mild thrombocytosis, an effect which was classically postulated to be to high erythropoietin levels in the body as a result of anemia, cross-reacting to activate thrombopoietin receptors in precursor cells which make platelets. (Such slightly high platelet counts present no danger, but are valuable as a marker). However, this mechanistic effect has been searched for and not corroborated.

The diagnosis of iron deficiency anemia will be suggested by appropriate history (e.g., anemia in a menstruating woman or an athlete engaged in long distance running), the presense of occult blood (i.e., hidden blood) in the stool, and often by other history. For example, known celiac disease can cause malabsorption of iron. A travel history to areas

in which hookworm and whipworm are endemic, may be helpful in guiding certain stool tests for parasites or their eggs.

Iron deficiency is diagnosed by diagnostic tests as a low serum ferritin, a low serum iron level, an elevated serum transferrin and a high total iron binding capacity (TIBC). Serum ferritin is the most sensitive lab test for iron deficiency anemia, however serum ferritin can be elevated by any type of chronic inflammation, and so is not always a reliable test of iron status if it is within normal limits (i.e., this test is meaningful if abnormal, but less meaningful if normal). The ratio of serum iron to TIBC (sometimes called iron saturation or transferrin saturation index or percent) is the most specific indicator of iron deficiency, when it is sufficiently low. The iron saturation (or transferrin saturation) of < 5% almost always indicates iron deficiency, while levels from 5% to 10% make the diagnosis of iron deficiency possible, but not definitive. Saturations over 12% (taken alone) make the diagnosis unlikely. Normal saturations are usually slightly higher for women (>12%) than for men (>15%), but this may simply indicate an overall slight poorer iron status for women in the "normal" population.

Change in lab values in iron deficiency anemia

Change	Parameter
Decrease	ferritin, hemoglobin, MCV
Increase	TIBC, transferrin, RDW

Iron deficient anemia and thalassemia minor present with many of the same lab results. It is very important not to treat a patient with thalassemia with an iron supplement as this can lead to hemochromatosis (accumulation of iron in various organs especially liver). A hemoglobin electrophoresis would provide useful evidence in distinguishing these two conditions, along with iron studies.

Gold standard

Traditionally, a definitive diagnosis requires a demonstration of depleted body iron stores by performing a bone marrow aspiration, with the marrow stained for iron. Because this is invasive and painful, while a clinical trial of iron supplementation is inexpensive and non-traumatic, patients are often treated based on clinical history and serum ferritin levels without a bone marrow biopsy. Furthermore, a study published April 2009 questions the value of stainable bone marrow iron following parenteral iron therapy.

Treatment

If the cause is dietary iron deficiency, eating more iron-rich foods such as beans and lentils or taking iron supplements, usually with iron(II) sulfate, ferrous gluconate, or iron amino acid chelate ferrous bisglycinate, synthetic chelate NaFerredetate EDTA will usually correct the anemia.

Recent research suggests the replacement dose of iron, at least in the elderly with iron deficiency, may be as little as 15 mg per day of elemental iron. An experiment done in a group of 130 anemia patients showed a 98% increase in iron count when using an iron supplement with an average of 100 mg of iron. Women who develop iron deficiency anemia in mid-pregnancy can be effectively treated with low doses of iron (20–40 mg per day). The lower dose is effective and produces fewer gastrointestinal complaints. There is evidence that the the body adapts to oral iron supplementation, so that iron is often effectively started at a comparitively low dose, then slowly increased.

There can be a great difference between iron intake and iron absorption, also known as bioavailability. Scientific studies indicate iron absorption problems when iron is taken in conjunction with milk, tea, coffee and other substances. There are already a number of proven solutions for this problem, including:

- Fortification with ascorbic acid, which increases bioavailability in both presence and absence of inhibiting substances, but which is subject to deterioration from moisture or heat. Ascorbic acid fortification is usually limited to sealed dried foods, but individuals can easily take ascorbic acid with basic iron supplement for the same benefits.
- Microencapsulation with lecithin, which binds and protects the iron particles from the action of inhibiting substances. The primary benefit over ascorbic acid is durability and shelf life, particularly for products like milk which undergo heat treatment.
- Using an iron amino acid chelate, such as NaFeEDTA, which similarly binds and protects the iron particles. A study performed by the Hematology Unit of the University of Chile indicates that chelated iron (ferrous bis-glycine chelate) can work with ascorbic acid to achieve even higher absorption levels
- Separating intake of iron and inhibiting substances by a couple of hours.
- Using non-dairy milk (such as soy, rice, or almond milk) or goats' milk instead of cows' milk.
- Gluten-free diet resolves some instances of iron-deficiency anemia, especially if the anemia is a result of celiac disease.
- It is believed that "heme iron", found only in animal foods such as meat, fish and poultry, is more easily absorbed than "non-heme" iron, found in plant foods and supplements.

Iron bioavailability comparisons require stringent controls, because the largest factor affecting bioavailability is the subject's existing iron levels. Informal studies on bioavailability usually do not take this factor into account, so exaggerated claims from health supplement companies based on this sort of evidence should be ignored. Scientific studies are still in progress to determine which approaches yield the best results and the lowest costs.

If anemia does not respond to oral treatments, it may be necessary to administer iron parenterally (e.g., as iron dextran) using a drip or hemodialysis. Parenteral iron involves risks of fever, chills, backache, myalgia, dizziness, syncope, rash and anaphylactic shock.

A follow up blood test is essential to demonstrate whether the treatment has been effective.

Iron supplements should be kept out of the reach of children, as iron-containing supplements are a frequent cause of poisoning in children.

Iron supplementation and infection risk

Because one of the functions of elevated ferritin (an acute phase reaction protein) in acute infections is thought to be to sequester iron from bacteria, it is generally throught that iron supplementation (which circumvents this mechanism) should be avoided in patients who have active bacterial infections. Replacement of iron stores is seldom such an emergency situation that it cannot wait for such infections to be treated.

Some studies have found that iron supplementation can lead to an increase in infectious disease morbidity in areas where bacterial infections are common. For example, children receiving iron-enriched foods have demonstrated an increased rate in diarrhea overall and enteropathogen shedding. Iron deficiency protects against infection by creating an unfavorable environment for bacterial growth. Nevertheless, while iron deficiency might lessen infections by certain pathogenic diseases, it also leads to a reduction in resistance to other strains of viral or bacterial infections, such as *Salmonella typhimurium* or *Entamoeba histolytica*. Overall, it is sometimes difficult to decide whether iron supplementation will be beneficial or harmful to an individual in an environment that is prone to many infectious diseases; however this is a different question than the question of supplementation in individuals who are already ill with a bacterial infection.

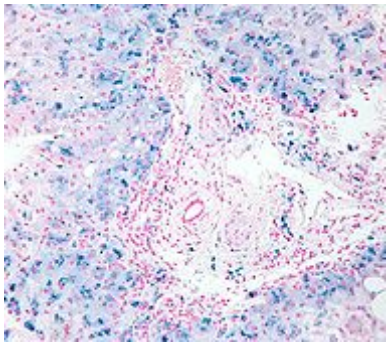
Effect of vitamin and mineral supplements

There is an observed correlation between serum retinol and hemoglobin levels. Women with a low serum retinol concentration are more likely to be iron-deficient and anemic, compared to those with normal to high levels of retinol. While vitamin A deficiency has an adverse effect on hemoglobin synthesis, even a slight increase in vitamin A intake can lead to a significant rise in hemoglobin levels. However, vitamin A is less effective in alleviating severe iron-deficiency anemia. Low levels of iron in the body cannot be relieved by vitamin A supplementation alone. Additionally, a low ascorbic acid stores in the body causes an impairment in the release of stored iron in the reticuloendothelial cells. Copper is necessary for iron uptake, and a copper deficiency can result in iron deficiency. Copper deficiency can sometimes be caused by excessive zinc or vitamin C supplementation.

Chapter- 8

Iron Overload

Iron overload



Micrograph of **haemosiderosis**. Liver biopsy. Iron stain.

ICD-10	R79.0
ICD-9	790.6
DiseasesDB	5581
MeSH	D019190

In medicine, **iron overload** indicates accumulation of iron in the body due to any cause.

Terminology

Haemochromatosis or haemosiderosis

Historically, the term **haemochromatosis** (spelled **hemochromatosis** in American English) was initially used to refer to what is now more specifically called haemochromatosis type 1 (or HFE-related hereditary haemochromatosis). Currently, haemochromatosis (without further specification) is mostly defined as iron overload with a hereditary/primary cause, or originating from a metabolic disorder. However, the term is currently also used more broadly to refer to any form of iron overload, thus requiring specification of the cause, for example *hereditary haemochromatosis*. Hereditary

haemochromatosis is an autosomal recessive disease with estimated prevalence in the population of 2 in 1,000 in Caucasians, with lower incidence in other races. The gene responsible for hereditary haemochromatosis (known as HFE gene) is located on chromosome 6; the majority of hereditary haemochromatosis patients have mutations in this HFE gene. Hereditary haemochromatosis is characterized by an accelerated rate of intestinal iron absorption and progressive iron deposition in various tissues that typically begins to be expressed in the third to fifth decades of life, but may occur in children. The most common presentation is hepatic cirrhosis in combination with hypopituitarism, cardiomyopathy, diabetes, arthritis, or hyperpigmentation. Because of the severe sequelae of this disease if left untreated and recognizing that treatment is relatively simple, early diagnosis before symptoms or signs appear is important.

