Hypochromic Anaemias and Iron Overload

- Iron deficiency is the most common cause of
- anaemia.
- It is the most important cause of a microcytic hypochromic anaemia, in which the two red cell indices MCV (mean corpuscular volume) and MCH (mean corpuscular haemoglobin) are reduced
- Blood film shows small (microcytic) and pale
- (hypochromic) red cells.
- This appearance is caused by a defect in haemoglobin synthesis (Fig. 3.1).
- The major differential diagnosis in microcytic
- hypochromic anaemia is thalassaemia and anaemia of chronic disease.

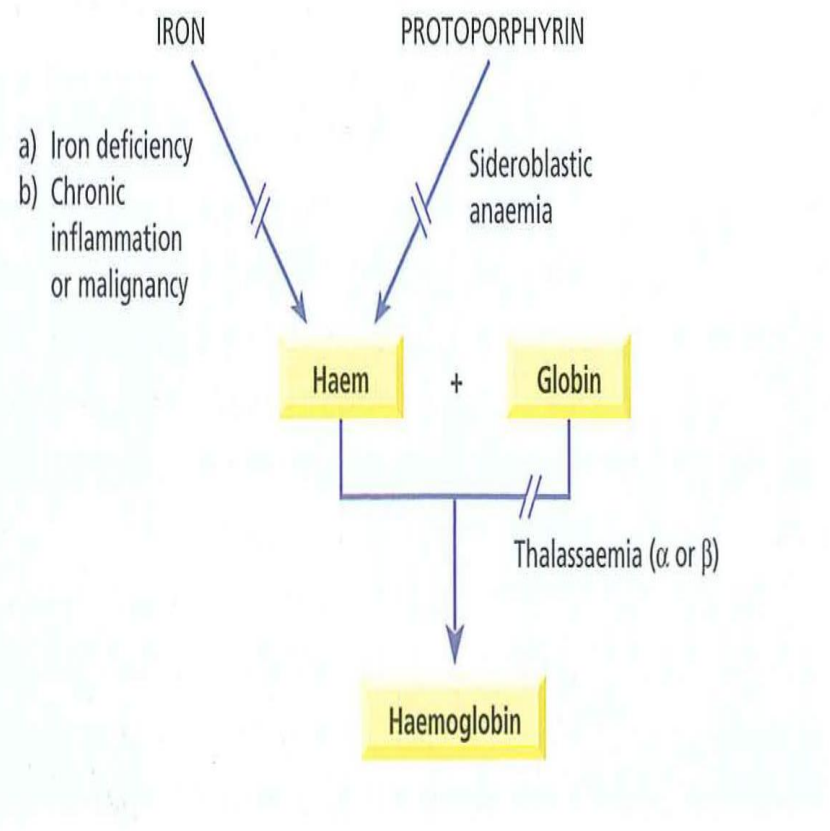

Fig. 3.1 The causes of a hypochromic, microcytic anaemia. These include lack of iron (iron deficiency) or of iron release from macrophages to serum (anaemia of chronic inflammation or malignancy), failure of protoporphyrin synthesis (sideroblastic anaemia) or of globin synthesis (α- or β-thalassaemia). Lead also inhibits haem and globin synthesis.

Nutritional and metabolic aspects of iron

- Iron is one of the most common elements in the Earth's crust, yet iron deficiency is the most common cause of anaemia, affecting about 500 million people worldwide.
- Body has a limited ability to absorb iron and frequently loses excess of iron as haemorrhages.

Body iron distribution and transport

The transport and storage of iron is largely mediated by three proteins:

- transferrin,
- the transferrin
- receptor 1 (TfR1) and ferritin.

- Transferrin can contain up to two atoms of iron. It delivers iron to tissues that have transferrin receptors, especially erythroblasts in the bone marrow which incorporate the iron into haemoglobin (Fig. 3.2). The transferrin is then reutilized.
- At the end of their life, red cells are broken down in the macrophages of the reticuloendothelial system (RES) and the iron is released from haemoglobin, enters the plasma and provides most of the iron on transferrin. Only a small proportion of plasma transferrin iron comes from dietary iron, absorbed through the duodenum and jejenum.

