UNIT 10 MINERALS (MICRO MINERALS): IRON, ZINC, COPPER, SELENIUM, CHROMIUM, MANGANESE, IODINE AND FLUORINE

Structure

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10.1 INTRODUCTION

The last unit focused on the macro minerals. Now in this unit we will study about the micro minerals, namely, iron, zinc, copper, selenium, chromium, manganese, iodine and fluorine. We will study the food sources, functions, metabolism and methods of assessing status of these important micro minerals.

Objectives

After studying this unit, you will be able to:

- differentiate between macro and micro minerals,
- list important food sources of micro minerals,
- describe the absorption and metabolic fate of each mineral,
- explain the nutritional and biochemical role of various micro minerals and relate them to physiological functions and symptoms of inadequate intakes, and
- select appropriate methods for assessing status.

10.2 MICRO MINERALS– AN OVERVIEW

Micro minerals are those *minerals, which comprise less than 0.01% of the total body weight and are required in concentrations of one part per million or less*. Initially, these minerals were also referred to as 'trace minerals' or 'trace elements' as their concentration in tissues were not easily quantified by early analytical methods. A trace element/mineral, as you may be aware, can be defined as *a chemical element present in minute quantities; especially one used by organisms and held essential to their physiology*. A micro mineral or a micro nutrient, on the other hand, *is an organic compound essential in minute amounts for the growth and health of an animal.*

Like macro minerals, micro minerals must also be present in the body in optimal range for normal functioning. Whenever, the concentration is too low or too high, the body

functions are impaired. The functions and routes of metabolism for some micro elements are well established both in animals and humans while for others, the data are available only from animal studies. They normally function as a cation (ion with a positive charge) complexed with organic ligands or chelators. Proteins are the most important chelators. Besides these, porphyrin (the ring structure present in haemoglobin) and corrins (the ring structure in vitamin B_{12}) are other important chelators. As components of enzymes and proteins, these minerals frequently participate in redox reactions (reactions which involve the transfer of electrons) with the metal often functioning as the electron carrier. However, minerals such as zinc and manganese along with macro elements calcium and magnesium, perform non-redox functions in proteins and enzymes. Since many of the micro minerals share common mechanism for absorption, they compete with each other for absorption in the small intestine. Thus, excess of one micro element can aggravate the deficiency of another. Iron and zinc are the best known examples.

With this basic overview, we shall get to know about micro minerals in greater detail in the subsequent section(s). We begin our study with iron.

10.3 IRON

Iron was a familiar metal even in the ancient civilization. In India, iron implements made their appearance in between 1300-1000 BC and in due course, iron was used in a variety of cookery utensils. The presence of leached iron, especially when acidic foods were cooked in such utensils, was considered to be a significant contributor to dietary iron. The most important clinical application of iron was described in the 17th century, for treating "chlorosis"– a condition that resulted from severe iron deficiency in adolescent females in whom the dietary iron intake was only 4-3 mg/day as against the average iron content of 8-11 mg/day in normal persons. Major aspects of iron metabolism were elucidated by 1960 and today iron is one of the most investigated minerals in nutrition. Let us read further to understand the importance of iron in maintaining good health.

We all associate iron with its presence in blood and that its deficiency results in low haemoglobin levels and hence anaemia. But is iron present only in blood? Of course not. In our subsequent discussion, we will learn about the iron stores in the human body.

Total Body Iron

In humans, the total quantity of iron in the body varies with haemoglobin concentration, body weight, gender and the amount of iron stored in various tissues. Approximate distribution of body iron is shown in Table 10.1.

Table 10.1: Distribution of body iron in different compartments

Compartment	Iron Content (mg)	Total Body Iron %
Haemoglobin Iron	2000	67
Storage Iron	Varies from 200–1000	6-27
Tissue Iron: Myoglobin	130	3.5
Enzyme Iron	8	0.2
Other-transport Iron & labile pool	83	2.28

Source: Modern Nutrition in Health and Disease 8th Ed., 1994.

You may have observed in Table 10.1 that maximum amount of iron is incorporated in haemoglobin. The amount of storage iron shall depend upon the dietary iron consumed and its bioavailability. It would be interesting to note here that iron can exist

in a number of oxidation states ranging from Fe^{2-} to Fe^{6+}. You must also remember that in the human body and food, it occurs generally as ferric (Fe^{3+}) and ferrous (Fe^{2+}) iron. We have so far discussed about the presence of iron in the body. Let us now quickly find out about the presence of iron in food i.e. learn about the food sources of iron.

Food Sources

Iron is found in foods in one of the two forms i.e. *haem* or *non-haem*. In the human diet, the primary sources of haem iron are the haemoglobin and myoglobin from consumption of meat, poultry and fish whereas non-haem iron is obtained from cereals, pulses, legumes, fruits and vegetables. Dietary non-haem iron accounts for about 85% of the total iron intake even among non-vegetarians. The good plant and animal food sources of iron are shown in the Table 10.2 (a) and (b).

Table 10.2 (a): Sources of haem iron and their content (mg)

Haem Iron Sources	Fe Content
Chicken liver	9.9
Chicken	1.2
Eggs	1.8
Salmon	0.9

Source: Indian Food Composition Tables by T. Longvah, R. Ananthan, K. Bhaskarachary and K. Venkaish, National Institute of Nutrition ICMR, 2017

Table 10.2(b): Sources of non-haem iron and their content (mg/100)

Non-haem Iron Sources	Fe Content
Cashew nut	5.9
Almonds	4.5
Raisins (Black)	6.8
Soybeans	8.29
Spinach	2.95
Wheat Whole	3.97
Kidney beans	6.30
Bitter Gourd	1.15
Lentil whole	7.57

Source: Indian Food Composition Tables by T. Longvah, R. Ananthan, K. Bhaskarachary and K. Venkaish, National Institute of Nutrition ICMR, 2017

Let us read further to find out as to how dietary iron is digested, absorbed, transported, utilized and excreted from the human system or in other words, how are adequate levels of iron maintained in different body compartments.

Metabolism of Iron

In this sub-section, we will study how body gets its iron supply, how iron is transported and utilized by the various tissues and how iron balance is maintained.

Like other minerals, we obtain iron from the diet, which is absorbed from the gastrointestinal tract. A unique feature of iron metabolism is that the body re-utilizes quantitatively the iron released from the degradation of erythrocytes, with very little being excreted. Hence, it is very frequently mentioned that once iron enters the body, the body holds on to it tenaciously. We will first learn how dietary iron is absorbed and then review how the iron is re-utilized.

Absorption of Iron

Before it can be absorbed, iron whether it is in the form of haem or non-haem. must be released from the food matrices where it is bond with other constituents. *Proteases* (the enzyme) in the stomach and small intestine hydrolyze haem iron from the globin portion of haemoglobin or myoglobin. In the case of non-haem iron, gastric secretion including HCl and pepsin aid its release from food components. Most non-haem iron is present in the ferric form which is reduced to ferrous form in the acidic environment of the stomach. However, as the ferrous iron passes into the small intestine (alkaline pH), some Fe^{2+} may be oxidized to become ferric iron. Following its liberation from food components, absorption takes place. Like other minerals, iron is also absorbed in duodenum and upper jejunum. The process of absorption is divided into three phases:

i) Iron uptake by enterocytes (epithelial cell of the superficial layer of the small and large intestine tissue)

ii) Intra enterocyte transport

iii) Storage and extra enterocyte transport.

The mechanism of absorption differs for non-haem and haem iron and therefore they will be dealt separately. Let us have a look at the non-haem iron absorption first.

a) *Mechanism of Non-haem Iron Absorption*

We will discuss all the three phases one by one.

i) *Uptake of iron by enterocytes*: Ferrous iron traverses the brush border of the intestine better than the ferric iron. The mechanism of absorption of the latter is not clear but it is postulated that it binds to luminal binding proteins. Mucin, a small protein made in the intestinal cells and released into the gastrointestinal tract, is thought to facilitate iron absorption. It binds multiple Fe^{3+} ions at an acidic pH and maintains its solubility even in alkaline pH and thus aids in its absorption. After traversing the brush border, iron binds to the receptor on the luminal surface of enterocyte and is transported inside the cell.

ii) *Intra enterocyte transport*: In the enterocyte, the absorbed iron can have one of the following metabolic fates:

- transported through the enterocyte into the blood, and
- stored in the enterocyte for future use or elimination.

Iron is transported through the enterocyte to the baso-lateral membrane by iron binding protein–*mobilferrin*. Mobilferrin can also bind to Ca, Cu and Zn. The multiple metal ion-binding properties of mobilferrin may be partially responsible for interactions between these minerals at absorptive surface.

The iron which is not transported across the cell for release is stored as *ferritin* in mucosal cells. If required for the body, it is released for transport. If not needed, the iron remains as ferritin and is excreted when mucosal cells are sloughed off in the lumen. Thus, ferritin in the enterocyte acts as an 'iron sink', trapping excess iron and removing it via intestinal excretion.

iii) *Extra enterocyte transfer*: Little is known about iron transport across the baso-lateral membrane. After crossing the baso-lateral membrane, it binds to plasma transport protein *transferrin*. Iron is oxidized before it can bind to transferrin. This is brought about by *ceruloplasmin*, a Cu-containing protein. The process has been depicted in Figure 10.1.

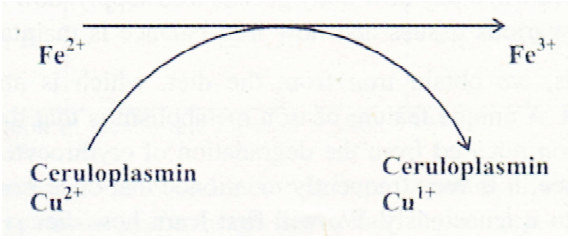


Figure 10.1: Oxidation of iron

Next, we shall review the mechanism of absorption of haem iron.

b) *Mechanism of Absorption of Haem Iron*

Haem iron is soluble in the alkaline environment of the intestine. It binds to the receptor on the enterocytes and is internalized. After entering the mucosal cell, haem is degraded to iron, carbon monoxide and bilirubin IXa by the enzyme *haemeoxygenase*. The liberated iron is then treated in the same manner as is the non-haem iron.

We have read in the previous units that whatever may be the quantity of a particular nutrient that we may consume, the entire amount may not get digested and absorbed (bioavailable) due to varied reasons. Let us see what factors affect the bioavailability of dietary iron.

Factors affecting Absorption of Dietary Iron

Haem iron is more bioavailable than non-haem iron because it is absorbed intact as a soluble complex by endocytosis (process whereby cells absorb material, molecules such as proteins, from outside by engulfing it with their cell membrane). Non-haem iron, on the other hand, forms insoluble complexes with many components concurrently present in the diet, rendering the iron unavailable for mucosal uptake. The absorption of iron also depends on the iron status of the individual and on the availability of an iron-binding mucosal transport protein (transferrin) to facilitate the uptake from the intestines.

There are mainly four factors that determine iron bioavailability/ absorption from the diet. These include:

i) Form of iron; whether haem or non-haem

ii) Solubility; specially of the non-haem iron compounds

iii) Other dietary factors; inhibitors and enhancers

iv) Iron status of the individual

Our subsequent discussions will elaborate upon each of these aspects.

i) *Form of Iron*: We have read earlier that iron in foods occurs either as haem or non-haem iron. Haem iron comprises of iron in combination with porphyrins and is found only in the flesh foods in the form of haemoglobin and myoglobin. Muscle meats are therefore good sources of haem iron. Haem iron is absorbed to a much greater extent than non-haem. Haem iron absorption is generally 2-3-fold higher than non-haem iron absorption. The average absorption of haem iron from meat-containing meals is about 25%. The absorption of haem iron can vary from about 40% during iron deficiency to about 10% during iron repletion. Haem iron can be degraded and converted to non-haem iron if foods are cooked at a high temperature for too long. Iron absorption is not affected by other dietary factors except calcium which has been shown to depress haem iron absorption. In addition to providing higher bioavailable iron, haem iron compounds also enhance non-haem iron absorption. Further, non-haem iron absorption in healthy adults may vary from less than 1% to about 0% depending on the composition of the diet.

The next factor that is being discussed is the solubility of the iron/its complex with other substances.

ii) *Solubility*: Solubility is crucial for non-haem iron absorption as the inorganic iron salts have to be solubilized in the intestine for the iron to be taken up by the muscosal cells. The acidic pH of the stomach makes iron soluble. However, as the chyme passes into the small intestine, the rising pH tends to precipitate iron as ferric hydroxide complexes. The presence of ascorbic acid and other organic acids in the small intestine solubilize to chelate the iron so that it can be absorbed. Ferrous salts are more soluble than ferric salts and are therefore better absorbed.

iii) *Inhibitors and Enhancers*: *Phytates* and *fibre* from whole grain cereals, *tannins* and *polyphenols* in tea, *oxalates* in green leafy vegetables like spinach and excess *calcium* taken as supplements can all *depress non-haem iron absorption* significantly, by forming insoluble components. The Indian vegetarian diet consisting predominantly of cereals and pulses, high in phytates, has a low iron bioavailability. This is further compromised when tea is drunk with a meal, as polyphenols in tea depress iron absorption. Iron absorption from wheat has been reported to be 5%. However, when tea is taken with a breakfast meal comprising of wheat chapattis and potato vegetable, the reported absorption has been only 1.8%. Ragi balls or sorghum breakfast with potato vegetable and tea resulted in only 0.8-0.9% absorption of iron.

On the contrary, *ascorbic acid is a potent enhancer* of iron absorption. Addition of orange juice containing 40-50 mg ascorbic acid to a breakfast meal consisting of bread, eggs and tea was found to increase iron absorption from 3.7% to 10%. Thus, ascorbic acid can counter the inhibitory effect of tannins or phytates, producing a 2-3 fold increase in iron absorption.

Thus, ascorbic acid can enhance iron absorption in a number of ways. Firstly, it reduces insoluble ferric iron to soluble ferrous iron; secondly, ascorbic acid forms low molecular weight chelates with iron that remain soluble in the intestine; thirdly, ascorbic acid-iron chelates preferentially release the iron for absorption to the brush border. Together, these mechanisms ensure that dietary iron is well absorbed in the presence of ascorbic acid.

Other factors known to enhance iron absorption are meat and flesh foods and some amino acids such as cysteine.

The best way to increase bioavailability of iron in Indian vegetarian diet is to consume adequate amounts of ascorbic acid rich fruits and vegetables with the meals, reduce phytate content by appropriate home levels processes such as germination and fermentation and avoid drinking tea with the meals.

Another factor which may determine the absorption of iron is the existing iron status of the individual. This is particularly relevant with respect to iron deficiency anaemia.

iv) *Iron Status of the Individual*: Lastly, iron status of the individual is a floury determinant of how much iron is absorbed. On a mixed diet with some haem iron, the overall absorption may approximate to 10% in normal subjects while it is about 20% in iron deficient subjects.

Table 10.3 lists the currently known dietary factors affecting iron absorption.

Table 10.3: Dietary factors affecting iron absorption

Increase Absorption	Decrease Absorption
• Gastric Acidity	• Increased intestinal motility
• Ascorbic Acid	• Phytates and oxalates
• Certain organic acids like citric, lactic and tartaric acid	• Iron-binding phenolic compounds such as ferrous pyrophosphate, ferrous citrate
• Animal proteins such as meat, fish, poultry	• Calcium, Phosphorus and Magnesium
• Sugars – Fructose, sorbitol	• Zinc, Manganese and Copper
• Physiological factors – pregnancy and growth	• Tannic acid in coffee and tea
• Depleted iron status	• High Iron status
	• Antacids
	• Achlorhydria, Hypochlorhydria
	• Poor fat digestion

So far we have discussed about the various aspects of iron absorption. However, it was also mentioned that once iron gets absorbed, it is utilized judiciously again and again by our body. What is the mechanism that regulates iron balance and absorption? Let us understand about it in detail.

Iron Balance and Regulation of Iron Absorption

The body has three unique mechanisms for maintaining iron balance.

The first is the continuous *reutilization of iron* from catabolized erythrocytes in the body. When an erythrocyte dies after about 120 days, it is usually degraded by the macrophages of the reticular endothelium. The iron is released and delivered to transferrin in the plasma, which brings the iron back to red blood cell precursors in the bone marrow or to other cells in different tissues. Uptake and distribution of iron in the body is regulated by the synthesis of transferrin receptors on the cell surface. This system for internal iron transport not only controls the rate of flow of iron to different tissues according to their needs, but also effectively prevents the appearance of free iron and the formation of free radicals in the circulation.