The term **haemosiderosis** is generally used to indicate the pathological effect of iron accumulation in any given organ, which mainly occurs in the form of haemosiderin. Sometimes, the simpler term **siderosis** is used instead.

Other definitions distinguishing haemochromatosis or haemosiderosis that are occasionally used include:

- Haemosiderosis is haemochromatosis caused by excessive blood transfusions, that is, haemosiderosis is a form of secondary haemochromatosis.
- Haemosiderosis is hemosiderin deposition within cells, while haemochromatosis is hemosiderin within cells AND interstitium.
- Haemosiderosis is iron overload that does not cause tissue damage, while haemochromatosis does.
- Haemosiderosis is arbitrarily differentiated from haemochromatosis by the reversible nature of the iron accumulation in the reticuloendothelial system.

Clinical presentation

Organs commonly affected by haemochromatosis are the liver, heart and endocrine glands.

Haemochromatosis may present with the following clinical syndromes:

- Cirrhosis of the liver
- Diabetes due to pancreatic islet cell failure
- Cardiomyopathy
- Arthritis (iron deposition in joints)
- Testicular failure
- Tanning of the skin

Causes

The causes can be distinguished between primary cases (hereditary or genetically determined) and less frequent secondary cases (acquired during life). People of Celtic (Irish, Scottish, Welsh) origin have a particularly high incidence of whom about 10% are carriers of the gene and 1% sufferers from the condition.

Primary haemochromatosis

The fact that most cases of haemochromatosis were inherited was well known for most of the 20th century, though they were incorrectly assumed to depend on a single gene. The overwhelming majority actually depend on mutations of the HFE gene discovered in 1996, but since then others have been discovered and sometimes are grouped together as "non-classical hereditary haemochromatosis", "non-HFE related hereditary haemochromatosis", or "non-HFE haemochromatosis".

Description	OMIM	Mutation
haemochromatosis type 1: "classical"-haemochromatosis	235200	HFE
Haemochromatosis type 2A: juvenile haemochromatosis	602390	Haemojuvelin ("HJV", also known as RGMc and HFE2)
Haemochromatosis type 2B: juvenile haemochromatosis	606464	hepcidin antimicrobial peptide (*HAMP*) or HFE2B
Haemochromatosis type 3	604250	transferrin receptor-2 (TFR2 or HFE3)
Haemochromatosis type 4/ African iron overload	604653	ferroportin (SLC11A3/SLC40A1)
Neonatal haemochromatosis	231100	(unknown)
Acaeruloplasminemia (very rare)	604290	caeruloplasmin
Congenital atransferrinaemia (very rare)	209300	transferrin
GRACILE syndrome (very rare)	603358	BCS1L

Most types of hereditary haemochromatosis have autosomal recessive inheritance, while type 4 has autosomal dominant inheritance.

Secondary haemochromatosis

- Severe chronic haemolysis of any cause, including intravascular haemolysis and ineffective erythropoiesis (haemolysis within the bone marrow).
- Multiple frequent blood transfusions (either whole blood or just red blood cells), which are usually needed either by individuals with hereditary anaemias (such as beta-thalassaemia major, sickle cell anaemia, and Diamond–Blackfan anaemia) or by older patients with severe acquired anaemias such as in myelodysplastic syndromes.
- Excess parenteral iron supplements, such as can acutely happen in iron poisoning

- Excess dietary iron
- Some disorders do not normally cause haemochromatosis on their own, but may do so in the presence of other predisposing factors. These include cirrhosis (especially related to alcohol abuse), steatohepatitis of any cause, porphyria cutanea tarda, prolonged haemodialysis, post-portacaval shunting.

Diagnosis

There are several methods available for diagnosing and monitoring iron loading including:

- Serum ferritin
- Liver biopsy
- HFE
- MRI

Serum ferritin is a low cost, readily available, and minimally invasive method for assessing body iron stores. However, the major problem with using it as an indicator of iron overload is that it can be elevated in a range of other medical conditions unrelated to iron levels including infection, inflammation, fever, liver disease, renal disease and cancer. Also, total iron binding capacity may be low, but can also be normal.

The standard of practice in diagnosis of hemochromatosis was recently reviewed by Pietrangelo. Positive HFE analysis confirms the clinical diagnosis of hemochromatosis in asymptomatic individuals with blood tests showing increased iron stores, or for predictive testing of individuals who have a family history of hemochromatosis. The alleles evaluated by HFE gene analysis are evident in ~80% of patients with hemochromatosis; a negative report for HFE gene does not rule out hemochromatosis. In a patient with negative HFE gene testing, elevated iron status for no other obvious reason, and family history of liver disease, additional evaluation of liver iron concentration is indicated. In this case, diagnosis of hemochromatosis is based on biochemical analysis and histologic examination of a liver biopsy. Assessment of the hepatic iron index (HII) is considered the "gold standard" for diagnosis of hemochromatosis.

MRI is emerging as an alternative to liver biopsy for measuring liver iron loading. For measuring liver iron concentrations, **R2-MRI** (also known as **FerriScan**) has been validated and is coming into use in medical centers. It is not recommended in practice guidelines at this time.

Prognosis

A third of those untreated develop hepatocellular carcinoma.

Treatment

Routine treatment in an otherwise healthy person consists of regularly scheduled phlebotomies (bloodletting). When first diagnosed, the phlebotomies may be fairly frequent, perhaps as often as once a week, until iron levels can be brought to within normal range. Once iron and other markers are within the normal range, phlebotomies may be scheduled every other month or every three months depending upon the patient's rate of iron loading.

For those unable to tolerate routine blood draws, there is a chelating agent available for use. The drug Deferoxamine binds with iron in the bloodstream and enhances its elimination via urine and faeces. Typical treatment for chronic iron overload requires subcutaneous injection over a period of 8–12 hours daily. Two newer iron chelating drugs which are licensed for use in patients who receive regular blood transfusions to treat thalassemia (and thus who develop iron overload as a result) are deferasirox and deferiprone.

Chapter- 9

Hemosiderosis

Hemosiderosis

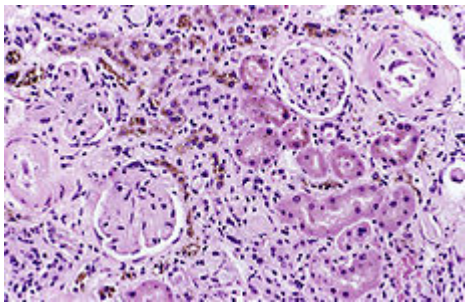


Image of a kidney viewed under a microscope. The brown areas contain hemosiderin

MeSH D006486

Hemosiderosis (AmE) or **haemosiderosis** (BrE) is a form of iron overload disorder resulting in the accumulation of hemosiderin.

Types include:

- Transfusion hemosiderosis
- Idiopathic pulmonary haemosiderosis

Transfusion hemosiderosis

Transfusional hemosiderosis is the accumulation of iron in the liver and/or heart but also endocrine organs, in patients who receive frequent blood transfusions (such as those with thalassemia, sickle cell disease, aplastic anemia or myelodysplastic syndrome).

Treatment

Treatment is by iron chelating agents: deferoxamine, deferiprone or deferasirox. If iron overload has caused end-organ damage, this is generally irreversible and may require transplantation.

Notable patients

Ted DeVita, the "bubble boy", died of transfusional iron overload from too many blood transfusions.

Idiopathic pulmonary haemosiderosis

Idiopathic pulmonary haemosiderosis (or **idiopathic pulmonary hemosiderosis**; **IPH**) is a lung disease of unknown cause that is characterized by alveolar capillary bleeding and accumulation of haemosiderin in the lungs. It is rare, with an incidence between 0.24 and 1.23 cases per million people.

History

The condition was first described as "brown lung induration" by Rudolf Virchow in 1864 in patients after their death. Wilhelm Ceelen later correlated his findings to the clinical symptoms of two children who died of IPH in 1931. The first living patient was diagnosed by Jan Waldenström in 1944. It has been given several names, including:

- Haemosiderin accumulation
- Pulmonary haemosiderosis
- Brown induration of lung
- Essential brown induration of lung
- Ceelen-Gellerstedt syndrome (after physicians Wilhelm Ceelen and Nils Gellerstedt)

Pathophysiology

Being idiopathic, IPH by definition has an unknown cause. It is thought to be an immune-mediated disease. The lung bleeding causes accumulation of iron, which in itself causes additional lung damage. Meanwhile, there is insufficient iron for inclusion into the haemoglobin molecules inside red blood cells which carry oxygen to body tissues for cellular respiration.