- Some iron is stored in the macrophages as ferritin and haemosiderin, the amount varying widely according to overall body iron status. Ferritin is a water-soluble protein-iron complex of molecular weight 465 000. It is made up of an outer protein shell, apoferritin, consisting of 22 subunits and an iron-phosphate-hydroxide core. It contains up to 20% of its weight as iron and is not visible by light microscopy. Each molecule of apoferritin may bind up to 4000-5000 atoms of iron.

- **Haemosiderin is an insoluble protein-iron complex containing approximately 37% iron by weight. It is derived from partial lysosomal digestion of aggregates of ferritin molecules and is visible in macrophages and other cells by light microscopy after staining by Perls' (Prussian blue) reaction.**

- **Iron in ferritin and haemosiderin is in the ferric form. It is mobilized after reduction to the ferrous form, vitamin C being involved. A copper-containing enzyme, caeruloplasmin, catalyses oxidation of the iron to the ferric form for binding to plasma transferrin.**

- **Iron is also present in muscle as myoglobin and in most cells of the body in iron-containing enzymes (e.g. cytochromes, succinic dehydrogenase, catalase) (Table 3.1). This tissue iron is less likely to become depleted than haemosiderin, ferritin and haemoglobin in states of iron deficiency, but some reduction of haem-containing enzymes may occur.**