The re-utilization of iron is a highly significant process. As mentioned earlier, the red blood cells (erythrocytes) contain two thirds of the total body iron. If $1/120^{th}$ of this is to be degraded daily, (note: life span of erythrocytes is 120 days) it results in the release of about 20 mg of iron daily within the body. Almost all of this is re-utilized for the synthesis of new haemoglobin and erythrocytes. Only an extremely small proportion i.e., about 1 mg is lost from the body to be replaced by dietary iron. The amount of iron released from erythrocytes and re-utilized for new haemoglobin is termed as *iron turnover* in the body.

The second mechanism involves access to the specific storage protein, *ferritin*. This protein stores iron in periods of relatively low need and releases it to meet excessive iron demands. This iron reservoir is especially important in the third trimester of pregnancy.

The third mechanism involves the regulation of absorption of iron from the intestines; decreasing body iron stores trigger increased iron absorption and increasing iron stores trigger decreased iron absorption. Iron absorption decreases until equilibrium is established between absorption and requirement.

Now we shall discuss the transport and storage of absorbed dietary iron in our body.

Transport and Storage

You have seen that transferrin binds both newly absorbed iron and iron released after degradation of haemoglobin. Transferrin is a glycoprotein and has two binding sites for Fe^{3+}. It acts as an iron transport protein. Normally, in plasma it is one-third saturated with ferric ions. It distributes iron throughout the body to wherever it is needed, mostly to erythrocyte precursors in the bone marrow. In iron deficiency, transferrin saturation is reduced while in iron overload, tansferrin saturation gets increased.

Any absorbed iron in excess of body needs is stored in the liver, in two forms, as *ferritin* and *haemosiderin*. Ferritin and haemosiderin are the two major iron storage proteins. The ratio of these two proteins in the liver varies according to the level of iron stored, with ferritin predominating at lower iron concentrations and haemosiderin at higher concentrations. Iron is released from these stores in times of need more readily from ferritin than haemosiderin.

Binding of iron by protein during storage and transport serves as a defense mechanism. How? If iron ions are left unbound, the redox activity of iron can lead to the generation

of harmful free radicals that can cause damage to the cells and their membranes.

We have been reading that once iron is absorbed, our body tries to use it conservatively and re-utilizes it again and again. What would happen then, if iron is consumed in excess of our requirements? Further, the iron absorption need not always be complete. Unabsorbed iron would get excreted. Let us read how iron gets excreted from the body.

Excretion

Our body has a limited capacity to excrete iron once it has been absorbed. Daily losses in adult man are between 0.9 to 1.05 mg. About 0.08 mg is lost via urine, 0.2 mg via skin and remaining in the faeces. Women in the reproductive age lose more iron owing to menstrual cycles.

Iron is unique among the minerals, that once absorbed the body holds onto it, and therefore, major regulation of iron balance is through absorption of iron rather than through excretion. The percentage of iron absorbed can vary from less than 1% to more than 50%, depending on the food eaten and the response of the regulatory mechanisms that reflects body's physiological need for iron. However, this regulatory mechanism is not perfect across the entire range of intakes.

Next, we shall discuss how iron is taken up by different tissues to perform various functions in the body.

Iron Uptake by Cells and its Functions

Iron participates in a large number of biochemical reactions. However, for iron to perform any function, it first needs to be taken up by the cells. Let us then first review iron uptake by cells.

Cell membranes contain a protein specific for binding transferrin called '*transferrin receptor*'. Transferrin containing two ferric ions, binds to this receptor. Thereafter, iron-transferrin-transferrin receptor complex is internalized by endoyctosis. Within the cell, iron is released from transferrin.

It has been shown that intracellular iron concentration more or less remains constant. This intracellular iron homeostasis is maintained by regulating the synthesis and action of proteins involved in the iron acquisition, utilization and storage. When intracellular iron is scarce, cell needs to increase its iron concentration. This is achieved by acquisition of plasma iron and mobilization of storage iron. Also, there is a need to prioritize utilization of iron so that iron is preferentially available for the synthesis of life sustaining iron-containing proteins. Therefore, whenever the intracellular iron concentration is low, the number of transferrin receptors on the cell increase. Further, it is postulated that iron concentration also regulates the synthesis of *apoferritin* and δ-*aminolevulinic acid synthase*. The latter is the key enzyme for haem synthesis.

Now that we have been acquainted to the mechanism involved in iron uptake by cells, let us focus on the functions of iron.

Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues. The general classification of the reactions in which iron is involved includes:

- Oxygen transport and storage
- Electron transfer
- Substrate oxidation– reduction

Four major classes of iron containing proteins carry out these reactions in the mammalian system. These are illustrated in Figure 10.2.

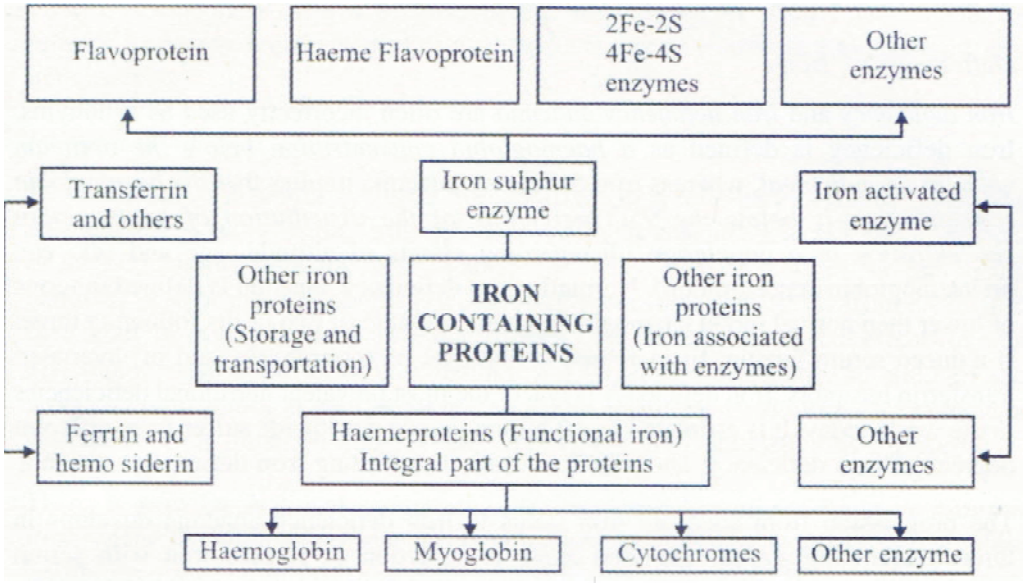


Figure 10.2: Classification of major mammalian iron containing proteins

Source: Beard and Dauson, 1997

Several iron-containing enzymes, the cytochromes, have one haem group and one globin protein chain. These enzymes act as electron carriers within the cell and their structures do not permit reversible loading and unloading of oxygen. Their role in the oxidative metabolism is to transfer energy within the cell and specifically in the mitochondria. Other key functions for the iron-containing enzymes (e.g. cytochrome P450) include the synthesis of steroid hormones and bile acids; detoxification of foreign substances in the liver; and signal controlling in some neurotransmitters, such as the dopamine and serotonin systems in the brain.

As a component of cytochromes and other enzymes of electron transport chain, it is critical for conversion of food into ATP. Iron-containing molecules ensure that macromolecules like carbohydrates and fats are oxidized to provide the energy necessary for all physiological processes and movements.

Iron is a component of many other tissue enzymes required for immune system functioning. Non-haem iron proteins, as we know, are responsible for a wide range of functions such as enzymes methane mono-oxygenase (oxidizes methane to methanol) and ribonucleotide reductase (reduces ribose to deoxyribose; DNA biosynthesis).

As a part of haemoglobin, iron is required for the transport of oxygen, to all cells in the body. Thus, haemoglobin is critical for cell respiration. Most of the iron in the body is present in the erythrocytes as haemoglobin, a molecule composed of four units, each containing one haem group and one protein chain. The structure of haemoglobin allows it to be fully loaded with oxygen in the lungs and partially unloaded in the tissues (e.g. in the muscles). The iron-containing oxygen storage protein in the muscles, *myoglobin*, is similar in structure to haemoglobin but has only one haem unit and one globin chain. As myoglobin, iron functions as a ready source of oxygen to the muscles.

Iron is thus crucial for the survival, growth and normal functioning of the human system. Let us now read about the consequences of deficiency and iron overload in the body.

Deficiency and Iron Overload

In the following discussion, we shall cover both the deficiency and the consequences of iron overload. We shall begin with iron deficiency.

Deficiency of Iron

Iron deficiency and *iron deficiency anaemia* are often incorrectly used as synonyms. Iron deficiency is defined as *a haemoglobin concentration below the optimum value in an individual*, whereas iron deficiency anaemia implies that *the haemoglobin concentration is below the 95th percentile of the distribution of haemoglobin concentration in a population* (disregarding effects of altitude, age and sex, etc. on haemoglobin concentration). Normally, iron deficiency anaemia is defined in terms of lower then normal blood haemoglobin levels and at least two of the following three: i) reduced serum ferritin, ii) increased erythrocyte protoporphyrin, and iii) increased transferrin receptors. Iron deficiency is one of the most prevalent nutritional deficiencies in the world today. It is estimated that 2 billion people worldwide suffer from different degrees of iron deficiency, about half of them, manifesting iron deficiency anaemia.

The progression from adequate iron status to iron deficiency anaemia develops in three overlapping stages. The *first stage* is depletion of storage iron with serum ferritin levels starting to decline. However, the transferrin saturation, erythrocyte protoporphyrin and haemoglobin are within normal limits. As iron stores get increasingly depleted, iron deficiency develops which is the *second stage*. During this stage, in addition to low serum ferritin levels, transferrin saturation is also reduced and erythrocyte protoporphyrin is elevated. Haemoglobin may be normal. Eventually when iron deficiency progresses to anaemia, haemoglobin levels start declining; this is the *third and final stage* of iron deficiency.

The functional effects of iron deficiency anaemia result from both a reduction in circulating haemoglobin and a reduction in iron-containing enzymes and myoglobin. These include:

- fatigue, restlessness and impaired work performance,
- disturbance in thermoregulation,
- impairment of certain key steps in immune response,
- adverse effects on psychomotor and mental development particularly in children, and
- increased maternal and perinatal mortality and morbidity .

Studies in animals have clearly shown a relationship between iron deficiency and brain functions. Several structures in the brain have high iron content. The observation that the lower iron content of the brain in iron-deficient growing rats cannot be increased by giving iron at a later date, strongly suggests that the supply of iron to brain cells takes place during an early phase of brain development and that, as such, early iron deficiency may lead to irreparable damage to brain cells. In humans, about 10% of brain-iron is present at birth; at the age of 10 years, the brain has only reached half its normal iron content, and optimal amounts are first reached between the ages of 20 and 30 years. Several groups have demonstrated a relationship between iron deficiency and attention, memory and learning in infants and small children. In the most recent well-controlled studies, no effect was noted from the administration of iron.

Iron deficiency also negatively influences the normal defence systems against infections. Several studies have observed a reduction in physical working capacity in human populations with longstanding iron deficiency, and demonstrated an improvement in working capacity in these populations after iron administration. Well-controlled studies in adolescent girls show that iron-deficiency without anaemia is associated with reduced physical endurance and changes in mood and ability to concentrate.

Considering the ill-effects of iron deficiency, preventing this problem is crucial. Populations most at-risk for iron deficiency are infants, children, adolescents and women of childbearing age, especially pregnant women. The weaning period in infants is especially critical because of the very high iron requirement needed in relation to energy requirement. Let us then focus our attention on prevention of iron deficiency.

Prevention of Iron Deficiency

Iron deficiency anaemia accounts for approximately one-half or more of all the anaemia's seen world wide. Iron deficiency without anaemia affects a large segment of the populations, as many as with anaemia. Thus, 70% or more of the pre-school children, 90% or more of pregnant women and adolescent girls suffer from either iron deficiency or iron deficiency anaemia in India. The serious functional effects of iron deficiency anaemia on learning, cognition and physical performance in children and productivity in adults, as well as, increased maternal and pre-natal mortality in pregnant women make it imperative to prevent and or treat iron deficiency as a priority.

There is a major National programme, the *National Nutritional Anaemia Control Programme* that aims to prevent and treat anaemia in pregnant women using a public health approach. Iron (100 mg elemental iron) and folic acid (0.5 mg) in the form of tablets are provided to all pregnant women for 100 days during a pregnancy through the ICDS.

Severely anaemic women are given two tablets a day for 100 days as a treatment. Medicinal iron in a suitable form proves useful in treating iron deficiency at individual levels. Long-term prevention of iron deficiency must depend on improving the bio-availability of iron and increasing the iron content of the diets. Studies have shown that consumption of fruits rich in ascorbic acid such as guavas with major meals can improve haemoglobin levels. Drinking tea with meals should be avoided. At least a gap of ½-2 hours is needed between a meal and tea for better iron absorption.

While the deficiency of iron is a common health problem; it is important to consider the causes of this problem. Nutritional iron deficiency implies that the diet cannot supply enough iron to cover the body's physiological requirements for this mineral. Worldwide, this is the most common cause of iron deficiency. In many tropical countries, infestations with hookworms lead to intestinal blood losses that in some individuals can be considerable.

Besides deficiency conditions, there can be situations (though rare) when there is excessive accumulation of iron in the body. Let us next discuss the consequences of iron toxicity.

Iron Overload/Toxicity

We have seen that absorption of iron is very effectively regulated. This prevents overload of the tissues with iron from diet/supplements in normal healthy individuals. However, an excessive body burden of iron can be produced by greater-than-normal absorption from the alimentary canal, by parenteral injection or by a combination of both. For instance, people with genetic defects develop iron overload as it occurs in *idiopathic haemochromatosis*. It is *a hereditary disorder of iron metabolism characterized by abnormally high iron absorption owing to a failure of the iron absorption control mechanism at the intestinal level*. High deposits of iron in the liver and the heart can lead to cirrhosis, hepatocellular cancer, congestive heart failure and eventual death.

African or Bantu siderosis, chronic liver disease, pancreatic insufficiency, shunt haemochromatosis and certain types of refractory anaemia have been found to be associated with iron overload. It has recently been shown that excess iron intake via overuse of iron supplements could pose a possible health risk. Cellular and tissue

injury due to free radical reactions appears to be the possible mechanism. Normally iron is bound tightly to the proteins. However, it is possible that excess iron intake permits some iron to be in a free form. Associated complications may include increased risk for bacterial infection, neoplasia, arthopathy, cardiomyopathy and endocrine dysfunction.

Next, we shall learn about the indicators of iron status in the human body. These indicators/values provide valuable information to plan the subsequent course of treatment and ensure proper rehabilitation.

Assessment of Iron Status

In view of widespread iron deficiency, it is important to have reliable and sensitive measures of iron status. Iron status can be assessed by a number of methods, which are suitable for different stages of iron deficiency. These are briefly discussed below:

i) *Serum Ferritin*: This method is indicative of iron stores. As we know, a long term negative iron balance first results in depletion of iron stores with a fall in serum ferritin levels. Plasma ferritin concentration of less than 30 microgram per litre is considered indicative of iron deficiency. In normal subjects, plasma ferritin averages 100 mcg/L. Values in excess of 250 mcg/L are indicative of iron overload.

ii) *Transferrin receptors*: As iron deficiency progresses into second stage, the number of transferrin receptors on the cell surface increase. Measurement of serum tranferrin receptors is thought to reflect transferrin receptors on immature red cells. Values more than 8.5 mg/L reflects iron deficiency.

iii) *Erythrocyte protoporphyrin*: In the early stages of iron deficiency, there is accumulation of free protoporphyrin (precursor of haemoglobin). Zinc protoporphyrin is usually measured. Levels more than 40 micro mol/mol haem is associated with iron deficiency.

iv) *Transferrin saturation*: As iron deficiency progresses, there is a decline in transferrin saturation. With deficiency, transferrin saturation reduces to less than 15-16%, is indicative of iron deficiency.

v) *Haemoglobin and Haematocrit*: In the final stages of iron deficiency, anaemia occurs. Haemoglobin and haematocrit levels indicate prevalence of anaemia. Haematocrit represents that *proportion of the total blood volume that is red blood cell and is expressed as percentage (%)*. Values of these two indicators, below which anaemia is considered to exist, according to age and sex is given in the Table 10.4.

Table 10.4 : Haemoglobin and haematocrit levels below which anaemia is present

Age/ Gender Group	Haemoglobin (g/l)	Haematocrit (mmol/L)
Children 59 m	110	6.83
Children 5-11 years	115	7.13
Children 12-14 years	120	7.45
Non-pregnant women (above 15 years of age)	120	7.45
Pregnant women	110	6.83
Men (above 15 years of age)	130	8.07

Source: WHO, 2001.

So, how much iron should be consumed in order to maintain an adequate iron nutriture? Let us read and find out.

Requirements

In Unit 1, we have already learnt about how recommended daily intakes are computed. The requirements for iron, as recommended by ICMR, for various age-groups, are given in Table 10.5. The recommended intakes are based on iron absorption of 6% in children and 8% in all men and women.