Idiopathic pulmonary haemosiderosis can occur either as a primary lung disorder or as the sequela to other pulmonary, cardiovascular or immune system disorder.

- PH1 involves PH with circulating anti-GMB antibodies.
- PH2 involves PH with immune complex disease such as systemic lupus erythematosus, SLE.

- PH3 involves no demonstrable immune system involvement.

Related or similar conditions

There are many pulmonary problems that may seem to mimic haemosiderosis but do not necessarily include the deposits of iron into the lung. The deposition of iron in the lungs, occurring in the form of haemosiderin, is the defining characteristic of this illness. These other conditions may occur separately or together with haemosiderosis.

- Pulmonary Fibrosis
- Adult Respiratory Distress Syndrome (ARDS)
- Immune Complex Disease
- intra-alveolar bleeding

Diagnosis

Clinically, IPH manifests as a triad of haemoptysis, diffuse parenchymal infiltrates on chest radiographs, and iron deficiency anaemia. It is diagnosed at an average age of 4.5 plus or minus 3.5 years, and it is twice as common in females. The clinical course of IPH is exceedingly variable, and most of the patients continue to have episodes of pulmonary haemorrhage despite therapy. Death may occur suddenly from acute pulmonary haemorrhage or after progressive pulmonary insufficiency resulting in chronic respiratory failure.

Treatment

Corticosteroids are the mainstay of treatment of IPH, though they are controversial and lack clear evidence in their favour. They are thought to decrease the frequency of haemorrhage, while other studies suggest that they do not have any effect on the course or prognosis of this disease. In either case, steroid therapy has significant side effects. Small trials have investigated the use of other medications, but none has emerged as a clear standard of care. This includes immune modulators such as hydroxychloroquine, azathioprine, and cyclophosphamide. 6-mercaptopurine as a long-term therapy may prevent pulmonary haemorrhage. A 2007 scientific letter. reports preliminary success in preventing pulmonary haemorrhage with the anti-oxidant N-acetylcysteine.

Prognosis

Death may occur rapidly with acute, massive pulmonary bleeding or over longer periods as the result of continued pulmonary failure and left heart failure. Historically, patients had an average survival of 2.5 years after diagnosis, but today 86% may survive beyond five years.

Hemosiderin deposition in the lungs is often seen after diffuse alveolar hemorrhage, which occurs in diseases such as Goodpasture's syndrome, Wegener's granulomatosis,

and idiopathic pulmonary haemosiderosis. Mitral stenosis can also lead to pulmonary hemosiderosis. Hemosiderin collects throughout the body in hemochromatosis. Hemosiderin deposition in the liver is a common feature of hemochromatosis and is the cause of liver failure in the disease. Deposition in the pancreas leads to diabetes and in the skin leads to hyperpigmentation. Hemosiderin deposition in the brain is seen after bleeds from any source, including chronic subdural hemorrhage, Cerebral arteriovenous malformations, cavernous hemangiomata. Hemosiderin collects in the skin and is slowly removed after bruising; hemosiderin may remain in some conditions such as stasis dermatitis. Hemosiderin in the kidneys have been associated with marked hemolysis and a rare blood disorder called paroxysmal nocturnal hemoglobinuria.

Hemosiderin may deposit in diseases associated with iron overload. These diseases are typically diseases in which chronic blood loss requires frequent blood transfusions, such as sickle cell anemia and thalassemia, though beta thalassemia minor has been associated with hemosiderin deposits in the liver in those with non-alcoholic fatty liver disease independent of any transfusions.

Treatment

Treatment for hemosiderin focuses on limiting the effects of the underlying disease leading to continued deposition. In hemochromatosis, this entails frequent phlebotomy. In diseases such as Wegener's granulomatosis, immune suppression is required. Limiting blood transfusions and institution of iron chelation therapy when iron overload is detected are important when managing sickle-cell anemia and other chronic hemolytic anemias.

Diagnosis

There are several methods available for diagnosing and monitoring hemosiderosis including:

- Serum ferritin
- Liver biopsy
- MRI

Serum ferritin is a low cost, readily available, and minimally invasive method for assessing body iron stores. However, the major problem with using it as an indicator of hemosiderosis is that it can be elevated in a range of other medical conditions unrelated to iron levels including infection, inflammation, fever, liver disease, renal disease and cancer.

While liver biopsies provide a direct measure of liver iron concentration, the small sample size relative to the size of the liver can lead to sampling errors given the heterogeneity of iron concentration within the liver. Furthermore, the invasive nature of liver biopsy and the associated risks of complications (which can range from pain,

haemorrhage, gallbladder perforation and other morbidities through to death in approx 1 in 10,000 cases) prevent it being used as a regular monitoring tool.

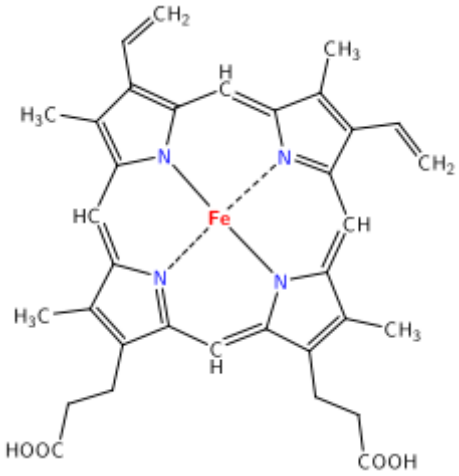
MRI is emerging as the method of choice for measuring liver iron loading due to the fact that it is non-invasive, safer and generally cheaper to perform than liver biopsy; does not suffer from problems with sampling variability; and can be used more frequently than performing liver biopsies.

For measuring liver iron concentrations, **R2-MRI** (also known as **FerriScan**) has been most extensively validated and widely adopted.

Chapter- 10

Iron Deficiency (Medicine)

Iron deficiency



Iron in heme

ICD-10	E61.1
ICD-9	280.9
DiseasesDB	6947
MedlinePlus	000584
eMedicine	med/1188

Iron deficiency (**sideropenia** or **hypoferremia**) is one of the most commonly known forms of nutritional deficiencies. In the human body, iron is present in all cells and has several vital functions—as a carrier of oxygen to the tissues from the lungs in the form of hemoglobin, as a transport medium for electrons within the cells in the form of cytochromes, and as an integral part of enzyme reactions in various tissues. Too little iron can interfere with these vital functions and lead to morbidity and death.

The direct consequence of iron deficiency is iron deficiency anemia. Groups that are most prone to developing this disease are children and pre-menopausal women.

Total body iron averages approximately 3.8 g in men and 2.3 g in women. In blood plasma, iron is carried tightly bound to the protein transferrin. Bacteria, like human cells, require iron for growth, and restricting its bioavailability in this way prevents their infectious growth. Indeed, during fever, one way of controlling bacterial growth is through temporary hypoferremia.

There are several mechanisms that control human iron metabolism and safeguard against iron deficiency. The main regulatory mechanism is situated in the gastrointestinal tract. When loss of iron is not sufficiently compensated by adequate intake after some time that is determined by the state of body iron storage, iron deficiency develops.

Causes

- chronic bleeding (hemoglobin contains iron)
    - excessive menstrual bleeding
    - non-menstrual bleeding
    - bleeding from the gastrointestinal tract (ulcers, hemorrhoids, etc.)
    - rarely, laryngological bleeding or from the respiratory tract
- inadequate intake (special diets low in dietary iron)
- substances (in diet or drugs) interfering with iron absorption
- malabsorption syndromes
- fever where it is adaptive to control bacterial infection
- blood donation

Though genetic defects causing iron deficiency have been studied in rodents, there are no known genetic disorders of human iron metabolism that directly cause iron deficiency.

Symptoms

Symptoms of iron deficiency can occur even before the condition has progressed to iron deficiency anaemia.

Symptoms of iron deficiency are not unique to iron deficiency (i.e. not pathognomonic). Iron is needed for many enzymes to function normally, so a wide range of symptoms may eventually emerge, either as the secondary result of the anemia, or as other primary results of iron deficiency. Symptoms of iron deficiency include:

- fatigue
- pallor
- hair loss
- irritability
- weakness

- pica
- brittle or grooved nails
- Plummer-Vinson syndrome: painful atrophy of the mucous membrane covering the tongue, the pharynx and the oesophagus
- Impaired immune function
- Pagophagia

Likely lab test results in people with iron deficiency

- A full blood count would likely reveal microcytic anemia

- Low serum ferritin
- Low serum iron
- High TIBC (total iron binding capacity)
- It is possible that the fecal occult blood test might be positive, if iron deficiency is the result of gastrointestinal bleeding.

As always, laboratory values have to be interpreted with the lab's reference values in mind and considering all aspects of the individual clinical situation.

Serum ferritin can be elevated in inflammatory conditions and so a normal serum ferritin may not always exclude iron deficiency.

Consequences

Continued iron deficiency may progress to anemia and worsening fatigue. Thrombocytosis, or an elevated platelet count, can also result. A lack of sufficient iron levels in the blood is a reason that some people cannot donate blood.