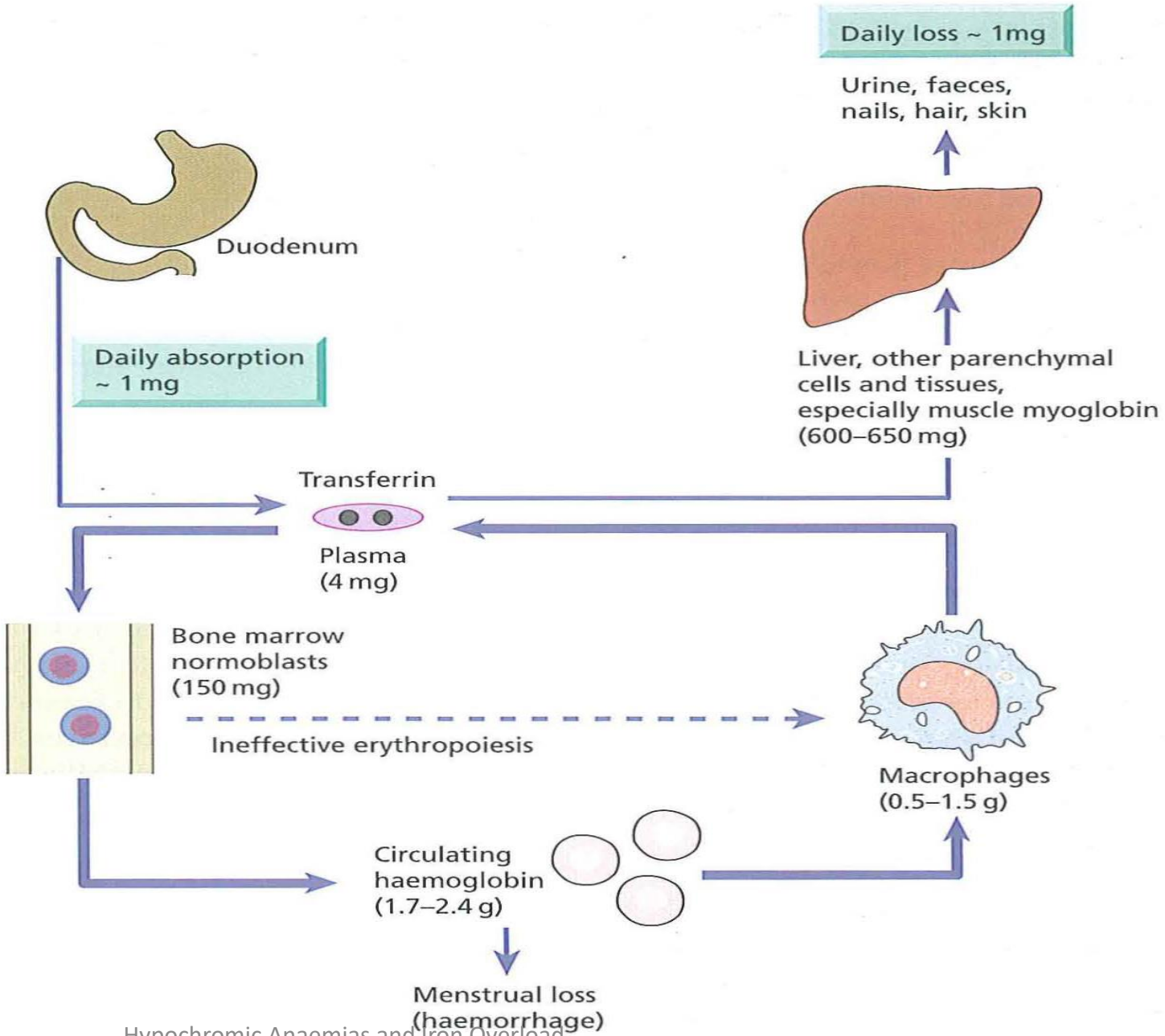

Fig. 3.2 Daily iron cycle. Most of the iron in the body is contained in circulating haemoglobin (Table 3.1) and is reutilized for haemoglobin synthesis after the red cells die. Iron is transferred from macrophages to plasma transferrin and so to bone marrow erythroblasts. Iron absorption is normally just sufficient to make up for iron loss. The dashed line indicates ineffective erythropoiesis.

Regulation of ferritin and transferrin receptor1 synthesis

- The levels of ferritin and TfRl are linked to iron status; iron overload causes a rise in tissue ferritin and a fall in TfRl
- In iron deficiency ferritin is low and TfRl increased.
- This linkage arises through the binding of an iron regulatory protein (lRP) to iron response elements (IREs) on the ferritin and TfRl mRNA molecules.
- Iron deficiency increases' the ability of IRP to bind to the IREs whereas iron overload reduces the binding.
- The site of IRP binding to IREs, whether upstream (5') or downstream (3') from the coding gene, determines whether the amount of mRNA and so protein produced is increased or decreased (Fig. 3.3).
- Upstream binding reduces translation whereas downstream binding stabilizes the mRNA, increasing protein translation.
- When plasma iron is raised and transferrin is saturated the amount of iron transferred to parenchymal cells is increased and this is the basis of the pathological changes associated with iron loading conditions.

HEPCIDIN

- **Hepcidin is a 25-amino acid polypeptide produced by liver cells.**
- **Both an acute phase protein and the major hormonal regulator of iron homeostasis (Fig, 3.4)**
- **Inhibits iron release from macrophages, intestinal epithelial cells and from placental syncytiotrophoblasts by its interaction with the transmembrane iron exporter ferroportin**
- **accelerates degradation of ferroportin mRNA**
- **Increased production of hepcidin is induced by inflammation via interleukin 6 (IL-6).**
- **Hepcidin synthesis and secretion are controlled by three proteins: HFE, hemojuvelin and transferrin receptor 2.**
- **Decreased production of hepcidin occurs in response to iron deficiency, hypoxia and ineffective erythropoiesis.**

<u>Transferrin receptor 2</u>

- **Senses the degree of saturation of transferrin and is a key regulator of hepcidin synthesis**
- **High saturation levels of transferrin stimulate hepcidin synthesis whereas low saturation levels as in iron deficiency reduce hepcidin synthesis.**

Dietary iron

- Iron is present in food as ferric hydroxides, ferricprotein and haem-protein complexes.
- Both the iron content and the proportion of iron absorbed differ from food to food.
- In general, meat-in particular liver -is a better source than vegetables, eggs or dairy foods.
- The average Western diet contains 10-15 mg iron daily from which only 5-10% is normally absorbed.
- The proportion can be increased to 20-30% in iron deficiency or pregnancy (Table 3,2) but even in these situations most dietary iron remains unabsorbed.