Table 10.5: ICMR recommendation for daily iron intake for Indians

Group	Body weight kg (Kg)	Pysiological Iron Requirement	Absorption assumed%	ICMR 2020[b]		TUL mg/d
				EAR (mg/d)	RDA (mg/d)	
Men	65.0	0.91	8	11	19	45
Women	55.0	1.20	8	15	29	45
Pregnant women	55.0[c]	2.55	8	21	27	45
Lactating women (0–6m)	55.0	1.28	8	16	23	45
Infants 0–6 m	5.4	--	--	--	--	40
6–12 m	8.5	0.33	15[d]	2	3	40
Children 1–3 y	11.7	0.38	6	6	8	40
4–6 y	18.3	0.49	6	8	11	40
7–9 y	25.3	0.61	6	10	15	40
Adolescents						
Boy 10–12 y	34.9	0.93	8	12	16	40
Girl 10–12 y	36.4	1.27	8	16	28	40
Boy 13–15 y	50.5	1.23	8	15	22	45
Girl 13–15 y	49.6	1.39	8	17	30	45
Boy 16–18 y	64.4	1.45	8	18	26	45
Girl 16–18 y	55.7	1.46	8	18	32	45

[b]EAR and RDA are rounded off to whole number;
[c]Pre-pregnancy weight: EAR and RDA is given as average of all 3 trimester for a woman having 12kg GWG
[d]Iron absorption reported for infants was 15%; TUL: Tolerable Upper Intake Level.
Source: Recommended Dietary Allowances for Indians NIN, ICMR, Hyderabad, India, 2020.

It is recommended *that the density of ascorbic acid should be at least 20 mg/1000 kcal* (3 times by weight to achieve 1:2 molar ratio to ascorbic acid) to ensure 5% iron absorption.

In this section we read about the food sources, metabolism, functions, deficiency, toxicity of iron, as well as, important indicators of iron status and the recommended dietary allowances for this nutrient crucial for our survival. Let us now attempt the questions given in check your progress exercise 1 to recapitulate the concepts we have learnt so far in this unit.

Check Your Progress Exercise 1

1) What percentage of iron is found in association with haemoglobin?

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2) List at least five sources of haem and non-haem iron.

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3) Enumerate a few dietary factors which affect iron absorption.

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4) How does our body maintain iron balance?

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5) What are the consequences of iron deficiency?

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6) List the various methods by which one can assess iron status.

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In our next section(s) we will read further about other micro minerals viz., zinc and copper.

10.4 ZINC

Zinc deficiency in humans was reported by *A.S. Prasad* among people consuming mostly breads and very little animal protein in Middle Eastern countries. Common manifestations of zinc deficiency were reduction in growth and appearance of skin lesions. In 1974, a genetic human disease–*acrodermatitis enteropathica* was related to an inability to absorb adequate zinc from the normal diet. The formal recognition of zinc as an essential nutrient came in 1974, when dietary allowances for nutrients were made.

In the biological systems, zinc is always found in the divalent (+2) state. Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30 mmol (2 g). Skeletal muscle accounts for approximately 60% of the total body content and bone mass, with a zinc concentration of 1.5-3 μmol/g (100-200 μg/g), for approximately 30%. The concentration of zinc in lean body mass is approximately 0.46 μmol/g (30 μg/g). Plasma zinc has a rapid turnover rate and it represents only about 0.l% of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye (4.2 μmol/g or 274 μg/g) and in prostatic fluids (4.6-7.7 mmol/l or 300-500 mg/L).

Let us next get to know about the food sources rich in zinc.

Food Sources

Zinc is normally associated with the protein and/or nucleic acid fraction of foods. Thus, foods high in proteins are good sources of zinc. Lean red meat, whole-grain cereals, pulses and legumes provide the highest concentrations of zinc: concentrations in such foods are generally in the range of 25-50 mg/kg (380-760 μmol/kg) raw weight. Processed cereals with low extraction rates, polished rice, and chicken, pork or meat with high fat content have moderate zinc content, typically between 10 and 25 mg/kg (150-380 μmol/kg). Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc, having concentrations <10 mg/kg (<150 μmol/kg) Saturated fats and oils, sugar and alcohol have very low zinc contents. Refer to Table 10.6, where sources of zinc along with content are given.

Table 10.6 : Zinc content of foods

Foods/ Food Groups	Zinc (mg/100 g) Edible Portion
Sea Food	
Oysters	17-91
Shrimp	1.1
Tung	0.5-0.8
Meat and Poultry	
Liver	3.1-3.9
Chicken	1.0-2.0
Beef	3.9-4.1
Pork	1.6-2.1
Eggs and dairy products	
Eggs	1.1
Milk	0.4
Cheeses	2.8-3.2
Pulses and Legumes	
Legumes (cooked)	0.6-1.0
Pulses/legumes (whole)	2.8-6.1
Bengal gram/red gram dhal	3.65

Grains and Cereal	
Wheat/wheat products	0.6-2.7
Raw Rice	1.21
Rice flakes	1.49
Ragi	2.53
Nuts and Oilseeds	
Gingelly seeds (white)	7.7
Almonds	3.6
Cashewnuts	5.34
Vegetables	0.08 - 1.90
Fruits	0.06 - 0.70

Source: Indian Food Composition Tables by T. Longvah, R. Ananthan, K. Bhaskarachary and K. Venkaish, National Institute of Nutrition ICMR, 2017

From Table 10.6, you can see that zinc is present in high amounts in nuts and red meat. Among seafood, oysters are very high in zinc. Other good animal sources include poultry, pork and dairy products. Among the foods of plant origin, legumes, whole grain cereals and vegetables (leafy vegetables and roots) are the good sources. Refining of cereals reduce the content to a large extent.

The important aspects of absorption, storage, transport and excretion of zinc shall now be dealt in detail.

Metabolism

Zinc has been found to play an important biological role in our body. Zinc ions can be chelated and precipitated by a number of chelating agents including some natural constituents of the diet. In order to take maximum benefit of this nutrient to enhance health, it is important to understand about its metabolism in detail. Let us begin with the absorption of zinc.

Absorption

Like iron, zinc also needs to be liberated from food prior to absorption. During digestive process; proteases, nucleases and hydrochloric acid all appear to release zinc bound to proteins and nucleic acids.

Zinc is absorbed throughout the small intestine, with absorption being most efficient in the jejunum. Zinc given as aqueous solution to fasting subjects is absorbed to the extent of 60-70%. However, absorption from solid diets is less efficient and varies widely depending upon the content of the zinc in the meal and the composition of the diet. Tentative estimates of absorption from different types of diet have been used for estimating requirements. These are:

a) Highly bioavailable diets (low in inhibitors, high in enhancers) 50-60%

b) Normal availability–a mixed diet 30%

c) Low availability diet (high in phytate, calcium and other inhibitors) 15%

Table 10.7 presents the criteria for categorizing diets according to the potential bioavailability of their zinc.

Table 10.7: Criteria of categorizing diets according to the potential bioavailability of their zinc

Normal Category[a]	Principal Dietary Characteristics
High availability	Refined diets low in cereal fibre, low in phytic acid content, and with phytate-zinc molar ratio <5; adequate protein content principally from non-vegetable sources, such as meats and fish. Includes semi-synthetic formula diets based on animal protein.
Moderate bioavailability	Mixed diets containing animal or fish protein. Lacto-ovo, ovo-vegetarian, or vegan diets not based primarily on unrefined cereal grains or high-extraction-rate flours. Phytate-zinc molar ratio of total diet within the range 5-15, or not exceeding 10 if more than 50% of the energy intake is accounted for by unfermented, unrefined cereal grains and flours and the diet is fortified with inorganic calcium salts (>1 g Ca^{2+}/day). Availability of zinc improves when the diet includes animal protein or milks, or other protein sources or milks.
Low availability	Diets high in unrefined, unfermented, and ungerminated cereal grain[b], especially when fortified with inorganic calcium salts and when intake of animal protein is negligible. Phytate-zinc molar ratio of total diet exceeds 15[c], high phytate, soya-protein products constitute the primary protein source. Diets in which singly or collectively, approximately 50% of the energy intake is accounted for by the following high–phytate foods; high-extraction-rate (<90%), heat, rice, maize, grains and flours, and millet; chapatti flours and tanok; and sorghum, cowpeas, pigeon peas, grams, kidney beans, black-eyed beans and groundnut flours. High intakes of inorganic calcium salts 1g Ca^{2+}/day), either as supplements or as adventitious contaminants (e.g. from calcareous geophagia), potentiate the inhibitory effects and low intakes of animal protein exacerbates these effects.

[a] At intakes adequate to meet the average normative requirements for absorbed zinc, the three availability levels correspond to 50%, 30% and 15% absorption. With higher zinc intakes, the fractional absorption is lower.

[b] Germination of cereal grains or fermentation (e.g. leavening) of many flours can reduce antagonistic potency of phytates; if done, the diet should then be classified as having moderate zinc availability.

[c] Vegetable diets with phytate-zinc ratios exceeding 30 are not unknown; for such diets, an assumption of 10% availability of zinc or less may be justified, especially if the intake of protein is low, that of inorganic, calcium salts is excessive (e.g. calcium salts providing <1.5 g Ca^{2+}/day), or both.

Source: Adapted from *Trace elements in human nutrition and health.* Geneva, World Health Organization, 1996.

Like other nutrients, zinc is also first absorbed in the enterocytes and then transported across the basolateral membrane.

Let us now see how zinc enters the enterocytes and what its fate in these cells is.

Zinc is absorbed into the enterocytes by a carrier-mediated process. Absorption by this process is efficient at low intakes. At high intakes, zinc appears to be absorbed by passive diffusion. Within the enterocytes, zinc has one of the following possible fates:

a) Used or stored within the enterocytes, and

b) Bound to the proteins such as *cysteine rich intestinal proteins* (CRIP) or *metallothionein*. Normally, initially absorbed zinc preferentially accumulates on CRIP. However, with the increased zinc concentrations, metallothionein concentrations rise. This is because the diets high in zinc appear to induce gene expression of metallothionein.

CRIP appears to mediate intracellular zinc transport while zinc bound to metallothionein is normally lost into the lumen with sloughing of these cells. These proteins can also bind other minerals especially copper in the enterocytes.

Zinc not bound to metallothionein or used within the cells is transported across the basolateral membrane with the help of *zinc transporters* (ZnTs). Many ZnTs have been identified in different tissues. ZnTs are found in enterocytes besides many other tissues. Look at Figure 10.3 for better clarity regarding transport of zinc. Here, as you can see, ZnT_1, binds to the unused and unbound Zn ions and transports it across the membrane.

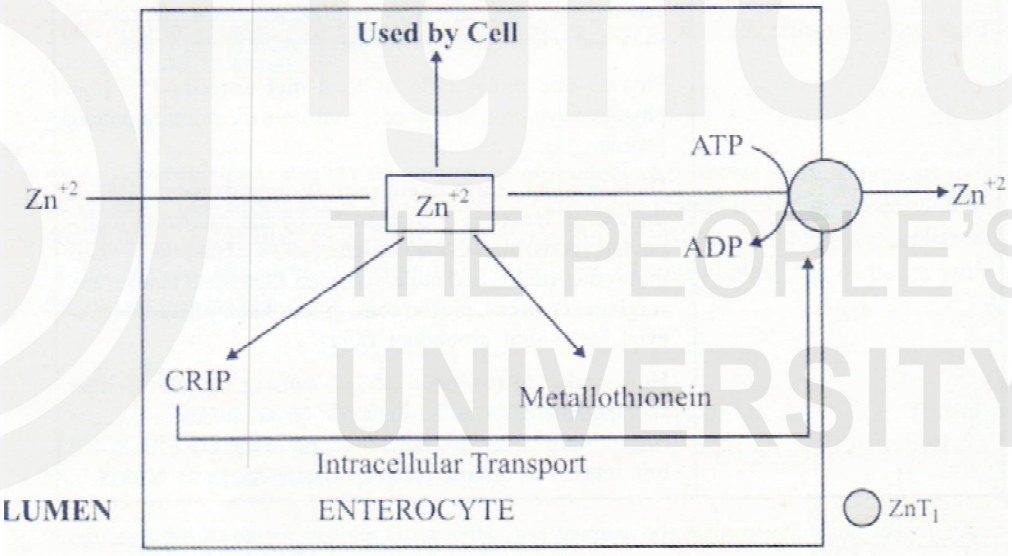


Figure 10.3: Enterocyte use and transport of zinc

The utilization of zinc depends on the overall composition of the diet. Experimental studies have identified a number of dietary factors as potential promoters or antagonists of zinc absorption. Let us learn about these factors.

Factors affecting Zn Absorption

In the last unit you have studied that absorption of various minerals (bioavailability) is influenced by number of factors. Similarly, in case of zinc, different constituents of the diet, commonly known as *dietary ligands* may bind to zinc and either inhibit or enhance its absorption. It has been observed that citric acid, picolinic acid, glutathione, amino acids especially histidine and cysteine and possibly lysine and glycine serve as ligands and appear to enhance zinc absorption, especially in the presence of inhibitors. Zinc histidine and cysteine complexes are absorbed 30-40% more efficiently than zinc sulphate. These two amino acids appear to be also the preferred ligands for zinc.

Isotope studies with human subjects have identified two factors that, together with the total zinc content of the diet, are major determinants of absorption and utilization of dietary zinc. The first is the content of *inositol hexaphosphate* (*phytate*) in the diet and the second is the level and source of *dietary protein*.

Phytates are present in whole-grain cereals and legumes and in smaller amounts in other vegetables. They have a strong potential for binding divalent cations and their depressive effect on zinc absorption has been demonstrated in humans. The molar ratio between phytates and zinc in meals or diets is a useful indicator of the effect of phytates in depressing zinc absorption. At molar ratios above the range of 6-10, zinc absorption starts to decline; at ratios above 15, absorption is typically less than 15%.

It has been observed that phytates, in the presence of high intraluminal calcium, has a greater inhibitory effect than phytates alone. Provisionally it has been suggested that if phytate to zinc molar ratio is greater than 15, the content of available zinc in the diet is likely to be low (less than 15%). Available evidence shows that only hexa and penta-phosphorylated forms of phytic acid inhibit zinc absorption. The phytate content can also be reduced by activating the *phytase* present in most phytate-containing foods or through the addition of microbial or fungal phytases. Phytases hydrolyze the phytate to lower inositol phosphates, resulting in improved zinc absorption. The activity of phytases in tropical cereals such as maize and sorghum is lower than that in wheat and rye. Germination of cereals and legumes increases phytase activity and addition of some germinated flour to ungerminated maize or sorghum followed by soaking at ambient temperature for 12-24 hours can reduce the phytate content substantially. Additional reduction can be achieved by the fermentation of porridge for weaning foods or dough for bread making. Thus, fermentation which promotes extensive degradation of dietary phytates can significantly improve the bioavailability of zinc.

The effect of phytate is, however, modified by the source and amount of dietary proteins consumed. Animal proteins improve zinc absorption from a phytate-containing diet. Zinc absorption from some legume-based diets (e.g. white beans and lupin protein) is comparable with that from animal protein-based diets despite a higher phytate content in the former.

As in case of iron, absorption of zinc generally is higher from foods of animal origin as compared to that from plant foods. Also, absorption appears to be enhanced by low zinc status, especially carrier-mediated mechanism. This indicates that the amount of zinc absorbed is homeostatically regulated.

What happens to zinc once it has been absorbed through the small intestine. Let us find out.

Transport and uptake by Cells

After absorption, zinc is bound to albumin and transported to the liver. In liver, it is concentrated and then transported to different tissues by various plasma proteins. Albumin transports 60% of the zinc, while remaining is transported by other compounds like α-2 macroglobulin, transferrin, immunoglobulin and two amino acids–histidine and cysteine.

Zinc is taken up by various tissues and is incorporated in different enzymes. Since zinc is an important component of various metallo-enzymes within the cells, enzyme synthesis and zinc uptake are correlated. However, mechanism of zinc uptake by various tissues is unknown. Multiple passive transport system including amino acid carrier systems have been proposed.

Next, we move over to the storage of zinc in the body.

Storage

Zinc is found in most organs, concentration being higher in liver, kidney, muscle, skin and bone. Zinc content of muscle, brain, lung and heart is relatively stable and does not respond to changes in dietary zinc intake. Similarly, release of zinc from bones is very slow and does not contribute zinc to other tissues during deprivation. When dietary zinc intake is insufficient, liver metallothionein zinc appears to be mobilized and redistributed. As dietary zinc intake decreases, liver and RBC metallothionein bound zinc reduces.

Zinc which is not absorbed by our gastrointestinal tract tends to get excreted by our body. Zinc may also get lost from our body due to damage to cells/tissues of our body or as a result of normal physiologic processes. The major routes of zinc excretion are highlighted in our subsequent discussions.