Treatment

Before any treatment is commenced there should be definitive diagnosis of the underlying cause for iron deficiency, particularly in older patients who are most susceptible to colorectal cancer and the gastrointestinal bleeding it often causes. In adults, 60% of patients with iron deficiency anemia may have underlying gastrointestinal disorders leading to chronic blood loss. It is likely that the cause of the iron deficiency will need treatment as well.

When iron deficiency has been diagnosed the condition can be treated with iron supplements, e.g. in the form of ferrous sulfate, ferrous gluconate, or amino acid chelate tablets. Recent research suggests the replacement dose of iron, at least in the elderly with iron deficiency, may be as little as 15 mg per day of elemental iron.

Food sources of iron

Mild iron deficiency can be prevented or corrected by eating iron-rich foods. Because iron is a requirement for most plants and animals, a wide range of foods provide iron. Good sources of dietary iron include red meat, poultry, lentils, beans, leaf vegetables, tofu, chickpeas, black-eyed peas, fortified bread, and fortified breakfast cereals. Iron in low amounts is found in molasses, teff and farina.

Iron from different foods is absorbed and processed differently by the body; for instance, iron in meat (heme iron source) is more easily broken down and absorbed than iron in grains and vegetables ("non-heme" iron source), but heme/hemoglobin from red meat has effects which may increase the likelihood of colorectal cancer. Minerals and chemicals in one type of food may inhibit absorption of iron from another type of food eaten at the same time. For example, oxalates and phytic acid form insoluble complexes which bind iron in the gut before it can be absorbed.

Because iron from plant sources is less easily absorbed than the heme-bound iron of animal sources, vegetarians and vegans should have a somewhat higher total daily iron intake than those who eat meat, fish or poultry. Legumes and dark-green leafy vegetables like broccoli, kale and oriental greens are especially good sources of iron for vegetarians and vegans. However, spinach and Swiss chard contain oxalates which bind iron making it almost entirely unavailable for absorption. Iron from nonheme sources is more readily absorbed if consumed with foods that contain either heme-bound iron or vitamin C. This is due to a hypothesised "meat factor" which enhances iron absorption.

Iron deficiency can have serious health consequences that diet may not be able to quickly correct, and iron supplementation is often necessary if the iron deficiency has become symptomatic.

Bioavailability and bacterial infection

Iron is needed for bacterial growth making its bioavailability an important factor in controlling infection. Blood plasma as a result carries iron tightly bound to transferrin, and only releases it to cells with appropriate cell markers thus preventing its access to bacteria. Between 15 and 20 percent of the protein content in human milk consists of lactoferrin that binds iron. As a comparison, in cow's milk, this is only 2 percent. As a result, breast fed babies have fewer infections. Lactoferrin is also concentrated in tears, saliva and at wounds to bind iron to limit bacterial growth. Egg white contains 12% conalbumin to withhold it from bacteria that get through the egg shell (for this reason prior to antibiotics, egg white was used to treat infections).

To reduce bacterial growth, plasma concentrations of iron are lowered in fever, and following surgery after open wounds where it acts as a protection against infection. Reflecting this link between iron bioavailability and bacterial growth, the taking of iron supplements can increase the risk of infection. A moderate iron deficiency, in contrast, can provide protection against acute infection.

Chapter- 11

Human Iron Metabolism Disorders

Iron metabolism disorder

Iron metabolism disorder

ICD-10	E83.1
ICD-9	275.0
MeSH	D019189

Genes involved in **iron metabolism disorders** include HFE and TFR2.

Hepcidin is the master regulator of iron metabolism and, therefore, most genetic forms of iron overload can be thought of as relative hepcidin deficiency in one way or another. For instance, a severe form of iron overload, juvenile hemochromatosis, is a result of severe hepcidin deficiency. The majority of cases are caused by mutations in the hemojuvelin gene (HJV or RGMc/repulsive guidance molecule c). The exceptions, people who have mutations in the gene for ferroportin, prove the rule: these people have plenty of hepcidin, but their cells lack the proper response to it. So, in people with ferroportin proteins that transport iron out of cells without responding to hepcidin's signals to stop, they have a deficiency in the action of hepcidin, if not in hepcidin itself.

But the exact mechanisms of most of the various forms of adult hemochromatosis, which make up most of the genetic iron overload disorders, remain unsolved. So while researchers have been able to identify genetic mutations causing several adult variants of hemochromatosis, they now must turn their attention to the normal function of these mutated genes.

These genes represent multiple steps along the pathway of iron regulation, from the body's ability to sense iron, to the body's ability to regulate uptake and storage. Working out the functions of each gene in this pathway will be an important tool for finding new methods of treating genetic disorders, as well as for understanding the basic workings of the pathway.

So though many mysteries of iron metabolism remain, the discovery of hepcidin already allows a much better understanding of the nature of iron regulation, and makes researchers optimistic that many more breakthroughs in this field are soon to come.

Neonatal hemochromatosis

Neonatal hemochromatosis	
OMIM	231100
DiseasesDB	34508

Neonatal Hemochromatosis is a rare and severe liver disease. Its characteristics are similar to hereditary hemochromatosis, where iron deposition causes damage to the liver and other organs and tissues.

Causes

The causes of neonatal hemochromatosis are still unknown, however recent research has led to the hypothesis that it is an alloimmune disease. Evidence supporting this hypothesis includes the high recurrence rate within sibships (>80%). This evidence along with other research indicates that Neonatal Hemochromatosis could be classified as a Congenital Alloimmune Hepatitis.

Treatment

Effective treatment of the disease has been confined to liver transplants. An antioxidant chelation cocktail has also been reported as having some success though its effectiveness cannot be confirmed.

Based on the alloimmune cause hypothesis, a new treatment involving high-dose immunoglobulin to pregnant mothers who have had a previous pregnancy with a confirmed neonatal hemochromatosis outcome, has provided very encouraging results.

Related conditions

Neonatal hemochromatosis is sometimes confused with juvenile hemochromatosis, which is a hereditary hemochromatosis, caused by mutations of a gene called hemojuvelin. While the symptoms and outcomes from these two diseases are similar, the causes appear to be different.

Aceruloplasminemia

Aceruloplasminemia	
ICD-10	E83.1
ICD-9	275.0
OMIM	604290
DiseasesDB	30055

Aceruloplasminemia is an autosomal recessive disorder of iron metabolism characterized by progressive neurodegeneration of the retina and basal ganglia and diabetes mellitus.

Iron accumulates in the pancreas, liver and brain. Accumulation in the eye may lead to retinal degeneration. The disease is caused by mutations in the ceruloplasmin gene.

Aceruloplasminemia belongs to the group of genetic disorders called neurodegeneration with brain iron accumulation (NBIA).

African iron overload

African iron overload	
OMIM	601195

African iron overload, formerly known as "Bantu siderosis", is an iron overload disorder first observed among people of African descent in Southern Africa.

Causes

Originally, this was blamed on ungalvanised barrels used to store home-made beer, which led to increased oxidation and increased iron levels in the beer. Further investigation has shown that only some people drinking this sort of beer get an iron overload syndrome, and that a similar syndrome occurred in people of African descent who have had no contact with this kind of beer (e.g., African Americans).

This led investigators to the discovery of a gene polymorphism in the gene for ferroportin, which predisposes some people of African descent to iron overload.

Polymorphisms in SLC40A1 have recently been investigated in Americans of African descent.

Atransferrinemia

Atransferrinemia

Violet, a 2-year-old European girl with atransferrinemia.

ICD-9	273.8
OMIM	209300
DiseasesDB	29538

Atransferrinemia, also called **familial hypotransferrinemia**, is an autosomal recessive metabolic disorder in which there is an absence of transferrin, a plasma protein that transports iron through the blood.