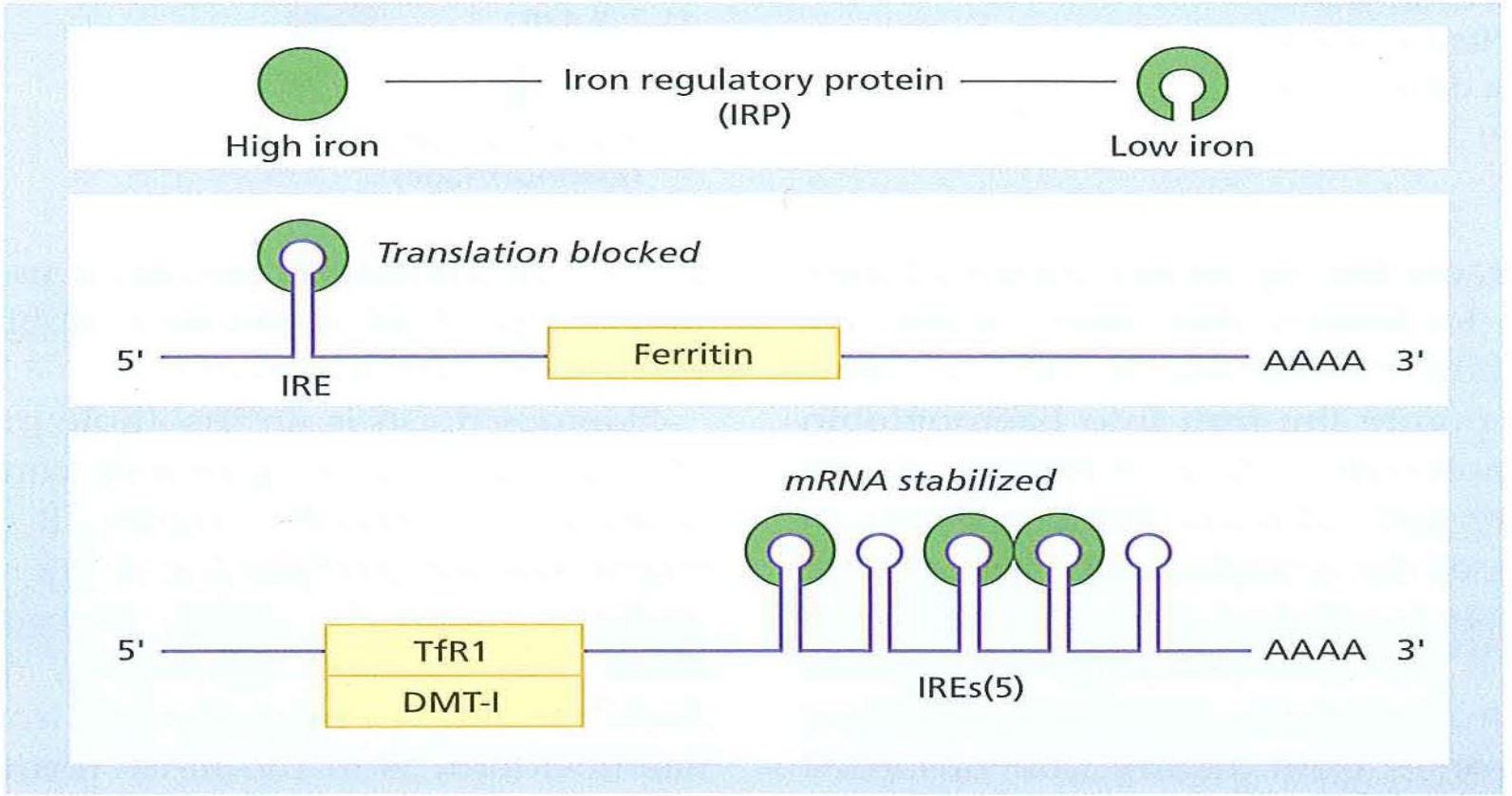

Fig. 3.3 Regulation of transferrin receptor 1 (TfR1), DMT-1 (divalent metal transporter) and ferritin expression by iron regulatory protein (IRP) sensing of intracellular iron levels. IRPs (●) are able to bind to stem-loop structures called iron response elements (IREs) (⌐) in transferrin receptor or ferritin mRNAs. IRP binding to the IRE within the 3′ untranslated region of TfR or DMT-1 mRNA leads to stabilization of the mRNA and increased protein synthesis whereas IRP binding to the IRE within the 5′ untranslated region of ferritin mRNA reduces translation. IRPs can exist in two states: at times of high iron levels the IRP binds iron and exhibits a reduced affinity for the IREs whereas when iron levels are low the binding of IRPs to IREs is increased. In this way synthesis of TfR, DMT-1 and ferritin is coordinated to physiological requirements.