Excretion

Zinc is excreted primarily through the following three routes:

i) *Gastrointestinal tract*: Majority of zinc is lost from the body in faeces. Endogenous zinc in the form of enzymes or metallo-proteins is secreted into the gastrointestinal tract by the salivary glands, intestinal mucosa, pancreas and liver. Some of this zinc is reabsorbed while some is excreted. Sloughed enterocytes also contribute to faecal zinc. Endogenous intestinal losses can vary from 7 µmol / day (0.5 mg/day) to more than 45 µmol / day (3 mg /day), depending on zinc intake – the higher the intake, the greater the losses.

ii) *Kidney*: Very small amount of zinc is excreted in the urine (0.3 - 0.7 mg/day), as most of the zinc filtered by the kidney is reabsorbed. Starvation and muscle catabolism increase zinc losses in urine.

iii) *Body surface*: Loss of zinc occurs due to the exfoliation of skin and sweating (0.7 -1.0 mg/day). Another route of zinc loss is hair, which contains 0.1-0.2 mg Zn/g hair. Strenuous exercise and elevated ambient temperatures can lead to high losses through perspiration.

Considerable scientific efforts have been carried out to improve our understanding regarding the biological and physiological role of zinc. The important functions of this mineral are highlighted in our subsequent discussions.

Functions

Zinc is an essential component of a large number of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids, as well as, in the metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components and membranes and in this way contributes to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus, in the process of genetic expression. Zinc also plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity. *Shankar* and *Prasad* have reviewed the role of zinc in immunity extensively. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms.

These divergent functions of zinc in the body can be grouped into three categories namely, catalytic, structural and regulatory. Some of the important functions are discussed below:

1) *Component of metalloenzymes*: Zinc is unique among the trace elements in that it is a part of enzymes for all six Enzyme Commission classes about which you may recall studying in the Nutritional Biochemistry Course (MFN-002) in Unit 4. As a component of these enzymes, it either provides structural integrity

to the enzyme or participates directly in the reaction at the catalytic site. Zinc is a component of over 300 metalloenzymes and is therefore vital for many fundamental life processes. For example, as a component of *carbonic anhydrase,* it helps in rapid disposal of carbon dioxide; as a part of *alcohol dehydrogenase,* it is involved in the conversion of alcohol to aldehyde such as conversion of retinol to retinal. It is also required for protein digestion since it's a component of *carboxypeptidase A* and *aminopeptidase*, the enzymes involved in the digestion of smaller peptides released after the action of the proteolytic enzymes pepsin, trypsin and chymotrypsin. *Superoxide dismutase* which catalyzes the removal of superoxide radical requires two atoms of both zinc and copper. Zinc has a structural role in this enzyme.

Delta amino levulinic acid dehydratase involved in haem synthesis also contains zinc. Similarly, DNA and RNA polymerase and deoxykinase involved in nucleic acid synthesis are zinc-dependent. Zinc also influences polysome conformation and is thus involved in protein biosynthesis.

2) *Transcription Factor*: Zinc is an important structural component of DNA-binding proteins also known as '*transcription factors*'. These transcription factors contain 'zinc fingers'. The term *zinc finger* is used mainly to denote the configuration of the protein, which looks like fingers. It contains a series of polypeptide loops resulting from twisting and coiling of the cysteine and histidine residues. Zinc is ligated to these two amino acids. The series of loops give rise to zinc fingers.

These zinc containing transcription factors bind to promoter sequences of specific genes and regulates transcription. Example of metallothionein mRNA is illustrated in Figure 10.4.

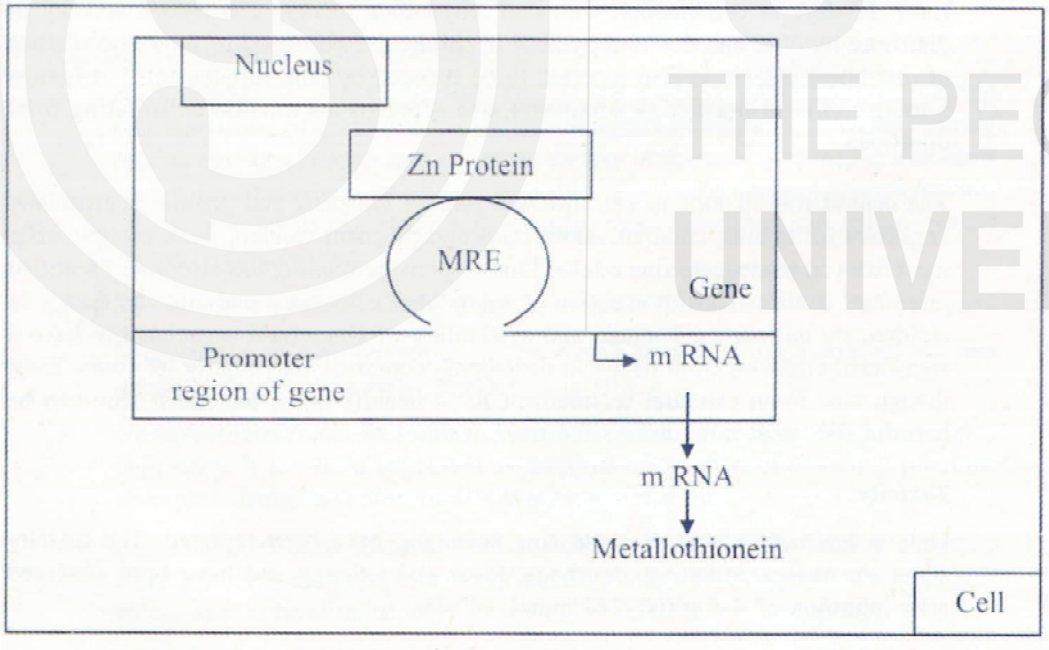


Figure 10.4: Interaction of transcription factor with zinc

Further, these DNA-binding proteins containing zinc fingers also bind to the hormones such as thyroxine, retinoic acid, 1,25-dihydroxycholecaliferol and other steroid hormones such as oestrogen and androgens. These proteins, with the hormones attached to them, bind to DNA and affect gene expression. More details regarding this will be covered under Unit 19.

Next, we come over to the consequences of zinc deficiency.

Deficiency

Zinc deficiency was identified for the first time in 1940 when malnourished Chinese patients were found to have low concentrations of zinc in blood during war time. The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia (loss of hair or baldness), impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes. The effects of marginal or mild zinc deficiency are less clear. A reduced growth rate and impairments of immune defence are so far the only clearly demonstrated signs of mild zinc deficiency in humans. Other effects, such as impaired taste and wound healing, which have been claimed to result from a low zinc intake, are less consistently observed.

The frequency and effects of such mild and moderate deficiency in human population have not been adequately investigated. Growth limiting mild zinc deficiency has been reported in otherwise healthy male American and Canadian infants and preschool children that responded to zinc supplement. In the small areas of Egypt and the Republic of Iran, growth failure in adolescents was found to be responsive to zinc supplements. Severe zinc deficiency in humans is rare.

Many studies have documented that zinc supplementation reduces morbidity from infectious diseases. Reduced activity of the zinc-dependent hormone *thymulin*, one of the factors responsible for reduced cell mediated immunity may contribute to the increased infectitious morbidity in zinc deficiency.

Diarrhoeal diseases are at the root of an estimated 2 million child deaths in developing countries annually. Studies have shown that an inexpensive 20 mg/day zinc supplement for 7-10 days in combination with oral rehydration therapy can reduce severity of diarrhoea by 40% and duration by 20% in children. Likelihood of future occurrence of diarrhoeal disease is also reported to be reduced by zinc supplements. It is now a routine clinical practice to administer zinc supplements to children suffering from diarrhoea.

The central role of zinc in *cell division, protein synthesis* and *growth* is especially important for infants, children, adolescents and pregnant women; these groups suffer most from an inadequate zinc intake. Zinc-responsive *stunting* has also been identified in several studies. Thus, prevention of suboptimal zinc status and zinc deficiency in children by an increased intake and availability of zinc could consequently have a significant effect on child health in developing countries, particularly like ours. Even though zinc is an essential requirement for a healthy body, too much zinc can be harmful. We shall now discuss the main features of zinc toxicity.

Toxicity

Only a few occurrences of acute zinc poisoning have been reported. The toxicity signs are nausea, vomiting, diarrhoea, fever and lethargy and have been observed after ingestion of 4-8 g (60-120 mmol) of zinc.

Gross acute zinc toxicity has been reported after consuming water stored in galvanized containers. Symptoms include nausea, vomiting and fever. These symptoms are observed after ingestion of 2 g or more of zinc.

Long-term zinc intakes higher than requirements could, however, interact with the metabolism of other trace elements. Copper seems to be especially sensitive to high zinc doses. A zinc intake of 50 mg/day (760 μmol) affects copper status. Because copper also has a central role in immune defence, these observations should be studied further before large-scale zinc supplementation programmes are undertaken. Any positive effects of zinc supplementation on growth or infectious diseases could be offset by associated negative effects on copper-related functions.

Intakes between 25 -50 mg zinc per day have been reported to interfere with metabolism of both iron and copper. FAO/WHO 2004 therefore recommended the upper level of zinc intake for an adult man at 45 mg / day (690 µmol/day) and extrapolated to other groups in relation to basal metabolic rate. For children, this extrapolation means an upper limit of intake of 23-28 mg /day (350-430 µmol / day), which is close to what has been used in some of the zinc supplementation studies. Except for excessive intakes of some types of seafood, such intakes are unlikely to be attained with most diets. Adventitious zinc in water from contaminated wells and from galvanized cooking utensils could also lead to high zinc intakes.

Clinical indices/parameters which can provide useful information regarding the zinc status in the human body have been elucidated next.

Assessment of Zn Status

Sensitive indices for assessing zinc status are unknown at present. Static indices, such as zinc concentration in plasma, blood cells and hair, and urinary zinc excretion are decreased in severe zinc deficiency. A number of conditions that are unrelated to zinc status can affect all these indices, especially zinc plasma levels. Food intake, stress situations such as fever, infection and pregnancy lower plasma zinc concentrations whereas, for example, longterm fasting increases it. However, on a population basis, reduced plasma zinc concentrations seem to be a marker for zinc-responsive growth reductions. A number of functional indices of zinc status have also been suggested, for example, wound healing, taste acuity and visual adaptation to the dark. Changes in these functions are, however, not specific to zinc and these indices have not been proven useful for identifying marginal zinc deficiency in humans thus far.

Let us review some of the assessment measures.

- *Measurement of zinc in plasma*: This is the most common method. Fasting concentrations of less than 70 µg/ litre suggests deficiency. However, fasting plasma zinc level decreases only when dietary intake is so low that homeostasis cannot be maintained. It should be noted that while making interpretations, plasma zinc levels can also be affected by stress infections and administration of oral contraceptives.
- *Measurement of zinc in RBCs and neutrophils*: This method is not common.
- *Metallothionein concentration*: Serum metallothionein concentrations are less sensitive to zinc deficiency than levels in RBCs.
- *Urinary zinc levels*: Excretion of zinc in urine decreases with severe zinc deficiency. It has been suggested as an alternate method of assessing oral zinc absorption using oral dose of 10-50 mg elemental zinc.
- *Hair zinc level*: Low zinc may be associated with chronic low intakes of dietary zinc. However, it is important that contamination of hair with shampoo, hair colour should be eliminated.
- *Measurement of activity of zinc-dependent enzymes*: In zinc deficiency, the activity of alkaline phosphatase declines faster than that of carbonic anhydrase.

It must be evident to you by now that zinc is an important element for maintaining health and performing important metabolic functions in the body. But how much amount of zinc would be required to maintain an optimum nutritional balance in the body i.e., prevent deficiency, as well as, toxicity. Let us focus on this aspect.

Requirement

Table 10.8 presents the recommendation for Zn, as recommended by ICMR (2020). From the EAR the RDA for adult man and woman is set at 17 and 13 mg/day, respectively and specific recommendations for all age groups are given in Table 10.8.

Table 10.8: EAR and RDA for Zinc (mg/d) in different age and physiological groups

Group	Body weight kg (Kg)[a]	Pysiological Iron Requirement	Absorption assumed%	ICMR 2020	
				EAR (mg/d)	RDA (mg/d)[b]
Men	65	3.25	23	14.1	17
Women	55	2.53	23	11.0	13.2
Pregnant women[c]	55	3.0	25	12.0	14.5
Lactating women (0–6m)	55	3.53	30	11.8	14.1
Infants 0–6 m	5.8	--	--	--	--
6–12 m	8.5	0.486	23	2.1	2.5
Children 1–3 y	12.9	0.64	23	2.8	3.3
4–6 y	18.3	0.85	23	3.7	4.5
7–9 y	25.3	1.13	23	4.9	5.9
Adolescents					
Boy 10–12 y	34.9	1.62	23	7.0	8.5
Girl 10–12 y	36.4	1.64	23	7.1	8.5
Boy 13–15 y	50.5	2.75	23	11.9	14.3
Girl 13–15 y	49.6	2.46	23	10.7	12.8
Boy 16–18 y	64.4	3.38	23	14.7	17.6
Girl 16–18 y	55.7	2.72	23	11.8	14.2

[a]Body weight ICMR 2020

[b]RDA was computed by multiplying EAR with a factor of 1.2.

[c]Pre-pregnancy weight; EAR and RDA is given as average of all 3 trimester for a women having 10 kg/GWG

Source: Recommended Dietary Allowances and EAR-Nutrient requirements for Indians NIN, ICMR, Hyderabad, India, 2020.

It must have been interesting to know about the ubiquitous presence and wide spectrum of properties of zinc. In-depth research over the past few years is unveiling the massive scope of zinc in maintaining good health. The next nutrient that we shall discuss is copper. However, before we proceed, answer the following questions for a quick recapitulation.

Check Your Progress Exercise 2

1) Why is zinc referred to as the most abundant intracellular trace element?

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2) Name a genetic disease which leads to an inability to absorb adequate zinc from diet?

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3) Describe the term 'Zinc Fingers'.

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4) Briefly discuss function of zinc that makes it a unique trace element.

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5) Enumerate any five techniques of assessing Zn status.

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6) What are the symptoms associated with prolonged intake of Zn?

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Our next topic of discussion is *copper* which is essential for all higher plants and animals. Though required in very small amounts, copper is essential for conducting important metabolic functions in the body. Critical evaluation and adequate understanding of its functions is essential for prompt and judicious treatment of patients with copper imbalance which is frequently critical and associated with high rates of mortality. Let us learn about this nutrient in detail.

10.5 COPPER

As early as the times of Hippocrates, copper compounds were used to treat various diseases. However, in the 20th century, it was noticed that animals fed milk diets developed anaemia, which could not be corrected by dietary iron alone. In 1928, *E.B. Hart* and co-workers demonstrated that rats which developed the milk diet anaemia required copper along with iron to correct anaemia. It is now known that copper is

a constituent of several enzymes and proteins, most of which catalyze oxidation–reduction reactions.

In the body, copper occurs in two oxidation states – Cu^{1+} (Cuprous) or Cu^{2+} (Cupric). The body of a healthy adult contains a little over 0.1 g of copper with concentration being high in liver, brain, heart, bone, hair and nails. About 25% of body copper is present in muscle, and 42% in the skeleton.

Next, we come over to the food sources of copper.

Food Sources

Foods containing more than 1 mg copper per 1000 kilocalories are considered high in copper and include green leafy vegetables, nuts, legumes, dried fruits, muscle meats and shellfish especially oysters. Let us look at the copper content of some important foodstuffs in Table 10.9. This information would be of great help in planning diets requiring copper restriction/enhanced intake.

Table 10.9: Copper content of some important foods

Food	Copper Content (mg/100 g)	Food	Copper Content (mg/100 g)
Dairy		*Vegetables*	
Egg, whole	0.07	Potato, brown (Big)	0.09
Cow milk, whole	0.03		
Paneer	1.10	Red Carrot	0.10
Meat, Fish, Poultry			
Liver, beef	1.23	Spinach	0.17
Chicken	0.25	Peas	0.2
Pork	0.22	Lettuce	0.14
Tuna	0.27	Tomato	0.06
Shrimp	0.30	Baby Corn	0.12
Grains		Cabbage	0.03
Wheat Semolina	0.46	*Fruits*	
Rice, white	0.23	Apple	0.04
Rice flakes	0.26	Banana	0.12
Whole wheat	0.49	Grapes (green)	0.07
Nuts		Peach	0.08
Almonds	1.08	Pear	0.08
		Pineapple	0.07
		Raisins	0.32
		Prunes	0.29

Source: Indian Food Composition Tables, NIN, ICMR, Hyderabad, India, 2017

Copper though present in small amounts in the food needs to be absorbed, transported, stored and excreted efficiently so as to be able to perform its host of functions some of which are critical for other metabolic functions in our body. A brief overview regarding the metabolism of copper is being discussed next.