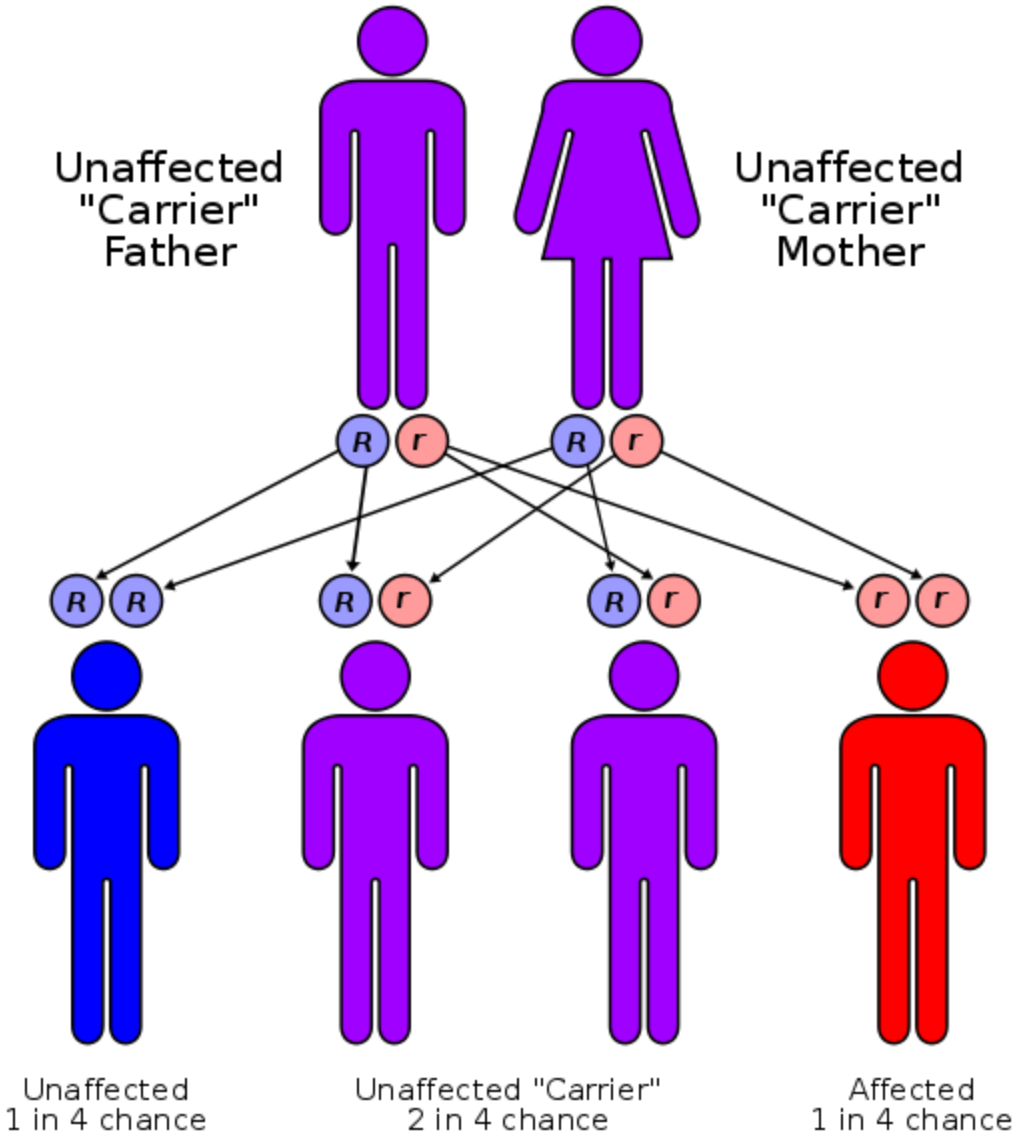
Atransferrinemia is characterized by anemia and hemosiderosis in the heart and liver. The iron damage to the heart can lead to heart failure. The anemia is typically microcytic and hypochromic (the red blood cells are abnormally small and pale). Atransferrinemia is extremely rare, with only eight cases documented worldwide.

Symptoms

Severe microcytic hypochromic anemia, growth retardation and recurrent infections are the first clinical signs of the disease. Iron overload occurs mainly in the liver, heart, pancreas, thyroid, kidney and bone joints, leading to mild to severe symptoms of liver

and heart failure, arthropathy and hypothyroidism. Death may occur due to heart failure or pneumonia.

Genetics



Atransferrinemia has an autosomal recessive pattern of inheritance, meaning both copies of the gene in each cell are defective.

A case study was done in 1961 on a 7-year-old girl who died of heart failure with atransferrinemia. The half-normal levels of transferrin in her parents' bloodstream supported the notion that this disorder is transferred in an autosomal recessive pattern. Atransferrinemia was reported in only eight patients in six families as of the year 2000. A

lack of scientific data and public outreach, however, have suggested that there is a higher number of current cases. Researchers used the first known case reported in the United States and identified mutations in the TF gene as a probable cause of the disorder.

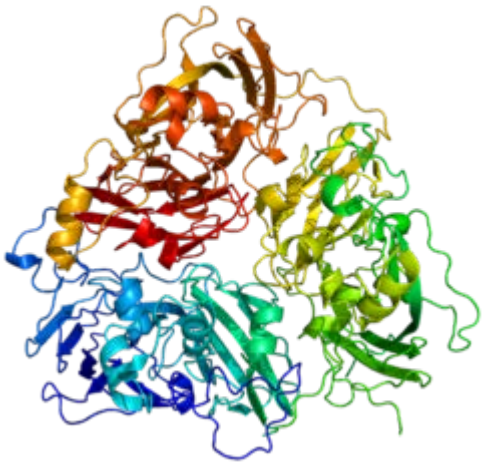
Treatment

Treatment with infusions of plasma or purified apotransferrin may stabilise or correct the anemia and growth defects.

Chapter- 12

Ceruloplasmin

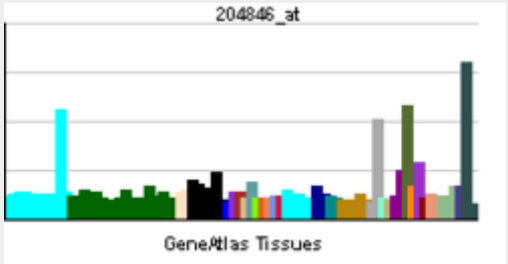
Ceruloplasmin (ferroxidase)



PDB rendering based on 1kcw.

Identifiers	
Symbols	CP; CP-2
External IDs	OMIM: 117700 MGI: 88476 HomoloGene: 75 GeneCards: CP Gene
EC number	1.16.3.1

RNA expression pattern



Orthologs		
Species	**Human**	**Mouse**
Entrez	1356	12870
Ensembl	ENSG00000047457	ENSMUSG00000003617
UniProt	P00450	Q2F3J4
RefSeq (mRNA)	NM_000096	NM_001042611
RefSeq (protein)	NP_000087	NP_001036076
Location (UCSC)	Chr 3: 150.37 - 150.42 Mb	Chr 3: 20.15 - 20.2 Mb

Ceruloplasmin (or **caeruloplasmin**) is a ferroxidase enzyme that in humans is encoded by the *CP* gene.

Ceruloplasmin is the major copper-carrying protein in the blood, and in addition plays a role in iron metabolism. It was first described in 1948. Another protein, hephaestin, is noted for its homology to ceruloplasmin, and also participates in iron and probably copper metabolism.

Function

It is an enzyme (EC 1.16.3.1) synthesized in the liver containing 6 atoms of copper in its structure. Ceruloplasmin carries about 70% of the total copper in human plasma while albumin carries about 15%. The rest is accounted for by macroglobulins. Albumin may be confused at times to have a greater importance as a copper carrier because it binds copper less tightly than ceruloplasmin. Ceruloplasmin exhibits a copper-dependent oxidase activity, which is associated with possible oxidation of Fe^{2+} (ferrous iron) into Fe^{3+} (ferric iron), therefore assisting in its transport in the plasma in association with transferrin, which can only carry iron in the ferric state. The molecular weight of human ceruloplasmin is reported to be 151kDa.

Pathology

Like any other plasma protein, levels drop in patients with hepatic disease due to reduced synthesizing capabilities.

- Mechanisms of low ceruplasmin levels:
    -
    - Gene expression genetically low: aceruloplasminemia
    - Copper levels are low in general:
        - Malnutrition/trace metal deficiency in the food source
    - Copper does not cross the intestinal barrier due to ATP7A deficiency in Menkes disease
    - Delivery of copper into the lumen of the ER-Golgi network is absent in hepatocyte due to absent ATP7B in Wilson's disease.

Copper availability doesn't affect the translation of the nascent protein. However, the apoenzyme without copper is unstable. Apoceruloplasmin is largely degraded intracellularly in the hepatocyte and the small amount that is released has a short circulation half life of 5 hours as compared to the 5.5 days for the holo-ceruloplasmin.

Mutations in the ceruloplasmin gene can lead to the rare genetic human disease aceruloplasminemia, characterized by iron overload in the brain, liver, pancreas, and retina.

Interpretation

Decreased levels

Lower-than-normal ceruloplasmin levels may indicate:

- Menkes disease (Menke's kinky hair syndrome) (very rare)
- Wilson's disease (a rare copper storage disease)
- Overdose of Vitamin C
- Copper deficiency
- Aceruloplasminemia

Elevated levels

Greater-than-normal ceruloplasmin levels may indicate or be noticed in:

- pregnancy
- oral contraceptive pill use
- lymphoma
- acute and chronic inflammation (it is an acute-phase reactant)
- rheumatoid arthritis
- Angina
- Alzheimer's disease
- Schizophrenia
- Obsessive-compulsive disorder

Regulation

A cis-regulatory element called the GAIT element is involved in the selective translational silencing of the Ceruloplasmin transcript. The silencing requires binding of a cytosolic inhibitor complex called IFN-gamma-activated inhibitor of translation (GAIT) to the GAIT element.

Chapter- 13

Iron Tests

Iron tests are groups of clinical chemistry laboratory blood tests that are used to evaluate body iron stores or the iron level in blood serum.