Iron absorption

- Organic dietary iron is partly absorbed as haem and partly broken down in the gut to inorganic iron.
- Absorption occurs through the duodenum. Haem is absorbed through a specific receptor, Hep-I, exposed on the apical membrane of the duodenal enterocyte. Haem is then digested to release iron.
- Inorganic iron absorption is favoured by factors such as acid and reducing agents that keep iron in the gut lumen in the Fe2+ rather than the Fe3+ state (Table 3.2).
- The protein DMT-I (divalent metal transporter) is involved in transfer of iron from the lumen of the gut across the enterocyte microvilli (Fig. 3.5). Ferroportin at the basolateral surface controls exit of iron from the cell into portal plasma.
- The amount of iron absorbed is partly regulated according to the body's needs by changing the levels of DMT-l according to the iron status of the duodenal villous crypt enterocyte.
- In iron deficiency less iron is delivered to the crypt cell from transferrin which is largely unsaturated with iron. The consequent iron deficiency in the crypt cell results in increased expression of DMT-l.

- The increased expression of DMT-1 results, when the enterocyte reaches the apical absorptive surface of the duodenal villous 24-48 h later, in increased transfer of iron from the gut lumen into the enterocyte.
- Hepcidin is also a major regulator by affecting ferroportin concentration.
- Low hepcidin levels in iron deficiency increase ferroportin levels and allow more iron to enter portal plasma.
- Thus, less iron is lost when the enterocyte is shed into the gut lumen from the apex of the villous.
- Ferrireductase present at the apical surface converts iron from the Fe3+ to Fe2+ state and another enzyme, hephaestin (which contains copper), converts Fe2+to Fe3+ at the basal surface prior to binding to transferrin.