Metabolism

In food, most copper is present as Cu^{2+} and some as Cu^{1+}. This copper is bound to organic compounds especially protein. Gastric HCl, pepsin and some proteolytic

enzymes aid in the release of copper. Released copper forms soluble complexes with amino acids, organic acids and other chelators which are readily absorbed mainly in the upper intestinal tract. Some copper is also absorbed from the stomach; however, gastric copper absorption contributes relatively little to the overall absorption.

As in the case of other minerals, copper absorption appears to occur by two mechanisms:

i) Saturable active mechanism, which operates when the copper concentration is low, and

ii) Passive diffusion, which occurs at a higher concentration.

Efficiency of absorption varies from 30-50% of ingested copper. Copper absorption is influenced by copper status. Absorption is significantly higher during periods of low dietary copper and vice-versa. Various dietary factors influence copper absorption.

Dietary components exerting positive effect include amino acids especially histidine, organic acids such as citric, gluconic, lactic, acetic and malic acids. Dietary components which inhibit absorption include high intakes of several nutrients such as zinc (as you may recall studying in the last section), iron, molybdenum, calcium, phosphorus and excessive intake of antacids.

Once copper is within the intestinal cell, it may be used by the cell, may be stored in the cell or may be transported across the basolateral membrane. Copper transport across the basolateral membrane into the plasma appears to occur by a carrier-mediated active transport, specific for copper.

Copper which is not absorbed is excreted in the faeces. So, what happens to the copper which is absorbed?

After absorption, ionic copper is tightly bound to plasma proteins, namely albumin and transcuprein and is transported via portal blood to the liver. Small amount of absorbed copper is also transported to other tissues especially kidney.

In the liver, copper is incorporated into ceruloplasmin, which is then released in the blood. Ceruloplasmin constitutes 95% of the total plasma copper. Ceruloplasmin then delivers copper to various tissues. Tissues can also acquire copper from albumin, transcuprein and low molecular weight copper compounds.

Copper enters the cell directly through channels or after binding to protein transporters. Ascorbic acid enhances copper transfer. Glutathione appears to serve as a transporter of copper within the cell. In the cell, copper is incorporated into various copper enzymes and proteins such as cytochrome oxidase.

Most absorbed copper is secreted by the liver into the bile to be excreted in the faeces. This process is the major regulator of copper elimination. Only small amount of copper (~10-50 mcg) is excreted through kidney. Thus, the absorption and excretion process of copper helps in maintaining optimum levels of this element in our body so that it can help in performing a number of metabolic reactions in the body. Let us then learn about the role of copper in our body.

Functions

Copper serves as a co-factor, as well as, an allosteric component of enzymes. In many enzymes, copper functions as an intermediate in electron transfer. The list of copper-containing enzymes with their role is given in Table 10.10.

Table 10.10: Copper-containing enzymes

Enzymes	Function
1) *Amine oxidases*	Found in tissues throughout body and catabolize physiologically active amines.
a) Monoamine oxidase	Acts on serotonin, norepinephrine, tyramine and dopamine.
b) Diamine oxidase	Inactivates histamine. Also inactivates polyamines involved in cell proliferation i.e. it may play a role in limiting excessive growth. High activity in intestine, kidney and maternal plasma.
c) Lysyloxidase	Deaminates the lysine of newly formed immature elastin and collagen after which crosslinks are formed.
d) Peptidylglycine α-amidating monooxygenase	It is a newly identified cupro-enzyme involved in the synthesis of number of bioactive peptide.
2) *Ferroxidases*	
a) Ferroxidase I	Also known as ceruloplasmin. It catalyzes oxidation of ferrous iron and plays a role in the transfer of iron from storage to sites of haemoglobin synthesis.
b) Ferroxidase II	Catalyzes oxidation of iron.
3) *Cytochrome C oxidase*	It is present in mitochondria of cells throughout the body. It is involved in electron transport chain, reduces oxygen to water and allows formation of ATP. Activity is highest in heart followed by brain, liver and kidney tissues.
4) *Dopamine β-hydroxylase*	It catalyzes the conversion of dopamine to neurotransmitter – norepinephrin in the brain. Its concentration is higher in grey matter than white matter of the brain. It is also present in adrenal gland.
5) *Superoxide Dismutase* (*SOD*)	Functions as a scavenger of superoxide radical and protects against oxidation Cu/Zn. SOD is present in most cells and protects intracellular components from damage. High amounts are found in brain, liver, thyroid, kidney and pituitary. Extracellular SOD is present in high amounts in lungs, thyroid and uterus.
6) *Tyrosinase*	It catalyzes the conversion of tyrosine to dopamine, and oxidation of dopamine to dopaquinone, steps in the synthesis of melanin.

After going through the functions enumerated in Table 10.7, you would have realized that copper plays an important function in processes fundamental to human health. Thus, copper plays a role in bone formation and integrity of connective tissue in the heart and vascular system. It is required for the normal functioning of central nervous system and cardiovascular system. It is involved in iron metabolism. Recent evidence suggests a role for copper in immune function. Some indices of immune function have been shown to decline with deficiency but were not reversed by increased copper intake.

In addition to the above, copper may have other roles, which may not involve enzymes. Copper appears to influence gene expression through binding to specific transcription factors. In some cases, copper has been shown to influence transcription by binding to transcription factor, which in turn binds to promoter sequence of DNA.

Although a very small amount of copper is required for performing the functions discussed above, its deficiency can result in serious consequences which are being discussed next.

Deficiency

Owing to the remarkable homeostatic mechanisms, copper deficiency in humans is rare. However, copper deficiency has been reported under special circumstances. The predisposing factors of copper deficiency are prematurity, low birth weight and malnutrition, especially when combined with feeding practices such as cow's milk or total parenteral nutrition. The most frequent symptoms are anaemia, neutropenia (abnormally high levels of a type of WBC's in blood) and bone fractures. Other less frequent symptoms include hypo-pigmentation, impaired growth, and an increased incidence of infections and abnormalities of glucose and cholesterol metabolism.

It has been proposed that sub-optimal copper intakes over long periods may be involved in the precipitation of chronic diseases such as cardiovascular disease and osteoporosis.

While on one hand, a low intake of copper can affect our health, a very high intake or abnormally high levels of copper in the body's tissues can also be damaging to several body processes. Let us read further to find out the effects of copper toxicity.

Toxicity

Acute copper toxicity in humans is rare and occurs due to inadvertent consumption of copper salts. Symptoms include vomiting, diarrhoea, haemolytic anaemia, renal and liver damage. Clinical symptoms of chronic copper toxicity appear when the capacity for protective copper binding in the liver is exceeded which include jaundice, hepatitis and liver cirrhosis.

Apart from an abnormally high or low intake, copper imbalance in various tissues may also develop as a consequence of genetic disturbances in the metabolism of copper. The most important one's being the *Menke's* and the *Wilson's disease.* We will learn about these diseases while studying about selenium in section 10.6.

Now that you have realized the importance of copper in our diet, let us now move on to the understanding of various assessment parameters of copper status.

Assessment of Copper Status

A reliable index to assess marginal copper status is currently not available. However, severe copper deficiency may be detected by one or more of the three parameters, low serum copper levels, low serum ceruloplasmin both of which respond to copper administration; and a decline in red cell super oxide dismutase activity. Reported normal range for these parameters are as follows:

Serum copper: 64-156 mcg/dl (10-24.6 mmoles/L),

Ceruloplasmin: 18 -40 mg/dl,

Erythrocyte SOD: 0.47 mg/g

Serum copper and ceruloplasmin are reduced to levels far below normal in severe copper deficiency. Erythrocyte SOD in severe copper deficiency has not been reported.

So, then what is the safe level of dietary intake for copper? Let us find out.

Requirements

Safe and adequate range for copper intake is 1.5-3 mg/day. The RDA of Copper is about 2 mg/day. Refere to Table 10.11 which presents the recommended intake for other minerals and trace elements.

Table 10.11: Recommended Intakes for other Minerals and Trace Elements

S.No.	Minerals/Trace Element	Recommended intake
1.	Copper	2 mg/day
2.	Manganese	4 mg/day
3.	Chromium	50 µg/day
4.	Selenium	40 µg/day

In this section, we learnt about the salient features of copper. In our next section, we shall discuss about yet another important nutrient viz., selenium. However, before we proceed, you must attempt the questions mentioned below to recapitulate your understanding of the concepts discussed so far.

Check Your Progress Exercise 3

1) List the organs which contain a high concentration of Cu.

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2) Which components aid in the release of Cu in the gastrointestinal tract?

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3) Enumerate the dietary component affecting Cu absorption.

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4) Name the various proteins which deliver copper to:

a) Liver and Kidney

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b) Other tissues

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5) Enlist any five copper-containing enzymes, giving their important physiological functions.

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6) Mention the pre-disposing factors of Cu deficiency.

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7) What is Wilson's disease?

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10.6 SELENIUM

The element selenium was discovered in 1817 in association with the element sulphur. However, selenium as an essential nutrient remained unrecognized for many years, although selenium toxicity in horses and cattle, "blind staggers" and "alkali disease" was known since the 1930s.

The first description of the dietary selenium deficiency in isolated populations in the People's Republic of China, was made in 1979. The disease known as *Keshan disease*, named for the country where it was first recognized, was characterized by cardiomyopathy affecting primarily children and young women. The disease was often fatal. The second selenium deficiency disease *Kashin-Beck* disease was reported in 1980. It was prevalent in China and Sino-Soviet border. Both the diseases were caused primarily due to selenium deficiency in the soil.

Selenium is a non metallic element and exists in several oxidation states which include $Se^{2+,}$ Se^{4+} and Se^{6+}. The chemistry of selenium is similar to that of sulphur. Selenium replaces sulphur to form organic compounds such as selenocysteine and selenomethionine. Total selenium content of the body varies from 3-15 mg depending on the dietary intake. Approximately 30% of tissue selenium is contained in the liver, 15% in kidney, 30% in muscle and 10% in blood plasma. Much of tissue selenium is found in proteins as selenoanalogues of sulphur amino acids; other metabolically active forms include selenotrisulphides and other acid-labile selenium compounds.

In the body, selenium can be bound to selenium-binding proteins. It can also be directly incorporated into selenoprotein during translation at the ribosome complex using a RNA specific for the amino acid–*selenocysteine*. Thus selenocysteine can be considered as the 21st amino acid in terms of ribosome-mediated protein synthesis. At least 15 selenoproteins have now been characterized. Table 10.12 provides a list of these selenoproteins. We will learn about them later in the function section.

Table 10.12: A selection of characterized selenoproteins

Protein	Tissue Distribution
Cytosolic GSHPx	All, including thyroid
Phospholipid hydroxide GSHPx	All, including thyroid
Gastrointestinal GSHPx	Gastrointestinal tract
Extracellular GSHPx	Plasma, thyroid
Thioredoxin reductase	All, including thyroid
Iodothyronine-delodinase (type 1)	Liver, kidneys, and thyroid
Iodothyronine-deiodinase (type 2)	Central nervous system and pituitary
Iodothyronine-deiodinase (type 3)	Brown adipose tissue, central nervous system, and placenta
Selenoprotein P	Plasma
Selenoprotein W	Muscle
Sperm capsule selenoprotein	Sperm tail

* GSHPx, glutathione peroxidase.

Next, we shall brief ourselves regarding the presence of selenium in food.

Food Sources

Environmental conditions and agricultural practices have a profound influence on the selenium content of many foods. Table 10.12(a) illustrates the wide range of selenium content of the principal food groups and the variability in the selenium content of dietary constituents in selected countries. This variability is exceeded only by that found in the iodine content of foods.

Table 10.12: The selenium contents of foods and diets

a) *Typical ranges of selenium concentrations (ng/g fresh weight) in food groups*

Food Group	India	United States	International Compilation
Cereals and cereal products	5-95	10-370	10-550
Meat, meat products, and eggs	40-120	100-810	10-360
Fish and marine	280-1080	400-1500	110-970
Fish and freshwater	–	–	180-680
Pulses	10-138	–	–
Dairy products	5-15	10-130	1-170
Fruits and vegetables	1-7	1-60	1-20

b) *Typical distribution of selenium in dietary constituents (µg/day) in selected countries*

Food Groups	China		India		Finland	United Kingdom
	Keshan Disease Area	Disease Free Areas	Low-income Vegetarian Diets	Low-income Conventional Diets		
Total diet	7.7	16.4	27.4	52.5	30.0	31.0
Cereals and cereal products	5.4	11.6	15.7	21.1	2.8	7.0
Pulses	–	–	3.9	3.6	1.1	–
Meat and eggs	0.6	2.2	–	3.7	9.2	10.0
Fish			–	18.4	9.5	4.0
Dairy products			6.9	4.8	6.5	3.0
Fruits and vegetables	1.7	2.6	0.9	0.9	0.5	6.0
other	–	–	–	–	1.1	3.0

Source: Vitamin and Mineral Requirement in Human Nutrition, FAO/WHO (2004)

Cercals and grams are major delay sources of selenium. Geographic differences in the content and availability of selenium from soils to food crops and animal products have a marked effect on the selenium status of entire communities. Refer to Table 10.12(b) which presents the typical distribution of selenium in dietary constituents in selected countries. As you would notice, the distribution of Keshan disease and Kashin-Beck disease in China reflects the distribution of soils from which selenium is poorly available to rice, maize, wheat and pasture grasses.

Selenium enters the food chain through plants. The concentration of selenium in plants is directly related to the concentration of the mineral in the soil on which plants were grown. Plant food like mustard and to a lesser content garlic and brocolli, accumulate selenium from the soil. Among the different trace elements, selenium varies greatly in its soil concentration. It has been suggested that <10 ng/g for grain selenium and <3 ng/g for water-soluble soil selenium could be used as indices to define deficient areas.

The absorption of selenium by plants is not only dependent on the concentration of selenium in the soil but also on pH, microbial activity, rainfall and the chemical form of selenium. Higher plants can absorb selenium as selenate and can synthesize selenomethionine and to a lesser extent, selenocysteine.

Owing to all above factors, the selenium content in food varies greatly. Overall, animal products, especially organ meats, are thought to contain more selenium than plant sources, as you may have noticed in Table 10.12 (a). Seafoods are also considered good sources, although availability of the mineral from fish, especially those containing mercury, cadmium is low.

Selenium occurs in foods in organic form, such as, selenomethionine, selenocysteine, selenocystine and Se-methyl selenomethionine. In general, plant foods contain greater proportion of organic selenium compounds. Inorganic forms include selenite (H_2SeO_3) and Selenate (H_2SeO_4). These forms are found in some vegetables.

Next, we shall discuss about the absorption, transport, storage and excretion of selenium.

Metabolism

Selenium compounds are generally very efficiently absorbed by humans and selenium absorption does not appear to be under homeostatic control. Selenium is mainly absorbed from the duodenum. Less absorption occurs in the jejunum and ileum. Inorganic forms of selenium (mainly selenate) are passively transported whereas organic forms are actively transported.

Almost 50-80% of dietary selenium is absorbed, with efficiency being higher for organic forms, as compared to inorganic. Among the organic forms, selenomethionine is better absorbed than selenocysteine. Among the inorganic forms, selenates are better absorbed than selenites. For example, absorption of the selenite form of selenium is greater than 80% whereas that of selenium as selenomethionine or as selenate may be greater than 90%. In addition, some dietary factors appear to influence the absorption of the element. Phytates and heavy metals, such as mercury through chelation and precipitation, hinder selenium absorption. Vitamins C, A and E, as well as, glutathione enhance the absorption.

Refer to Figure 10.5 for a better understanding of selenium absorption.