Other terms used for the same tests are **iron panel**, **iron profile**, **iron indices** or **iron status**.

Tests

- Serum iron
- Ferritin
- Transferrin
- Total iron-binding capacity (TIBC)
- Transferrin saturation (Iron saturation of transferrin)
- Unsaturated iron binding capacity (UIBC)
- Transferrin receptor (TfR)

Brief Description of Some Iron Tests: -

Serum iron

Serum iron is a medical laboratory test that measures the amount of circulating iron that is bound to transferrin. Clinicians order this laboratory test when they are concerned about iron deficiency, which can cause anemia and other problems.

65% of the iron in the body is bound up in hemoglobin molecules in red blood cells. About 4% is bound up in myoglobin molecules. Around 30% of the iron in the body is stored as ferritin or hemosiderin in the spleen, the bone marrow and the liver. Small amounts of iron can be found in other molecules in cells throughout the body. None of this iron is directly accessible by testing the serum.

However, some iron is circulating in the serum. Transferrin is a molecule produced by the liver that binds one or two iron(III) ions; transferrin is essential if stored iron is to be moved and used.

Most of the time, about 30% of the available sites on the transferrin molecule are filled. The test for serum iron uses blood drawn from veins to measure the iron molecules that are bound to transferrin, and circulating in the blood.

The extent to which sites on transferrin molecules are filled by iron ions can be another helpful clinical indicator, known as percent transferrin saturation. Another lab test saturates the sample to measure the total amount of transferrin; this test is called total iron-binding capacity (TIBC). These three tests are generally done at the same time, and taken together are an important part of the diagnostic process for conditions such as anemia, iron deficiency anemia, anemia of chronic disesases and Haemochromatosis.

Normal values

Normal reference ranges are:

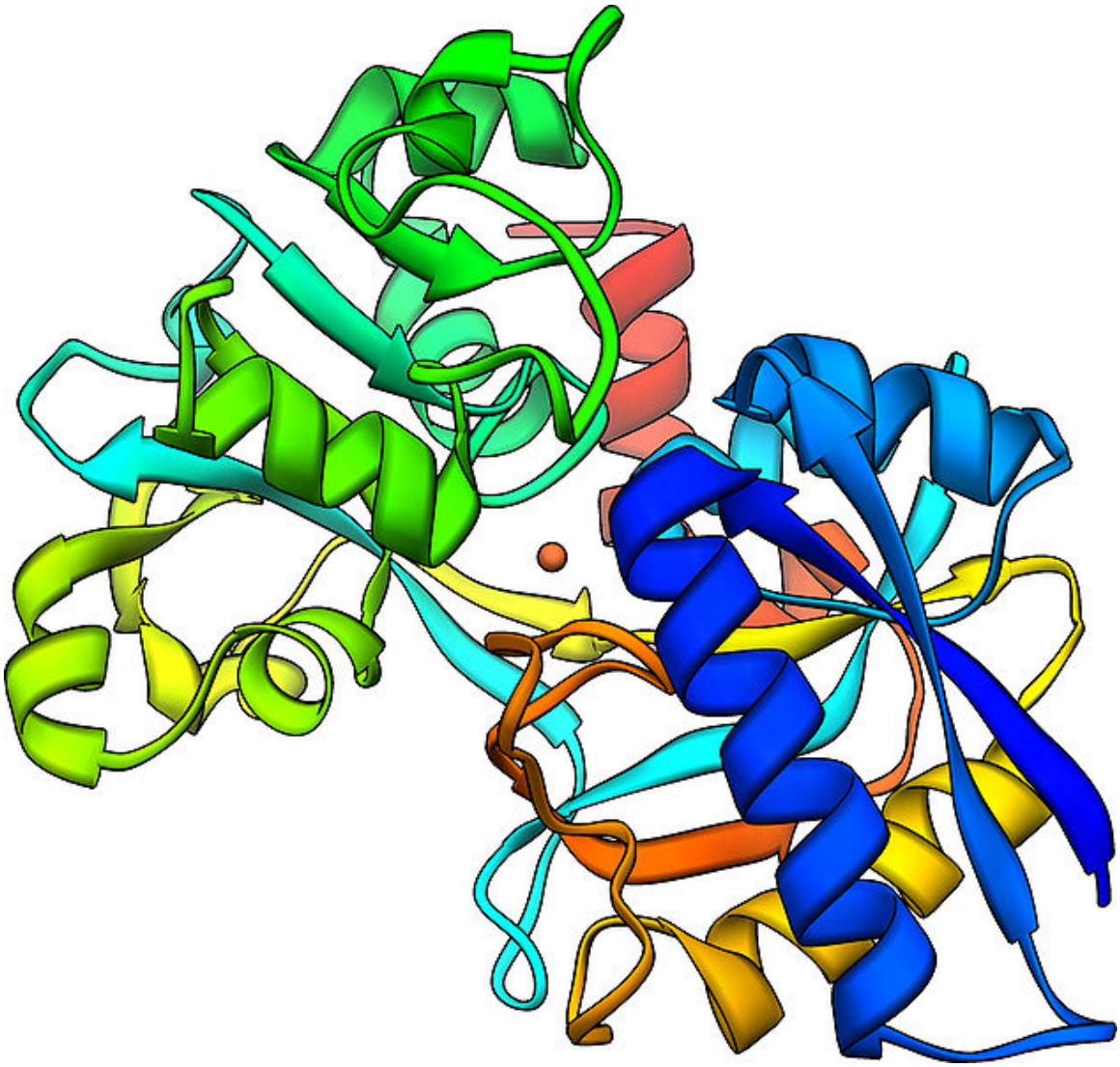
- Serum Iron (SI):
  - Men: 65 to 176 µg/dL
  - Women: 50 to 170 µg/dL
  - Newborns: 100 to 250 µg/dL
  - Children: 50 to 120 µg/dL
- TIBC: 240–450 µg/dL
- Transferrin saturation: 20–50%

µg/dL = micrograms per deciliter.

Laboratories often use different units and "normal" may vary by population and the lab techniques used; look at the individual laboratory reference values to interpret a specific test (for instance, your own).

Reference ranges for blood tests, comparing blood content of iron and related compounds (shown in brown and orange) with other constituents.

Total iron-binding capacity



transferrin

Total iron-binding capacity (TIBC) is a medical laboratory test which measures the blood's capacity to bind iron with transferrin. It is performed by drawing blood and measuring the maximum amount of iron that it can carry, which indirectly measures transferrin since transferrin is the most dynamic carrier. TIBC is less expensive than a direct measurement of transferrin.

The TIBC should not be confused with the UIBC, or "unsaturated iron binding capacity". The UIBC is calculated by subtracting the serum iron from the TIBC.

Interpretation

Taken together with serum iron and percent transferrin saturation clinicians usually perform this test when they are concerned about anemia, iron deficiency or iron

deficiency anemia. However, because the liver produces transferrin, alterations in function (such as cirrhosis, hepatitis, or liver failure) must be considered when performing this test. It can also be an indirect test of liver function, but is rarely used for this purpose.

The percent transferrin saturation (i.e., the result of the formula of serum iron/TIBC x 100) can also be a useful indicator.

Condition	Serum iron	Transferrin and TIBC	Percent transferrin saturation
iron deficiency anemia	Low	High. The liver produces more transferrin, presumably attempting to maximize use of the little iron that is available.	Low, as there is insufficient iron.
anemia of chronic disease	Low, as the body holds iron intracellularly with ferritin.	Low. The body produces less transferrin (but more ferritin), presumably to keep iron away from pathogens that require it for their metabolism.	Normal
pregnancy or use of hormonal contraception, but without iron deficiency	Normal	High. The liver increases the production of transferrin, thus raising TIBC.	Low, as there is excess transferrin with normal serum iron levels.

These examples demonstrate that to properly understand a value for TIBC, one also must know the serum iron, the percent transferrin saturation, and the individual clinical situation.

Usual values

Reference ranges for blood tests, comparing blood content of iron and related compounds (shown in brown and orange) with other constituents.

Normal reference ranges are:

- Serum iron: Male 65–177 µg/dL (11.6–31.7 µmol/L); Female 50–170 µg/dL (9.0–30.4 µmol/L)
- TIBC: 250–370 µg/dL (45-66 µmol/L)
- Transferrin saturation: Male 20–50%; Female 15–50%
- Serum ferritin: Male 20-250 µg/L, Female 15-150 µg/L

µg/dL = micrograms per deciliter; µmol/L = micromoles per litre.

Laboratories often use different units and "normal" may vary by population and the lab techniques used. Look at the individual laboratory reference values to interpret a specific test (for instance, your own).

Transferrin saturation

Transferrin saturation, measured as a percentage, is a medical laboratory value. It is the ratio of serum iron and total iron-binding capacity, multiplied by 100. For an explanation of some clinical situations in which this ratio is important. The three results are usually reported together.

Usual values

Normal reference ranges are:

- Serum iron: 60–170 µg/dl (10–30µmol/L)
- TIBC: 240–450 µg/dl
- Transferrin saturation: 15–50% (males), 12–45% (females)

µg/dl = micrograms per deciliter.