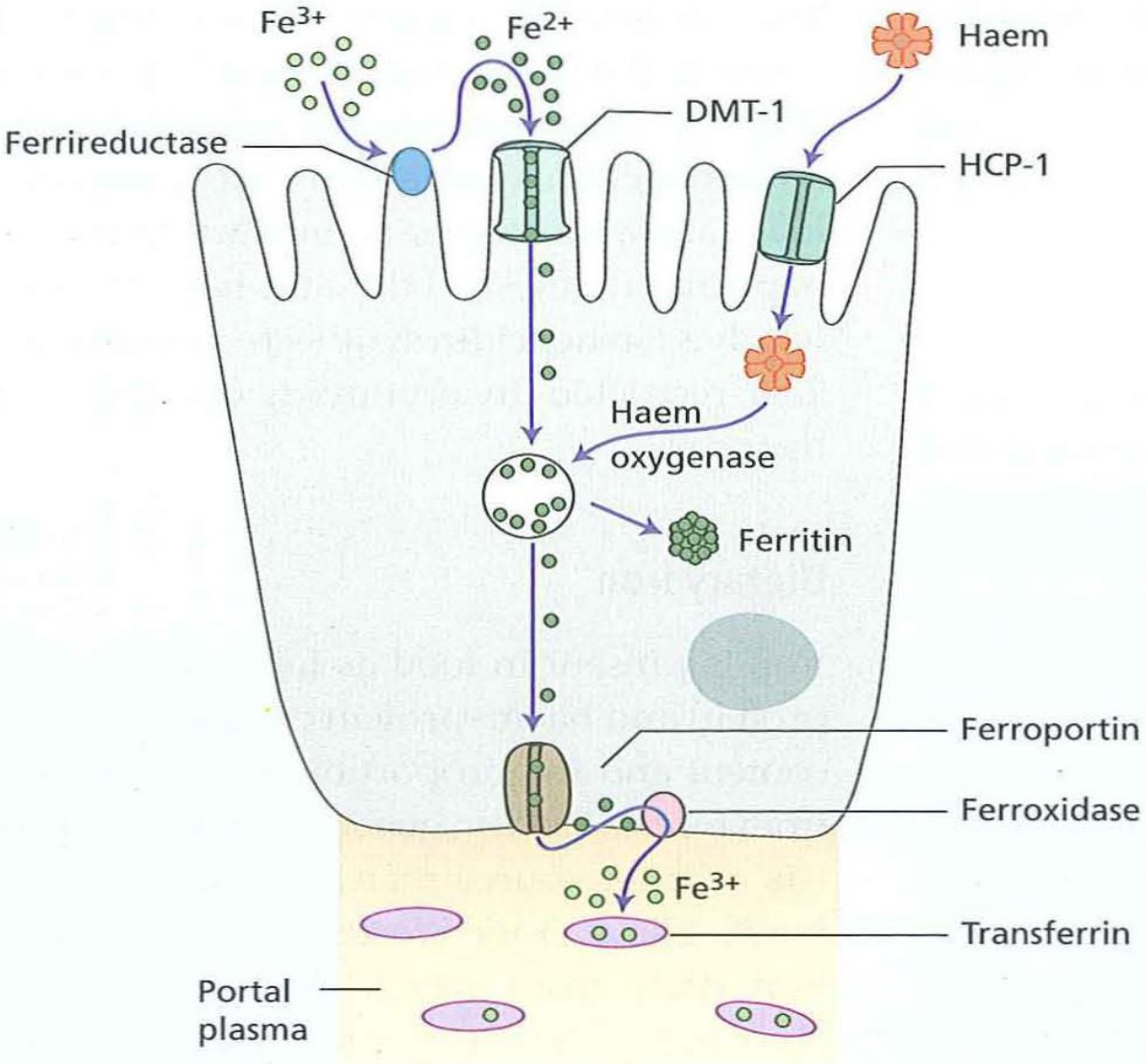

Fig. 3.5 The regulation of iron-absorption. Dietary ferric (Fe^{3+}) iron is reduced to Fe^{2+} and its entry to the enterocyte is through the divalent cation binder DMT-1. Its export into portal plasma is controlled by ferroportin. It is oxidized before binding to transferrin in plasma. Haem is absorbed after binding to its receptor protein HCP-1.

Table 3.2 Iron absorption.

Factors favouring absorption	Factors reducing absorption
Haem iron	Inorganic iron
Ferrous form (Fe^{2+})	Ferric form (Fe^{3+})
Acids (HCl, vitamin C)	Alkalis—antacids, pancreatic secretions
Solubilizing agents (e.g. sugars, amino acids)	Precipitating agents—phytates, phosphates
Iron deficiency	Iron excess
Ineffective erythropoiesis	Decreased erythropoiesis
Pregnancy	Infection
Hereditary haemochromatosis	Tea
Increased expression of DMT-1 and ferroportin in duodenal enterocytes	Decreased expression of DMT-1 and ferroportin in duodenal enterocytes
	Increased hepcidin

Iron requirements

- **The amount of iron required each day to compensate for losses from the body and for growth varies with age and sex.**
- **it is highest in pregnancy, adolescent and menstruating females (Table 3.3).**
- **Therefore these groups are particularly likely to develop iron deficiency if there is additional iron loss or prolonged reduced intake.**

Table 3.3 Estimated daily iron requirements. Units are mg/day.

	Urine, sweat, faeces	Menses	Pregnancy	Growth	Total
Adult male	0.5–1				0.5–1
Postmenopausal female	0.5–1				0.5–1
Menstruating female*	0.5–1	0.5–1			1–2
Pregnant female*	0.5–1		1–2		1.5–3
Children (average)	0.5			0.6	1.1
Female (age 12–15)*	0.5–1	0.5–1		0.6	1.6–2.6

* These groups are more likely to develop iron deficiency.

IRON DEFICIENCY

When iron deficiency is developing the reticuloendothelial stores (haemosiderin and ferritin) become completely depleted before anaemia occurs.

As the condition develops the patient may develop the general symptoms and signs of anaemia and also show a painless glossitis, angular stomatitis, brittle, ridged or spoon nails (koilonychia), dysphagia as a result of pharyngeal webs (PatersonKelly or Plummer-Vinson syndrome) unusual dietary cravings (pica).

The cause of the epithelial cell changes is not clear but may be related to reduction of iron in iron-containing enzymes.

In children, iron deficiency is particularly significant as it can cause irritability, poor cognitive function and a decline in psychomotor development.