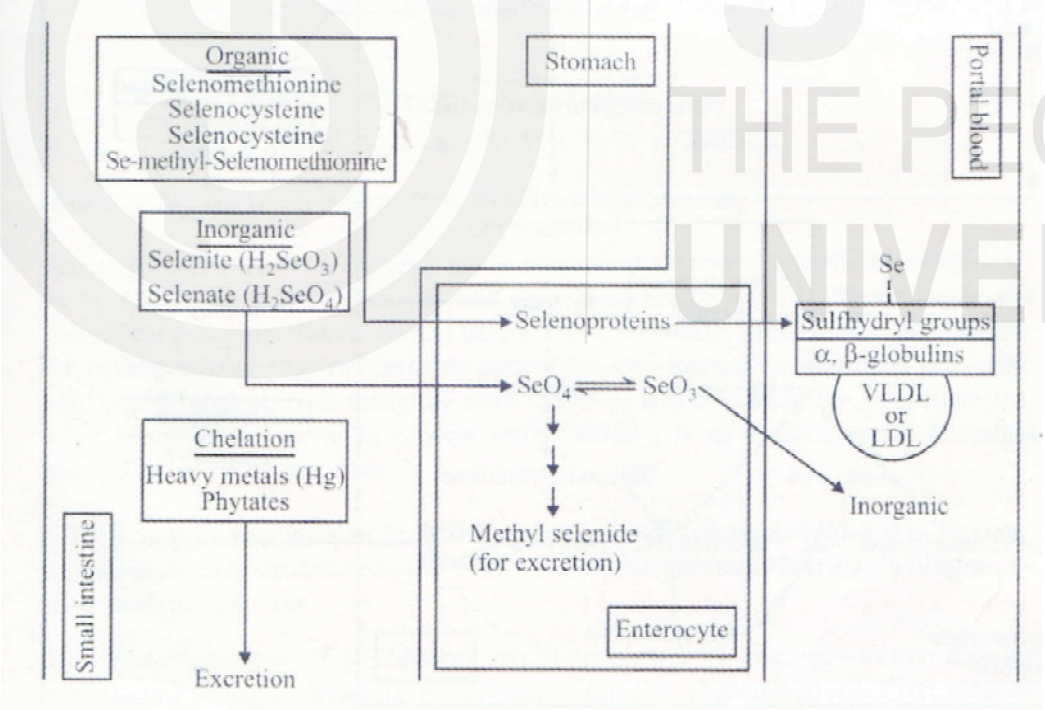


Figure 10.5: Absorption and transport of selenium

As you may have noticed in Figure 10.5, after absorption selenium binds to sulphydryl groups in α and β globulins of VLDL and LDL to be transported to the different tissues. Liver and kidneys appear to be the major target organs.

Within tissues such as liver, organic, as well as, inorganic selenium compounds have different fates. This is briefly discussed herewith:

1) *Selenomethionine* obtained from the diet may be:

- stored as such in amino acid pool,
- used for protein synthesis, and
- catabolized to selenocysteine.

2) *Selenocysteine* obtained from the diet or after catabolism of selenomethionine is degraded to yield free elemental selenium. This elemental selenium may be:

- attached to tRNA charged with serine to be incorporated in selenium dependent enzymes, and
- converted into selenite which may be stored or excreted.

3) *Selenate* from the diet is converted to *selenit*e. Selenite is further converted to *selenide*. Selenide may be:

- converted to selenophosphate to yield free selenium, which is incorporated into enzymes, and
- excreted as methyl selenide.

The above discussion can be clearly understood after going through Figure 10.6, which illustrates the metabolic fate of selenium.

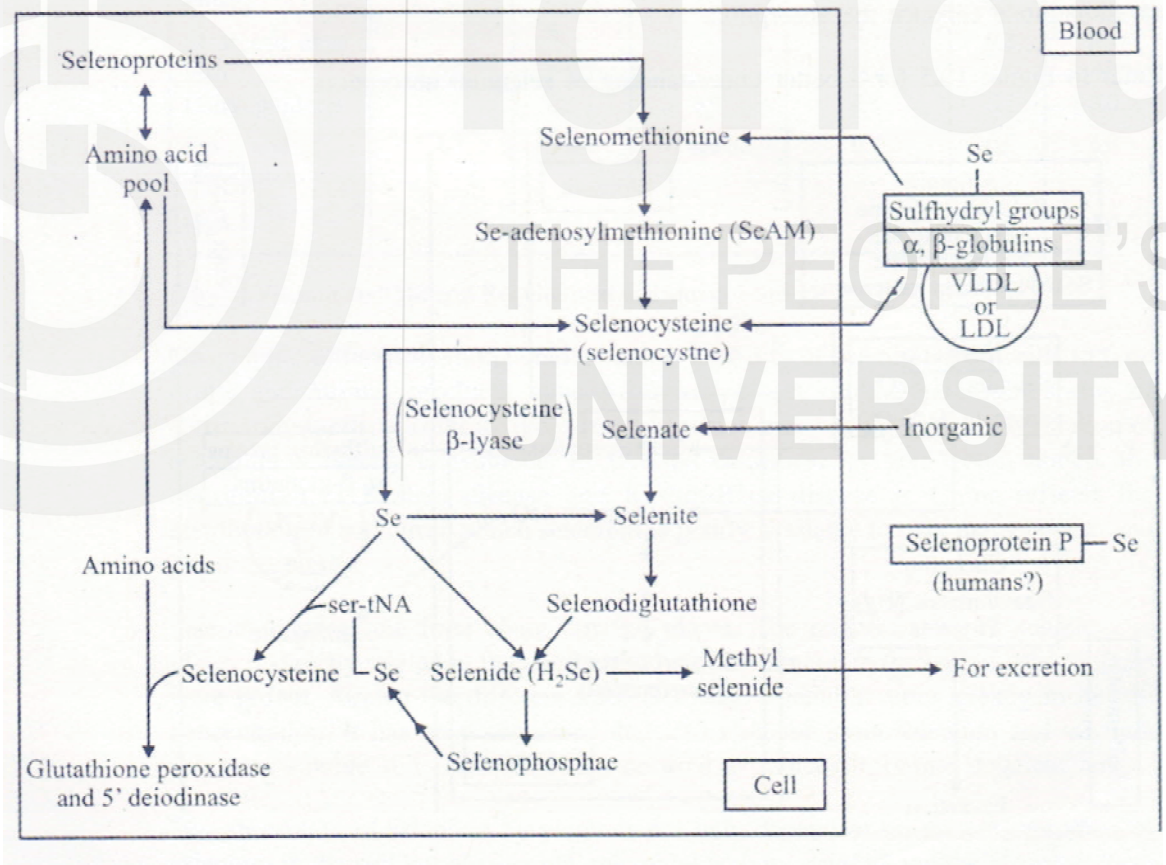


Figure 10.6: Metabolism of selenium in tissues

4) Selenium is excreted from the body almost equally in the urine (as methyl selenium) and faeces (unabsorbed selenium, biliary, pancreatic and intestinal secretion). Unlike copper, selenium is rapidly excreted in urine. Selenium losses through lungs and skin also contribute to daily selenium excretion.

After having read about the presence of selenium in human body and its association with several disease conditions, it must be evident that selenium plays an important

role in maintaining our health. The most salient functions of selenium have been discussed next.

Functions

Until recently, the only known metabolic role of selenium in humans was as a component of glutathione peroxidase which along with vitamin E and superoxide dismutase forms a part of the anti-oxidant defense system. However, more selenoproteins are being discovered and currently it is estimated that 50-100 selenoproteins are present in animals. Selenoproteins in animals and humans are involved in protection from oxidative damage, maintaining adequate thyroid hormone status and protection from injury by a heavy metal like mercury.

Three major enzyme systems in which selenium plays an important role have been identified in humans. These include:

a) Glutathione peroxidases,

b) Iodothyronine deiodonases, and

c) Selenoproteins P and W

Let us study them in greater detail.

a) *Glutathione peroxidases*: The role of selenium in the cytosolic enzyme, glutathione peroxidase (GSHPx), was first illustrated in 1973. Four selenium dependent glutathione peroxidases have been identified and named as Glutathione peroxidases 1-4 ($GSHP_{xs}$ 1-4). During stress, infection, or tissue injury, selenoenzymes may protect against the damaging effects of hydrogen peroxide or oxygen-rich free radicals. This family of enzymes catalyzes the destruction of hydrogen peroxide or lipid hydroperoxides according to the following general reactions:

$$H_2O_2 + 2GSH \longrightarrow 2H_2O + GSSG$$

$$ROOH + 2GSH \longrightarrow ROH + H_2O + GSSG$$

where, GSH is glutathione and GSSG is its oxidized form.

Thus, from the reaction above, it is evident that the main role of glutathione peroxidases is to reduce hydrogen peroxide and free hydroperoxides in different cells and tissues by using glutathione (GSH) as the hydrogen donor. Thus, the reactive species of hydroperoxide free radicals are converted into innocuous molecules of water. $GSHP_{x-1}$ is present in virtually all cells, $GSHP_{x-2}$ is localized in the gastrointestinal tract, $GSHP_{x-3}$ is present in plasma while $GSHP_{x-4}$ is most abundant in testis but present in other tissues also.

$GSHP_{x-4}$ plays a major role in protecting against lipid peroxidation as it is the only intracellular enzyme that can reduce fatty acid hydro peroxide, $GSHP_{x-3}$ in plasma can also perform this role.

b) *Iodothyronine Deiodinases*: Another group of selenoproteins are the iodothyronine deiodinases essential for the conversion of thyroxine or tetraiodothyronine (T_4) to its physiologically active form tri-iodothyronine (T_3). Three types of iodothyronine deiodinases have been identified, all of them being selenoproteins. When one iodine is removed from T_4, it is converted T_3. T_3 is more active than T_4. Thus, one of the deiodinase enzymes is involved in activating T_4. When one or more iodine is removed from T_3, the resulting molecules do not have enzyme activity. Therefore, another selenium-dependent deiodinase inactivates T_3.

Type I iodothyronine deiodinase (a selenoprotein) is found in liver, kidney and thyroid tissue. The major role of this enzyme is to provide T_3 to peripheral tissues by

deiodinating T_4 secreted by the thyroid gland. Selenium deficiency causes a decline in the Type I deiodinase enzyme activity, but it may not result in hypothyroidism as there is a compensatory increase in plasma T_4 levels. *Type II iodothyronine deiodinase*, also a selenoprotein, is present in the brain, pituitary and placenta. The major function of this enzyme is to regulate T_3 levels in these tissues, and control the secretion of thyroid stimulating hormone. Type II enzyme activity is also reduced in selenium deficiency. *Type III iodothyronine deiodinase*, another selenoprotein, is involved mainly in degradation of the T_4 and T_3. How this enzyme is affected in selenium deficiency is not fully investigated.

Thus, these selenoprotein enzymes regulate and maintain thyroid levels. Animal studies have shown that a combined deficiency of selenium and iodine produces much more severe hypothyroidism compared to iodine deficiency alone. Further, maternal deficiency of selenium and iodine is implicated in cretinism in newborn – the most severe outcome of thyroid hormone deficiency during pregnancy.

c) *Selenoproteins P and W*: The third group comprises of *selenoprotein P,* an extracellular constituent with multiple selenocysteine molecules. This has an antioxidant role, deactivating free radicals. *Selenoprotein W*, present in the muscle has a suggested role in muscular degeneration seen in combined selenium and vitamin E deficiency. Selenoprotein W gets reduced during selenium deficiency.

Another group of selenium-containing enzymes is the *thioredoxin reductases.* The selenoenzyme thioredoxin reductase is involved in disposal of the products of oxidative metabolism. It contains two selenocysteine groups per molecule and is a major component of a *redox system* with a multiplicity of functions, among which is the capacity to degrade locally excessive and potentially toxic concentrations of peroxide and hydroperoxides likely to induce cell death and tissue atrophy.

These selenoproteins catalyze the NADPH–dependent reduction of oxidized thioredoxin. Reduced thioredoxin provides reducing equivalents for various redox-dependent systems, such as, ribonucleotide reductase essential for DNA synthesis, redox regulation of transcription factors. Besides, these proteins have important functions in regulating cell growth and inhibiting apoptosis.

The above discussion clearly indicates the importance of selenium in human nutrition. Let us now find out how selenium status can have an impact on our health. We shall begin with the state of deficiency and then discuss the consequences of toxic levels of selenium.

Deficiency

Selenium deficiency has been linked to two regional human diseases: *Keshan disease* and *Kashin Beck's disease*.

Let us understand what these diseases are and their characteristic features.

Keshan disease: It is a cardiomyopathy (disease of the myocardium, involving heart muscle) that was identified to affect children and women of child bearing age in China. Sudden onset of insufficient heart function is characteristic of the acute form of this disease while in chronic Keshan disease, heart enlargement and insufficiency exist. Intervention trials comprising more than a million subjects in China has demonstrated the protective effect of selenium against Keshan's disease. It is important to note that selenium supplements cannot however reverse cardiac failure if it has occurred.

Kashin Beck disease: Kashin Beck disease was identified to affect growing children in parts of Siberian Russia and China. It is characterized by osteoarthritis involving degeneration and necrosis of the joints and epiphyseal-plate cartilages of legs and arms. It is possible that apart from selenium deficiency, many other factors may be contributing to the development of Kashin Beck's disease.

Suboptimal selenium status may be widespread in human population. It is accompanied by loss of immuno-competence with the impairment of both cell-mediated immunity and β-cell function. The early preclinical stages of development of human immunodeficiency virus (HIV) infection are accompanied by a very marked decline in plasma selenium. Subclinical malnutrition assumes increased significance during the development of acquired immune deficiency syndrome (AIDS). Selenium supplementation in subjects has been shown to mark immuno-stimulant effects including increased proliferation of activated T-cells. In addition, as selenium has well recognized anti-oxidant and anti-inflammatory roles, other oxidative stress or inflammatory conditions such as rheumatoid arthritis, ulcerative colitis, pancreatitis may also benefit from selenium supplementation.

Further, enhancement of the virulence of virus due to selenium deficiency has been reported. There is a growing evidence that suboptimal selenium status may also increase risk of cancer and cardiovascular disease. However, much work is still needed in these aspects.

Toxicity

There is a narrow margin between the beneficial and harmful intakes of selenium. The level at which selenosis occurs is not well-defined but threshold for toxicity appears to be 850-900 µg per day. Symptoms of chronic toxicity include brittle hair and nails, skin lesions with secondary infections and garlic odour in the breath. Chronic selenium poisoning in people is characterized primarily by loss of hair and changes in finger nail morphology. In some cases, skin lesions may occur.

Next, we shall learn about the parameters indicative of selenium status.

Assessment of Selenium Status

Blood glutathione (GSH) peroxidase activity is directly related to blood selenium up to a level of 1.27 µmoles/L. Beyond this point, the activity of the enzyme plateaus and therefore cannot be used for assessing selenium status. As of now, GSH peroxidase remains a useful index over the assessment of usual dietary intakes but is limited by the peak level reached at 1.27 mmol/L. Plasma selenium level is an index of short term status, as it has been shown to respond to selenium supplementation more rapidly in deficient individuals than whole blood selenium. Hair and nail selenium are not as yet established as valid parameters, although they are being investigated.

So what level of intake should be maintained to ensure the maintenance of optimum selenium levels in plasma? Let us find out.

Requirements

ICMR (2020) recommends 40 µg/day as adequate intake of selenium Refer to Table 10.14

Before we proceed to study chromiun, let us recapitulate what we have learnt so far by answering the check your progress exercise 4.

Check Your Progress Exercise 4

1) Name an enzyme which constitutes selenium as its integral part.

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2) Of all trace elements, selenium content in food varies greatly. Why?

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3) What are the factors which hinder selenium absorption?

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4) Enumerate the important functions of selenoenzymes. Name a test to indicate short-term changes in dietary intake of selenium.

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5) Write a short note on selenium deficiency.

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Now get down to the study of chromium.

10.7 CHROMIUM

As you will go through this section, you will realize that compared to other minerals, the essentiality of chromium was recognized very late. Let us briefly review its history.

By the year 1948, chromium was recognized as a consistent component of plant and animal tissue. In 1950, it was recognized as an element which potentiated insulin action and restored normal glucose tolerance in rats.

In humans, studies were initiated between 1964–68, wherein chromium supplementation was shown to improve impaired glucose tolerance. Despite these studies, the essentiality of chromium in human nutrition was documented as late as in 1977, when a female patient on total parenteral nutrition (TPN) developed diabetes – like symptoms that were refractory to insulin. Chromium supplementation was shown to alleviate these symptoms and insulin was no longer required. Subsequent studies confirmed these findings.

Chromium also exists in several oxidation states from Cr^{2-} to Cr^{6+} however Cr^{3+} or the trivalent form is also the biologically important one. Cr^{6+}, which is consumed in small amounts, comes from industrial sources. In the acidic environment of the stomach, Cr^{6+} is converted to Cr^{3+}.

Unlike other minerals, chromium is present in small amounts in human body. The kidneys, followed by spleen, liver, lungs, heart and skeletal muscle are the tissues with greatest chromium concentration.

Let us review the rich and poor sources of chromium.

Food Sources

Chromium occurs in trivalent form in foods. Good sources of chromium include whole grains, spices and condiments, meats especially organ meats, see food (oysters) mushrooms, cheese, prunes and tea followed by egg, butter and tubers like potato. Brewer's yeast has a high content of biologically active organically complexed form known as the *Glucose Tolerance Factor* (*GTF*). Chromium complexes with nicotinic acid and amino acids to form GTF.

We shall now brief upon the absorption, transport, storage and excretion of chromium from our body.