Laboratories often use different units and "normal" may vary by population and the lab techniques used. Look at the individual laboratory reference values to interpret a specific test (for instance, your own).

Reference ranges for blood tests, comparing blood content of iron and related compounds (shown in brown and orange) with other constituents.

Transferrin receptor

transferrin receptor (p90, CD71)

Identifiers	
Symbol	TFRC
Alt. symbols	CD71, TFR1
Entrez	7037
HUGO	11763
OMIM	190010
RefSeq	NM_003234
UniProt	P02786
Other data	
Locus	Chr. 3 *q29*

transferrin receptor 2

Identifiers	
Symbol	TFR2
Alt. symbols	HFE3, TFRC2
Entrez	7036
HUGO	11762
OMIM	604720
RefSeq	NM_003227
UniProt	Q9UP52
Other data	
Locus	Chr. 7 *q22*

Transferrin receptor (TfR) is a carrier protein for transferrin. It is needed for the import of iron into the cell and is regulated in response to intracellular iron concentration. It imports iron by internalizing the transferrin-iron complex through receptor-mediated endocytosis.

Regulation

Low iron concentrations promote increased levels of transferrin receptor, to increase iron intake into the cell. Thus, transferrin receptor maintains cellular iron homeostasis.

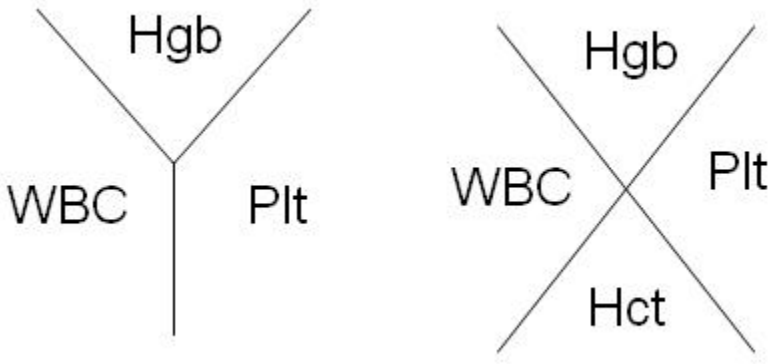
TfR production in the cell is regulated according to iron levels by iron response/ regulatory element binding protein (IRE-BP), also referred to as Iron Regulatory Protein (IRP). This protein binds to the hairpin like structure (IRE) that is in the 3' UTR of the TfR receptor. Once binding occurs, degradation of mRNA of IRE is inhibited.

Related tests

- Complete blood count (CBC), especially:
  - Hemoglobin, EVF or total red blood cells (RBC count)
  - MCV
  - MCH or MCHC

Brief Description of Some Related Iron Tests: -

Complete blood count



Schematics (also sometimes called "Fishbones") of shorthand for complete blood count commonly used by clinicians and healthcare providers. The shorthand on the right is used more often in the US. Hgb=Hemoglobin, WBC=White blood cells, Plt=Platelets, Hct=Hematocrit.

A **complete blood count** (**CBC**), also known as **full blood count** (**FBC**) or **full blood exam** (**FBE**) or **blood panel**, is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC.

Alexander Vastem is widely regarded as being the first person to use the complete blood count for clinical purposes. Reference ranges used today stem from his clinical trials in the early 1960s.

The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdictions.

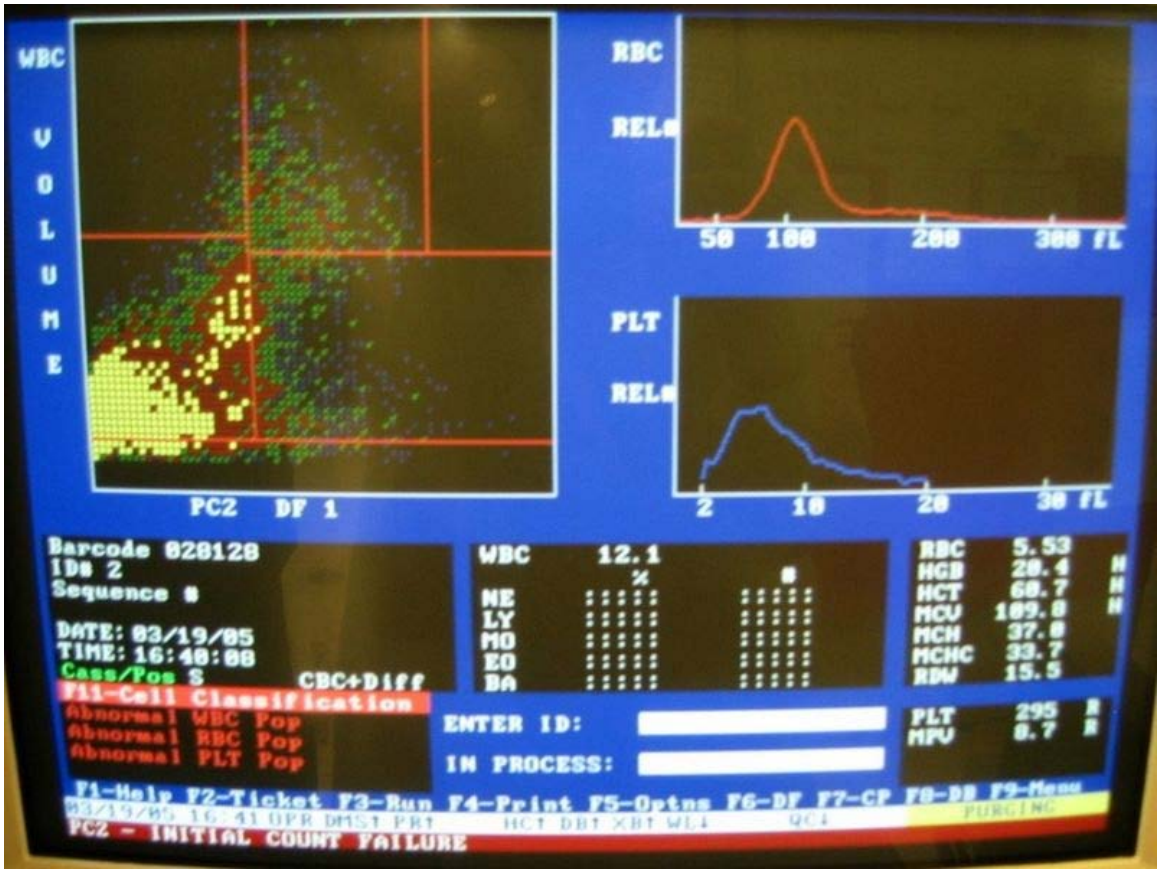
Methods

Samples

A phlebotomist collects the specimen, in this case blood is drawn in a test tube containing an anticoagulant (EDTA, sometimes citrate) to stop it from clotting, and transported to a laboratory.

In the past, counting the cells in a patient's blood was performed manually, by viewing a slide prepared with a sample of the patient's blood under a microscope (a blood film, or peripheral smear). Nowadays, this process is generally automated by use of an automated analyzer, with only approximately 30% samples now being examined manually.

Automated blood count



Complete blood count performed by an automated analyser. Differentials missing.

The blood is well mixed (though not shaken) and placed on a rack in the analyzer. This instrument has many different components to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The results are printed out or sent to a computer for review.

Blood counting machines aspirate a very small amount of the specimen through narrow tubing. Within this tubing, there are sensors that count the number of cells going through it, and can identify the type of cell; this is flow cytometry. The two main sensors used are light detectors, and electrical impedance. One way the instrument can tell what type of

blood cell is present is by size. Other instruments measure different characteristics of the cells to categorize them.

Because an automated cell counter samples and counts so many cells, the results are very precise. However, certain abnormal cells in the blood may be identified incorrectly, and require manual review of the instrument's results and identifying any abnormal cells the instrument could not categorize.

In addition to counting, measuring and analyzing red blood cells, white blood cells and platelets, automated hematology analyzers also measure the amount of hemoglobin in the blood and within each red blood cell. This information can be very helpful to a physician who, for example, is trying to identify the cause of a patient's anemia. If the red cells are smaller or larger than normal, or if there's a lot of variation in the size of the red cells, this data can help guide the direction of further testing and expedite the diagnostic process so patients can get the treatment they need quickly.

Automated blood counting machines include the Medonic M Series, Beckman Coulter LH series, Sysmex XE-2100, Siemens ADVIA 120 & 2120, the Abbott Cell-Dyn series, and the Mindray BC series.