Cauases of Iron Deficiency

- **Chronic blood loss, (especially uterine or from GIT) is the dominant cause. In contrast, in developed countries dietary deficiency is rarely a cause on its own.**
- **Half a litre of whole blood contains approximately 250 mg of iron and, negative iron balance is usual in chronic blood loss.**
- **Increased demands during infancy, adolescence, pregnancy, lactation and in menstruating women account for the high risk of anaemia.**
- **Newborn infants have a store of iron derived from delayed clamping of the cord and the breakdown of excess red cells. From 3 to 6 months there is a tendency for negative iron balance because of growth. From 6 months supplemented formula milk and mixed feeding, particularly with iron-fortified foods, prevents iron deficiency.**
- **In pregnancy increased iron is needed for an increased maternal red cell mass of approximately 35%, transfer of 300 mg of iron to the fetus and because of blood loss at delivery. Menorrhagia (a loss of 80 mL or more of blood at each cycle) is difficult to assess clinically, although the loss of clots, the use of large numbers of pads or tampons or prolonged periods all suggest excessive loss.**
- **It has been estimated to take 8 years for a normal adult male to develop iron deficiency anaemia solely as a result of a poor diet or malabsorption resulting in no iron intake at all.**
- **In clinical practice inadequate intake or. malabsorption are only rarely the sole cause of iron deficiency anaemia although in developing countries iron deficiency may occur as a result of a life-long poor diet, consisting mainly of cereals and vegetables. Gluten-induced enteropathy, partial or total gastrectomy and atrophic gastritis (often autoinumme and with *Helicobacter pylori* infection) may, however, predispose to iron deficiency.**

Serum iron and total iron-binding capacity

- The serum iron falls and total iron-binding capacity (TIBC) rises so that the TIBC is less than 10% saturated.
- This contrasts both with the anaemia of chronic disorders when the serum iron and the TIBC are both reduced and with other hypochromic anaemias where the serum iron is normal or even raised.
- **Serum transferrin receptor:** Transferrin receptor is shed from cells into plasma. The level of serum transferrin receptor (sTfR) is increased in iron deficiency anaemia but not in the anaemia of chronic disease or thalassaemia trait. The level is also raised if the overall level of erythropoiesis is increased.
- **Serum ferritin**
- A small fraction of body ferritin circulates in the serum, the concentration being related to tissue, particularly reticuloendothelial, iron stores. The normal range in men is higher than in women. In iron deficiency anaemia the serum ferritin is very low while a raised serum ferritin indicates iron overload or excess release of ferritin from damaged tissues or an acute phase response (e.g. in inflammation). The serum ferritin is normal or raised in the anaemia of chronic disorders.

Sideroblatic anaemia

- This is a refractory anaemia with hypochromic cells in the peripheral blood and increased marrow iron; presence of many pathological ring sideroblasts in the bone marrow
- Abnormal erythroblasts containing numerous iron granules arranged in a ring or collar around the nucleus instead of the few randomly distributed iron granules seen when normal erythroblasts are stained for iron. Sideroblastic anaemia is diagnosed when 15% or more of marrow erythroblasts are ring sideroblasts.
- Sideroblastic anaemia is classified into different types and the common link is a defect in haem synthesis.
- In the hereditary forms the anaemia is usually characterized by a markedly hypochromic and microcytic blood picture. The most common mutations are in the δ-aminolaevulinic acid synthase (ALA-S) gene which is on the X chromosome.
- Pyridoxal-6-phosphate is a coenzyme for ALA-S.
- Other rare types include mitochondrial defects, thiamine-responsive and other autosomal defects.

Lead poisoning and Iron Overload

- **Lead poisoning**
- Lead inhibits both haem and globin synthesis at a number of points.
- In addition it interferes with the breakdown of RNA by inhibiting the enzyme pyrimidine 5' nucleotidase, causing accumulation of denatured RNA in red cells
- the RNA giving an appearance called basophilic stippling on the ordinary (Romanowsky) stain
- The anaemia may be hypochromic or haemolytic,
- **Bo**ne marrow may show ring sideroblasts.
- Free erythrocyte protoporphyrin is raised.

- **Iron overload**
- There is no physiological mechanism for eliminating excess iron from the body
- Iron absorption is normally carefully regulated to avoid accumulation.
- Iron overload can occur in disorders associated with excessive absorption or chronic blood transfusion.
- Excessive iron deposition in tissues can cause serious damage to organs, particularly the heart, liver and endocrine organs.