Metabolism

Chromium appears to be absorbed throughout the small intestine, with absorption being higher in jejunum. The mechanism of absorption has not been well defined but appears to involve processes other than simple diffusion. At normal dietary intakes (10-40 mcg/day), the absorption ranges from 0.4 to 3.0% with absorption being higher at lower intakes. As you have studied for other minerals, even in the case of chromium, an inverse relation between intake and absorption appears to be a basal control mechanism to maintain the body levels of chromium.

As compared to healthy individuals, insulin-dependent diabetic patients absorb 2-4 times more chromium. It appears that these patients have an impaired ability to convert inorganic form to usable form and therefore require higher chromium. Like other trace minerals, absorption of chromium is also influenced by some factors. Enhancers and inhibitors are listed in the Table 10.13.

Table 10.13: Factors influencing absorption

Enhancers	Inhibitors
Ascorbic acid Picolinate (forms stable ipophillic ligand) Methionine and histidine (can chelate or and make it available better).	Antacids Phytates

After absorption, chromium binds to plasma proteins for transportation. Both transferrin and albumin are capable of binding absorbed Cr. It has been suggested that transferrin is the main binder of newly absorbed chromium and albumin assumes the role of chromium acceptor and transporter if transferrin binding sites are unavailable.

You have studied that transferrin has two metal binding sites, one is primarily for iron and the second is involved in chromium transport. During conditions of iron excess or iron overload such as iron storage diseases, all the metal transport sites on transferrin are occupied by iron. This may explain the high incidence of diabetes in haemochromatosis patients, which may be induced by chromium deficiency.

Although transferrin and albumin play the major roles in transportation, other plasma proteins such as α and β globulins and lipoproteins are also involved.

As you will go through the next section on 'Functions', you will realize that only organically complexed chromium i.e. GTF is active. It appears that absorbed inorganic chromium is transported to the liver, which is postulated to be the possible site for synthesis of metabolically active molecule. This molecule is held in a body pool and released as needed.

Most ingested chromium is excreted in faeces. Inorganic chromium is excreted primarily by the kidney, with small amounts being excreted through hair, sweat and bile. Organically bound chromium is excreted through bile.

The biologically active form of chromium performs several functions; the important ones are being subsequently discussed.

Functions

Active chromium as GTF potentiates the action of insulin and thus influences carbohydrate, lipid and insulin metabolism.

Let us first study the mechanism by which chromium potentiates insulin function.

Role in Insulin Formation

You are aware that insulin receptors are present in many cells with their concentration being highest in adipocytes (cells present in adipose tissue) and hepatocytes (liver cells). You also know that insulin receptor has two extracellular alpha-subunits and two extracellular beta-subunits. It is the alpha-subunit to which insulin binds. Once insulin binds to the alpha-subunit of the receptor, a specific phosphorylation of the beta-subunit occurs through a cascade of phosphorylation reactions. This leads to increased insulin sensitivity. The enzyme partly responsible for this phosphorylation is the '*insulin receptor tyrosine kinase*'. This enzyme is activated by chromium. In rats, removal of chromium has been shown to result in the loss of kinase-potentiating activity. Besides activating the kinase, chromium also inhibits *phosphotyrosine phosphatase*– an enzyme responsible for inactivation of insulin receptor.

The activation of 'insulin receptor tyrosine kinase' and inhibition of 'insulin receptor tyrosine phosphatase' by chromium would lead to an increased phosphorylation of the insulin receptor, which is associated with increased insulin sensitivity.

Since chromium improves insulin function, it is suggested that chromium may play a role in glucose and lipid metabolism. Let us now review these functions:

Role in Glucose, Lipid and Nucleic Acid Metabolism

Chromium leads to a decrease in blood glucose concentration in people with elevated glucose levels and an increase in those with low blood glucose levels. It shows no effect in the subjects with normal blood glucose levels.

Owing to its role in improving glucose tolerance, many studies having been conducted to see the effect of chromium supplementation in patients with impaired glucose tolerance, and Type 2 diabetes, however, results of different studies have been varied. From the results of various studies, it appears that supplementation level of 200 mcg/day as chromium chloride ($CrCl_3$) did not have any beneficial effect: Positive effects were observed in studies using 400 mcg Cr/day as $CrCl_3$. Almost all the studies employing more bioavailable Cr picolinate have reported favourable effects with greater effect reported at 1000 mcg/day than at 200 mcg/day. Also human studies include subjects of diverse genetic and nutritional backgrounds living in environments of varying degrees of stress, all of which may affect chromium metabolism.

Similarly, improved insulin function is also associated with improved lipid profile. Although number of beneficial effects of chromium on lipid profiles have been reported, these responses are not consistent from study to study. Overall, chromium appears to reduce levels of total cholesterol, LDL cholesterol and triglycerides in blood and increase level of HDL cholesterol.

Another proposed role for chromium is in relation to nucleic acid metabolism. It is postulated that Cr^{3+} is involved in maintaining the structural integrity of nuclear strands and in the regulation of gene expression.

It must be evident from the discussions above that chromium is important for glucose, fat, protein and especially nucleic acid metabolism. Thus, its low or excessive intake over a period of time may result in the development of metabolic changes in several nutrients. Let us read further to know as to what happens when chromium intake is above or below our requirements.

Deficiency

Hallmark of marginal chromium deficiency is *impaired glucose tolerance*. Individuals receiving TPN without chromium have been shown to develop symptoms of deficiency such as impaired glucose tolerance with high blood glucose level and glucose excretion in urine. Peripheral neuropathy has also been reported which was reversed with chromium supplementation.

Chromium deficiency results in insulin resistance characterized by hyperinsulinemia. Hyperinsulinemia is implicated as a risk factor for coronary heart disease.

Toxicity

Trivalent chromium, the form of chromium found in foods and supplements, is least toxic. Oral supplements upto 800 to 1000 mcg per day appear to be safe. However, hexavalent chromium often found in paints, welding fumes and other industrial settings is very toxic. Inhalation of Cr^{6+} may result in respiratory disease while direct contact results in dermatitis and skin ulceration. Liver damage can also occur.

Let us then learn how to assess the chromium status.

Assessment of Chromium Status

No specific tests are currently available, which could help us to determine chromium status. Another reason being the chromium content of physiological fluids is not indicative of its status. Also urinary chromium, hair chromium concentrations and fasting plasma chromium tests do not show consistent and reliable results.

So what level of dietary intake would suffice for our body's requirement and shall not cause any toxic effects? Let us find out this next.

Requirements of Chromium

The Recommended Dietary Allowance (RDA) for chromium is 50 μg/day as highlighted in Table 10.14.

10.8 MANGANESE

Manganese (Mn) is a transition element and can assume 11 different oxidation states, from – 3 to + 7. However, in living tissues, it is found in the + 2, +3 and +4 oxidation states. An adult man weighing 70 kg is estimated to contain 10-20 mg of the metal, with 25% of the total body stores in the skeleton. Relatively high amounts of the minerals are also present in liver, pancreas and intestine.

Although not much work has been done to identify the usual dietary intake of Mn among Indians in different age groups, the diet can be estimated to be a poor or good source of Mn by knowing the food sources of this element. So let us recapitulate on the same.

Food Sources

The food sources of manganese along with their content are tabulated in Table 10.14. Here, you can see that whole cereals, nuts, leafy vegetables and tea are good sources of Mn. Indian diets high in foods of plant origin supply on an average 8.3 mg of Mn/day.

Table 10.14: Manganese content of selected foods and beverages

Foods/Food Group	Manganese Content (mg/100 g)
Whole grains	3.19
Flour, whole grain	2.98
Flour, white	0.63
Bengal gram dal	1.68
Almonds	2.54
Carrots	0.20
Capsicum (green)	0.10
Apple	0.05
Cow Milk	0.01

Source: Recommended Dietary Allowances for Indians, NIN, ICMR, Hyderabad, India, 2010.

Let us next learn about the absorption, transport, storage and excretion of Mn i.e. the metabolism of manganese.

Metabolism

Intestinal absorption of Mn occurs throughout the length of the small intestine although the exact mechanism of absorption is not clearly established.

Ingested Mn is thought to be converted into Mn^{3+} in the duodenum. Results of the studies suggest that mucosal uptake could be a rapidly saturable process, which appears to be mediated by a high-affinity, low-capacity active transport system.

Available evidence also suggests that mucosal transport occurs through a non-saturable simple diffusion process. It appears that both processes might be involved in the absorption of mineral and may operate simultaneously.

Absorption of Mn from the diet is very low. On the basis of Mn retention, it has been estimated that adult humans absorb 4.8% of ingested manganese.

Let us now see which factors influence Mn absorption.

Major factors which may influence the absorption of this mineral include:

- Absorption decreases with increasing intake.
- Percent absorption is higher among women as compared to men.
- Increased dietary iron depresses Mn absorption whereas iron deficiency increases its absorption. This could be possibly due to the competition for similar binding and absorption sites between non-haem iron and Mn.
- High levels of dietary calcium, phosphorus and phytate impair the intestinal uptake of the element but these have been shown to be of limited significance.

Let us now study the fate of Mn which is absorbed.

After absorption, Mn is complexed with albumin and transported to the liver, which is the key organ in its metabolism. In the liver, Mn is found in both rapid and slow exchanging pools. The former is the precursor of biliary Mn, which is excreted in the faeces. The latter serves as the source of Mn for the liver and extrahepatic tissues. Mn becomes bound as Mn^{2+} to α-macroglobulin before traversing the liver. From the liver, some Mn^{2+} appears to be oxidized by ceruloplasmin to Mn^{3+} and complexes with transferrin. Transferrin bound Mn^{3+} is taken up by the extrahepatic tissues.

Mn is found in most organs and tissues and preferentially accumulates in the mitochondria. There is no storage form for Mn. Bone contains substantial amount of mineral but there is no mechanism to release it and thus bone Mn is considered as passive storage. It is released only as a result of normal bone turnover or in situations of accelerating bone resorption.

Mn is almost totally excreted in the faeces (92%). Excess absorbed Mn is quickly excreted by the liver into the bile to maintain homeostasis. Only trace amounts are excreted in urine.

Let us now briefly review some important functions of Mn.

Functions

Like other microminerals, Mn also functions in mammalian enzyme systems. It can function both as an integral part of metalloenzymes and as an enzyme activator. Manganese containing metalloenzymes are few, as shown in Table 10.15, whereas enzymes activated by Mn are much larger in number. Most of these metal activations by Mn are non-specific, as magnesium (Mg) can substitute for Mn. There are a few exceptions where Mn be specifically needed for activation. Examples include, activation of *glycosyl transferases, phosphoenol/phruvate carboxykinase* and *glutamine synthetase*.

Glutamine synthetase found in high concentration in the brain catalyzes the following reaction:

NH_3 + Glutamate + ATP → Glutamine + ADP + Pi

Thus, glutamate synthetase converts potentially toxic ammonia into glutamine and helps in the removal of ammonia (NH_3) as it is generated. It is interesting to note that even in severe Mn deficiency in animals, brain glutamine synthetase activity is maintained normal, suggesting that this enzyme has a high priority among the enzymes activated by Mn or that Mg can replace Mn.

Table 10.15: Mn activated enzymes and Mn containing metalloenzymes

Mn Activated Enzymes	Mn Containing Metalloenzymes
• Hydrolases • Kinases • Decarboxylases • Transferases • Lyases • Oxidoreductases • Ligases	• Arginase • Pyruvate carboxylase • Superoxide – Dismutase

You have seen that Mn is involved in a number of enzyme-catalyzed reactions. Therefore, it performs many important functions. These are briefly discussed herewith:

1) *Antioxidant activity*: As Mn is a component of mitochondrial Superoxide Dismutase (SOD), it can protect against oxidative damage. In-vitro experiments have indicated that Mn scavenged superoxide radicals at nanomolar concentration whereas hydroxy radicals were scavenged at macromolar concentrations. Thus, Mn

deficiency could damage mitochondrial membrane by depressing the activity of SOD. Although, a little work has been done in humans, depressed activity of the enzyme has been reported in animals.

2) *Carbohydrate metabolism*: Mn is required for carbohydrate metabolism. Enzymes pyruvate carboxylase and phosphoenol pyruvate carboxy kinase involved in gluconeogenesis require Mn for optimal function.

   Further, animal studies strongly suggest a role for Mn in regulation of insulin transcription and / or in insulin mRNA turnover. Mn-deficient animals have been shown to exhibit a diabetic response to oral glucose challenges characterized primarily by impaired insulin production.

3) *Integrity of cartilage*: Mn plays an important role in proteoglycan biosynthesis, which is essential for the integrity of cartilage. Bone defects have been observed in birds, rats and mice. This has been ascribed to a reduction in the activities of several Mn-dependent glycosyl transferases.

It must be clear by now that though Mn is classified as a trace element; it is involved in the regulation of several enzyme activities and other important functions. However, what would happen during sub-optimal intake of Mn? Read further to find out.

Deficiency

Mn deficiency has been observed in many species of animals and symptoms include: impaired growth, skeletal abnormalities, depressed reproductive function and defects in lipids and carbohydrate metabolism.

With respect to humans, there is a little evidence of Mn deficiency as this mineral is widely distributed in a variety of foods. However, limited studies have reported symptoms of its deficiency after consuming experimental diets deficient in Mn. These included dermatitis, depressed growth of hair and nail, hypocholesterolemia and weight loss. Please note that sample size was very small in these limited experimental studies.

Evidence is accumulating that Mn deficiency may be present in selected groups. It has been reported in patients on long-term parenteral nutrition when the solutions were low in Mn content. Modest supplementation of iron can result in lowering of lymphocyte Mn-SOD activity in humans. In view of high frequency of iron supplementation by some groups, it is worthwhile to find out the incidence of Fe-supplementation-induced reductions in Mn status.

Mn deprivation has been associated with osteoporosis, diabetes, epilepsy, atherosclerosis and impaired wound healing.

While a low Mn level in body tissues can affect the human health adversely, a higher than normal intake may also influence several functions. The consequences of toxicity are being discussed next.

Toxicity

Manganese is considered least toxic of the trace minerals through oral intake. However, some people may be at a risk to develop toxicity. For example, individuals with impaired biliary and/or hepatic dysfunction are more susceptible, as dietary Mn is cleared by the liver. Similarly, total parenteral nutrition (TPN) bypasses the normal homeostatic mechanisms of the liver and gut. Therefore, patients receiving long-term parenteral nutrition are also at a risk.

Manganese toxicity, however, occurs primarily in industrial workers exposed to excess airborne Mn such as in case of industries manufacturing steel, alloys and iron products. Airborne Mn is also contributed by the Mn containing antiknock compounds in gasoline fuel. Majority of Mn toxicity cases have been reported from

individuals exposed to airborne Mn in industrial areas in excess of 5 mg per cubic meter (m^3). Mn toxicity is a serious health hazard; in its severe form it results in serious psychiatric symptoms such as hyperirritability, violent acts, hallucinations and poor coordination. Several abnormalities occur in the central nervous system, the morphological lesions being similar to Parkinson's disease. In workers exposed to less than 1 mg/m^3, impaired motor coordination and impaired memory have been reported. The symptoms of Mn toxicity are apparently due to excessive tissue oxidative damage by Mn.

So which clinical indicators can help in identifying the Mn status of an individual? Read and find out.

Assessment of Mn Status

The body Mn status has not been yet established by laboratory tests. Though the normal range of serum Mn concentration is found out to be 0.04 to 1.4 mcg/dl, it has been shown that Mn supplementation significantly increased lymphocyte SOD activity and serum Mn concentrations.

You must have understood by now that an optimum intake of Mn is imperative for maintaining good health. However, what level of dietary intake per day would help in maintaining equilibrium between the intake and requirements? Let us find out.

Requirements

You have studied in Table 10.14 the RDA for certain other minerals and trace elements. ICMR (2020) recommends an intake of 4 mg/day for manganese.

In this section, you studied about the nutritional significance of chromium and magnesium for maintaining human health. In our next section, two very important nutrients viz., iodine and fluorine shall be dealt in detail. However, before we proceed, you must perform the check your progress exercise 5. You may have to read certain aspects again to clear your concepts.

Check Your Progress Exercise 5

1) Why do IDDM patients absorb 2-4 times more chromium?

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2) What are the factors that enhance Cr absorption?

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3) How does chromium deficiency induces high incidence of diabetes in haemochromatosis patients?

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4) List the factors influencing Mn absorption.

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5) Give the important functions of Mn.

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6) What is the main storage form of Mn? Describe the metabolic fate of bone Mn.

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7) Briefly explain the phases of Mn toxicity.

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In the following sections, we shall deal with two very important nutrients i.e. iodine and fluorine, the dietary supply of which is necessary in view of some very important functions that they perform. We shall begin with iodine.