Manual blood count

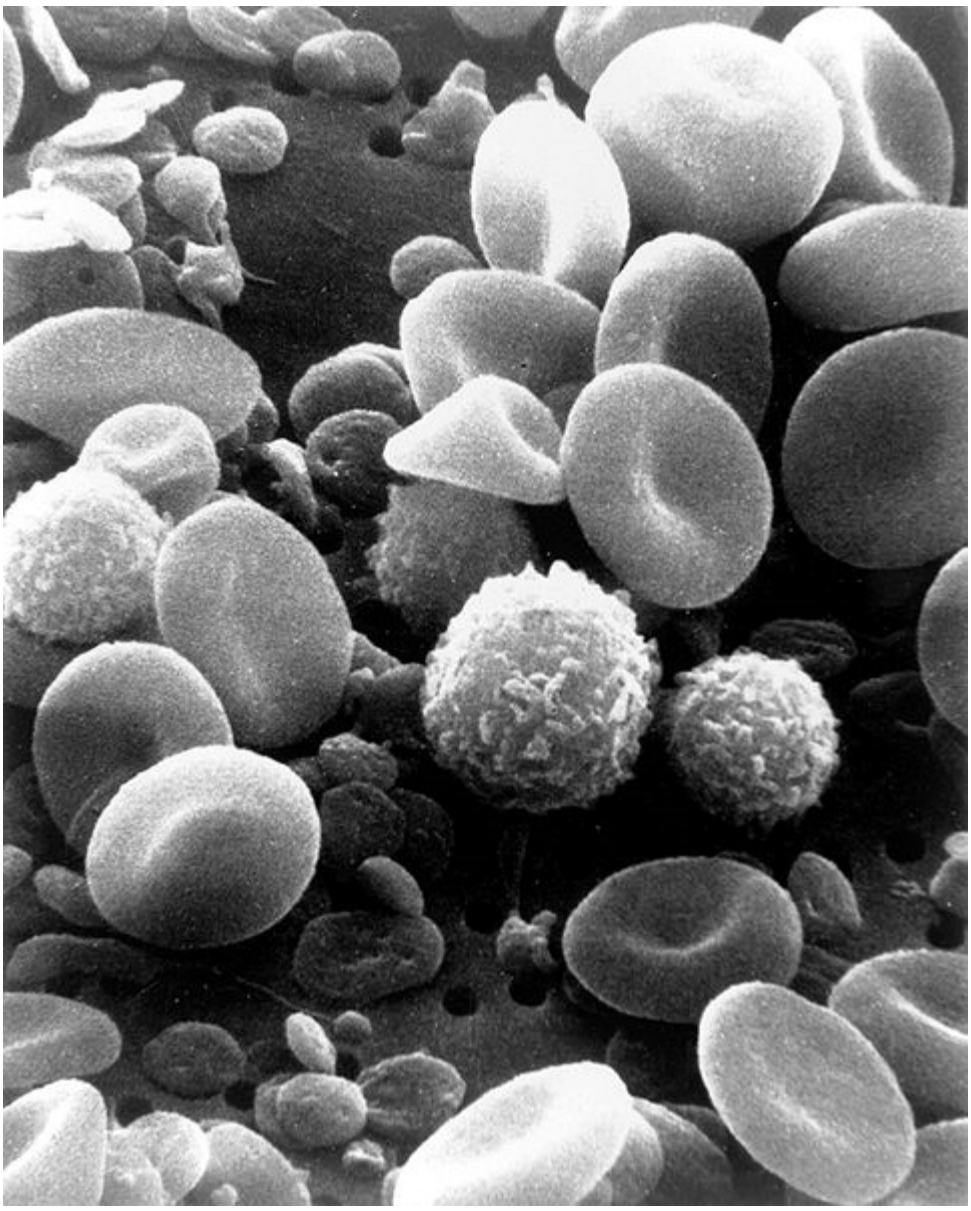
Counting chambers that hold a specified volume of diluted blood (as there are far too many cells if it is not diluted) are used to calculate the number of red and white cells per litre of blood.

To identify the numbers of different white cells, a blood film is made, and a large number of white cells (at least 100) are counted. This gives the percentage of cells that are of each type. By multiplying the percentage with the total number of white blood cells, the absolute number of each type of white cell can be obtained.

The advantage of manual counting is that automated analysers are not reliable at counting abnormal cells. That is, cells that are not present in normal patients and are only seen in the peripheral blood with certain haematological conditions. Manual counting is subject to sampling error because so few cells are counted compared with automated analysis.

Medical technicians examine blood film via a microscope for 30% of CBCs, not only to find abnormal white cells, but also because variation in the shape of red cells is an important diagnostic tool. Although automated analysers give fast, reliable results regarding how many red cells, the average size of the red cell, and the variation in size of the red cells, they don't detect cells' shapes. Also, some normal patients' platelets will clump in EDTA anticoagulated blood, which causes automatic analysers to give a falsely low platelet count. The technician viewing the slide in these cases will see clumps of platelets and can estimate if there are low, normal, or high numbers of platelets.

Results



A scanning electron microscope (SEM) image of normal circulating human blood. One can see red blood cells, several knobby white blood cells including lymphocytes, a monocyte, a neutrophil, and many small disc-shaped platelets.

A complete blood count will normally include:

Red cells

- Total red blood cells — The number of red cells is given as an absolute number per litre.
- Hemoglobin - The amount of hemoglobin in the blood, expressed in grams per decilitre. (Low hemoglobin is called anemia.)

- Hematocrit or packed cell volume (PCV) - This is the fraction of whole blood volume that consists of red blood cells.
- Red blood cell indices
    - Mean corpuscular volume (MCV) - the average volume of the red cells, measured in femtolitres. Anemia is classified as microcytic or macrocytic based on whether this value is above or below the expected normal range. Other conditions that can affect MCV include thalassemia, reticulocytosis and alcoholism.
    - Mean corpuscular hemoglobin (MCH) - the average amount of hemoglobin per red blood cell, in picograms.
    - Mean corpuscular hemoglobin concentration (MCHC) - the average concentration of hemoglobin in the cells.
- Red blood cell distribution width (RDW) - a measure of the variation of the RBC population

White cells

- Total white blood cells - All the white cell types are given as a percentage and as an absolute number per litre.

A complete blood count with differential will also include:

- Neutrophil granulocytes — May indicate bacterial infection. May also be raised in acute viral infections.Because of the segmented appearance of the nucleus, neutrophils are sometimes referred to as "segs." The nucleus of less mature neutrophils is not segmented, but has a band or rod-like shape. Less mature neutrophils — those that have recently been released from the bone marrow into the bloodstream — are known as "bands" or "stabs". Stab is a German term for rod.
- Lymphocytes — Higher with some viral infections such as glandular fever and. Also raised in chronic lymphocytic leukemia (CLL). Can be decreased by HIV infection. In adults, lymphocytes are the second most common WBC type after neutrophils. In young children under age 8, lymphocytes are more common than neutrophils.
- Monocytes — May be raised in bacterial infection, tuberculosis, malaria, Rocky Mountain spotted fever, monocytic leukemia, chronic ulcerative colitis and regional enteritis
- Eosinophil granulocytes — Increased in parasitic infections, asthma, or allergic reaction.
- Basophil granulocytes- May be increased in bone marrow related conditions such as leukemia or lymphoma.

A manual count will also give information about other cells that are not normally present in peripheral blood, but may be released in certain disease processes.

Platelets

- Platelet numbers are given, as well as information about their size and the range of sizes in the blood.
- Mean platelet volume (MPV) - a measurement of the average size of platelets.

Interpretation

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream. For example:

Type of Cell	Increase	Decrease
Red Blood Cells (RBC)	erythrocytosis or polycythemia	anemia or erythroblastopenia
White Blood Cells (WBC):	leukocytosis	leukopenia
-- lymphocytes	-- lymphocytosis	-- lymphocytopenia
-- granulocytes:	-- granulocytosis	-- granulocytopenia or agranulocytosis
-- --neutrophils	-- --neutrophilia	-- --neutropenia
-- --eosinophils	-- --eosinophilia	-- --eosinopenia
-- --basophils	-- --basophilia	-- --basopenia
Platelets	thrombocytosis	thrombocytopenia
All cell lines	-	pancytopenia

Many disease states are heralded by changes in the blood count:

- leukocytosis can be a sign of infection.
- thrombocytopenia can result from drug toxicity.
- pancytopenia is generally as the result of decreased production from the bone marrow, and is a common complication of cancer chemotherapy.

Diagnosis

IRON PANEL	Serum iron	Transferrin and TIBC	Transferrin saturation	Ferritin	Transferrin receptor	Related tests	
						Hb	MCV
Iron deficiency anemia	↓	↑	↓	↓	↑	↓	↓
Iron overload (hemochromatosis)	↑	↓	↑	↑	↓*	Normal	
Anemia of chronic disease	↓	↓	↓	↑*	↓*	↓	

Porphyria cutanea tarda (PCT)	↑	↓	↑	↑	**Normal**		
Thalassemia	↑	↓	↑	↑	↑	↓	
Sideroblastic anemia	↑	↓	↑	↑	↑*	↓	
Megaloblastic anemia	↑	↓	↑	↑	↑	↓	↑
Hemolytic anemia	↑*	↓*	↑*	↑*	↑	↓	
Pregnancy or use of hormonal contraception	**Normal**	↑	↓				