10.9 IODINE

Iodine derives the nutritional importance as a constituent of thyroid hormones, 3,5,3',5' tetraiodo-thyronine (thyroxine or T_4) and 3,5,3' tri iodo-thyronine (T_3).The thyroid hormones are indispensable for normal growth and development in humans and animals. Synthesis of the iodine containing thyroid hormones occurs exclusively in the thyroid gland. Goitre was known to the ancient Indians, Chinese, Greeks and Romans. Iodine as an element was discovered only in 1811; however, its presence in the thyroid gland was discovered by *Bauman* et, al in 1895. The relation between iodine deficiency and enlargement of the thyroid gland or goitre was shown early in the 20th century when it was reported by *David Marine* that the thyroid gland became *hyperplastic* (increase in number of normal cells in an organ and therefore an increase in volume/ size of the organ) with low level of iodine in the body. Subsequently in 1922, *Marine and Kimball* demonstrated that administration of small amounts of iodine could prevent or substantially reduce endemic goitre among school children in Ohio.

Introduction of iodized salt as a public health measure to prevent goitre was first introduced in Switzerland and Michigan. Following this, the incidence of goitre and cretinism fell rapidly in these countries. Another major development for the population at-risk of severe iodine deficiency in inaccessible mountainous areas, was the iodized

oil (1 ml containing 480 mg iodine) which can be given once in three years. Oral iodized oil is also effective but the effects may last only for one year.

Iodine is a non-metallic element of the halogen group with common oxidation states of $-I^{-1}$ (iodides), I^{+5} ,KI03 (iodates), I^{+7} KI04 (periodates) and less common states of +1 (iodine monochloride) and +3 (iodine trichloride). In humans, iodine is typically found and functions in its ionic form, iodide (I^{-1}).

About 15-20 mg iodine is found in human body, of which 70-80% is present in the thyroid gland. The thyroid gland weighs 15-25 grams and has a remarkable ability to concentrate iodine. In the iodine deficient individual, enlarged thyroid gland may contain only 1 mg iodine.

So, how can we consume adequate amounts of iodine in our diet? Let us get to know about the food sources, next.

Food Sources

Please note that unlike other minerals studied so far, like selenium, the iodine concentration in foods is highly variable and also depends on the concentration of iodine content of soil in that region. The iodine present in the upper crust of the earth is leached by glaciation and repeated flooding, and is carried to the sea. Seawater is, therefore, a rich source of iodine. The seaweed located near coral reefs has an inherent biological capacity to concentrate iodine from the sea. The average iodine content of foods (fresh and dry basis) is given in Table 10.16.

The amount of iodide in drinking water is an indicator of the iodide content of the rocks and soils of a region and it parallels the incidence of iodine deficiency among the inhabitants of that region. In general, iodine deficient areas have water iodine levels below 2 mcg/L as in Nepal and Sub-Himalayan India (0.1-1.2 mcg/L) compared with levels of 9 mcg/L in the city of Delhi, which is not iodine deficient.

Table 10.16: Average iodine content of foods (mg/kg)

Food	Fresh Basis		Dry Basis	
	Mean	Range	Mean	Range
Fish (fresh water)	30	17-40	116	68-194
Fish (marine)	832	163-3180	3715	471-4591
Shellfish	798	308-1300	3866	1292-4987
Meat	50	27-97	-	-
Milk	47	35-56	-	-
Eggs	93	-	-	-
Cereal grains	47	22-12	65	34-92
Fruits	18	10-29	154	62-277
Legumes	30	23-36	234	223-245
Vegetables	29	12-201	385	204-1636

Source: Koutras DA, Matovinovic J, Vought R. The ecology of iodine. In: Stanbury JB, Hetzel BS, eds. *Endemic goitre and endemic cretinism. Iodine nutrition in health and disease*. New Delhi, Wiley Eastern Limited, 1985:185-195.

In addition to water, iodine is also contributed by sea foods, as mentioned above. However, a large difference in the content exists between sea water fish and fresh water fish. Sea fish contain about 300-30,000 mcg iodine/kg in contrast to only 20-40 mcg iodine/kg in fresh water fish.

Also, food additives used as bread dough oxidizers or conditioners can contribute to the iodine content of the diet.

You must be acquainted with the physiological significance of iodine by now. Let us find out how the dietary iodine that we consume gets absorbed, transported, stored and if required, excreted from our body.

Metabolism

Now, we will very briefly study how iodine is absorbed, distributed in the body and excreted out.

Like other nutrients, dietary iodide is either found free or bound to amino acids. It is primarily found as *iodide* or *iodate*. The latter form is reduced to iodide by glutathione in the gut. Iodide is rapidly and completely absorbed throughout the gastrointestinal tract and very little iodine appears in faeces.

Iodine bound to amino acids is also absorbed but less efficiently. The thyroid hormones: thyroxine (T_4) and triiodothyronine (T_3) are also absorbed unaltered. Therefore, T_4 medication can be administered orally.

After absorption, free iodide appears in the blood and circulates to all tissues. Thyroid gland traps most of the ingested iodide (80%). This is achieved against an iodide gradient (often 40 to 50 times plasma concentration) by sodium-dependent active transport system. This mechanism is regulated by thyroid stimulating hormone (TSH) secreted by pituitary. Thyroid gland takes up almost 120 mcg of iodide per day. Other tissues such as salivary glands, gastric mucosa, choroid plexus and mammary glands also concentrate the element by a similar active mechanism.

Several sulphur-containing compounds such as thiocyanate, isothiocyanate and goitrin inhibit active transport mechanism by competing for uptake with iodide. Thus, iodide uptake by thyroid gland may be reduced. These are called *goitrogens* and their goitrogenic activity can be overcome by iodine supplementation. Refer to Box 10.1 for better understanding of goitrogens.

Box 10.1	**Goitrogens**
Goitrogens are substances that interfere with iodide metabolism in any way that inhibits thyroid hormone synthesis. As a result, there is augmentation in TSH release and subsequent thyroid gland enlargement. These active goitrogens are released by plant enzymes from thioglucosides or cyanogenic glucosides found in cassava, kale, cabbage, broccoli, turnips, rapeseeds and mustards. Most important of these is cassava, which can be detoxified by soaking in water and cooking it well. Tobacco smoke also contributes thiocynate.	

Unutilized iodide is excreted via kidneys, which forms the major route of iodide excretion (80-90 %). The urinary output of iodide correlates closely with the plasma iodide concentration and has been used to monitor iodide status. Some iodide is also lost in sweat, especially in the hot tropical regions.

Iodine, as we all know, performs some very important functions in our body particularly those pertaining to the thyroid gland. We will now discuss the functions of iodine in detail.

Functions

Iodine is an essential constituent of the thyroid hormones: thyroxine (T_4) and triiodothyronine (T_3), which have a key role in growth and development. Let us first briefly review how these hormones are synthesized and released from the thyroid gland.

Biosynthesis and Secretion of Thyroid Hormones

Histologically, the functional cells of the thyroid gland are arranged in follicles, which surround a central lumen containing a colloid in which the hormones are stored in the form of *thyroglobulin*. Thyroglobulin (refer to Figure 10.7) is a glycoprotein and is synthesized in the follicular cell as prothyroglobulin and the tyrosine units are iodinated in the intact protein.

As you may have noticed in Figure 10.7, the iodide actively transported into the cells from extracellular fluid (ECF) is released from the thyroid cells into the colloid follicle where it is oxidized by *thyroperoxidase* in the presence of hydrogen peroxide.

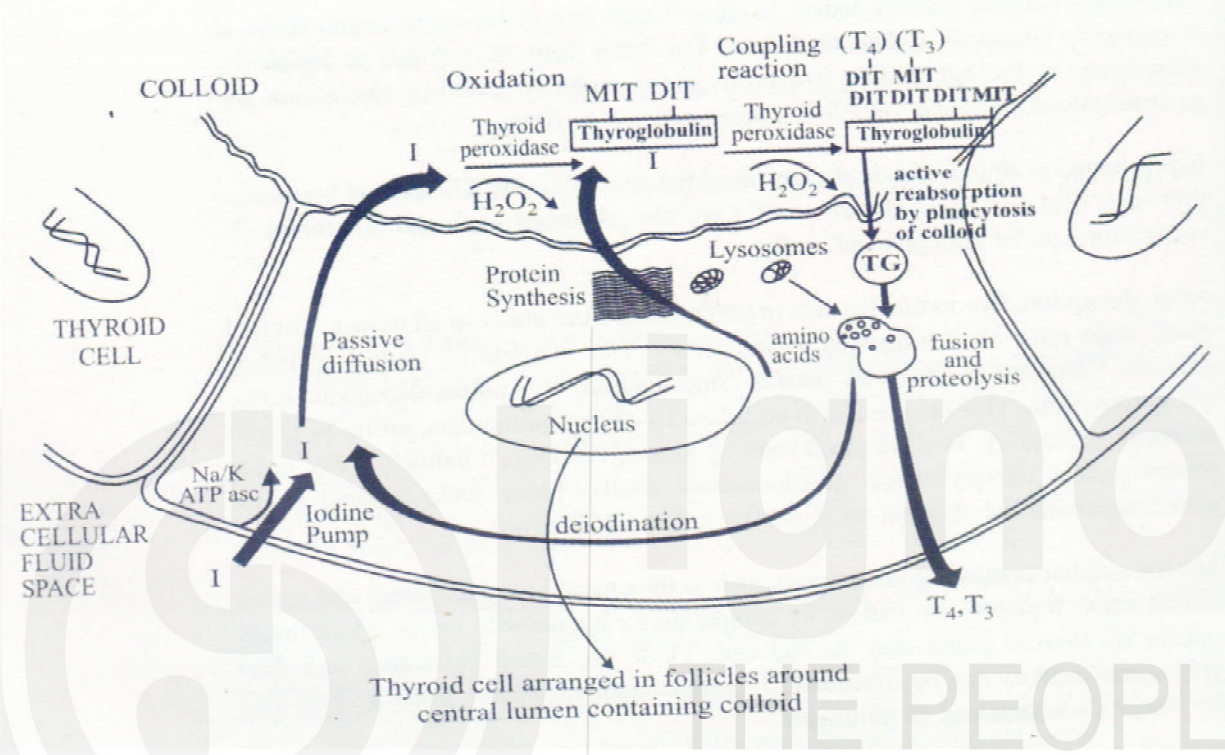


Figure 10.7: Biosynthesis and secretion of thyroid hormones

The oxidized iodine is then combined with the amino acid tyrosine in thyroglobulin to form mono and di-iodotyrosines (MIT/DIT), which are again catalyzed by thyroperoxidase. These are then coupled to form triiodo to thyronine and tetraiodo-thyroxine. The iodinated thyroglobulin is absorbed back into the cells by a process known as *pinocytosis*. The iodinated thyroglobulin is hydrolyzed within the cells by the cellular proteolytic enzymes to release T_4 and T_3 into the blood circulation. Un-utilized mono- and di-iodotyrosines are not released into the blood but are conserved within the gland for further incorporation into thyroglobulin.

In blood, these hormones bind to transport proteins mainly thyroxine-binding-protein and are distributed to the target cells in the peripheral tissues. All phases of biosynthesis and secretion of thyroid hormones are stimulated by thyroid-secretary hormone (TSH), which is secreted by anterior pituitary gland in response to low levels of thyroid hormones.

Let us next review the physiological functions and metabolic effects of thyroid hormones, in order to understand the importance of iodine in humans.

Physiologic Functions of Iodine

Thyroid hormone performs multiple functions as regulator of cell activity and growth. The hormone has crucial metabolic roles in the foetus, and in the infant post-natally. It promotes growth and maturation of peripheral tissues in the human embryo, the

most visible effect seen in the skeletal growth. Delayed bone development has been seen in hormone deficient human embryos. Thyroid hormone influences neuronal cell growth and dendrite development in the embryo. A major effect of foetal iodine deficiency is *cretinism,* characterized by mental deficiency and deaf mutism.

Postnatally, linear growth, i.e. stature and bone maturation are critically dependent on thyroid hormone. Both are retarded when there is a deficiency of the hormone due to low iodide intakes. The hormone plays an important role in the provision of energy to most cells in the body; the best indicator of this is the energy available for utilization in the basal state, i.e. the basal metabolic rate. In thyroid hormone deficiency, the BMR is lower, slowing the overall cellular activities. Iron deficiency in children is characteristically associated with goitre.

In the endemic iodine deficient regions of India, school children have been shown to have general IQs 10 points lower than children in non-iodine deficient areas. A high degree of apathy has also been noted in adults living in the iodine deficient areas in India. Even domestic animals in these areas have been reported to display apathetic behaviour. Reduced mental function is widely prevalent in thyroid hormone deficiency in the iodine deficient endemic areas, highlighting the key role of this hormone in neuronal and brain development and function. Iodine deficiency is a major obstacle to human and social development and should be prevented as a priority.

Some important aspects of the metabolic influences exerted by thyroid hormones are being highlighted in the subsequent text.

Metabolic Effects of Thyroid Hormones

Although (T_4) is quantitatively predominant, (T_3) is the more active form. The mechanism of action of thyroid hormones appears to involve binding to nuclear receptors, which, in turn, alter gene expression in pituitary, liver, heart, kidney and most crucially, the brain cells.

Overall, thyroid hormones stimulate synthesis of enzymes, oxygen consumption and basal metabolic rate (BMR) and thereby affect heart rate, respiratory rate, mobilization and metabolism of carbohydrates, lipogenesis and a wide variety of other physiological activities. They are necessary for the normal nervous system development and linear growth. Directly or indirectly, most organs are under the influence of these substances.

It is probable that iodine has additional roles to that of thyroid hormones activity, e.g. in antibiotic and anticancer activity, but these are poorly understood.

The deiodination of (T_4) and (T_3) takes place in extrathyroidal tissues, mainly liver.

Let us now proceed to learn about the health effects of a low iodine intake which continues to be a serious public health problem even today despite concerted efforts being laid down by our government to alleviate this nutritional deficiency disorder.

Deficiency

Iodine deficiency affects all populations at all stages of life, from the intrauterine stage to old age. However, pregnant women, lactating women, women of reproductive age, and children younger than 3 years of age are considered the most important groups in which to diagnose and treat iodine deficiency, because iodine deficiency occurring during foetal and neonatal growth and development leads to irreversible damage of the brain and central nervous system and, consequently, to irreversible mental retardation. Thus, its deficiency causes a wide spectrum of disorders. These include:

- *Mild goitre*, i.e., a larger thyroid gland than normal. The mildest form of goitre ranges from those only detectable by touch (palpation) to very large goitre that

can cause breathing problems. The enlargement of glands occurs from stimulation of thyroid cells by TSH and without ability to increase hormones production owing to iodine deficiency.

- The most severe form is *endemic cretinism*, which is characterized by congenital, severe irreversible mental and growth retardation.
- *Hypothyroidism*, which is accompanied by low BMR, apathy, slow reflex relaxation time with slow movements, cold intolerance and myxoedema (skin and subcutaneous tissues are thickened because of accumulation of mucin and become dry and swollen).

Collectively, these manifestations of iodine deficiency are termed '*Iodine Deficiency Disorders*' (IDD) about which you may recall studying in the Public Nutrition Course (MFN-006) in Unit 3.

The symptoms of IDD differ depending on the life stage at which iodine deficiency occurs. For example, iodine deficiency in foetus has most severe consequences and results in cretinism. There is severe mental retardation, deaf-mutism (defects of hearing and speech), squint, disorders of stance and gait and stunted growth.

However, varying degrees of intellectual or growth retardation are apparent when iodine deficiency occurs in infancy or childhood and adolescence.

Apart from cretinism, hypothyroidism and goitre, other features linked to IDD are the decreased fertility rates, increased stillbirths and spontaneous abortion rates and increased perinatal and infant mortality.

Epidemiological studies have indicated that an ingestion of 100-200 mg of iodine daily is sufficient to prevent deficiency except among individuals suffering from a genetic disorder. Revised standards proposed by Food Safety and Standard Authority of India (FSSAI) on mandatory fortification of all edible salt with iodine at 15-30 ppm (15-30 mg/kg), as part of universal salt iodization strategy to prevent iodine deficiency disorders, is in place in India. Therefore, daily intake of 10 g of iodized salt should be able to provide about 150-300 µg of iodine (if adequately iodized and stored properly), in addition to iodine present in foods consumed.

Toxicity

A wide range of iodine intakes is tolerated by most individuals, owing to the ability of the thyroid to regulate total body iodine. This tolerance to huge doses of iodine in healthy iodine-replete adults is the reason why WHO stated in 1994 that, "Daily iodine intakes of up to 1 mg, i.e. 1000 µg, appear to be entirely safe". This statement, of course, does not include neonates and young infants.

Over 2 mg iodine/day for long periods should be regarded as excessive or potentially harmful to most people. Such high intakes are unlikely to arise from natural foods, except for diets that are very high in seafood and/or seaweed or comprising foods contaminated with iodine. In contrast to iodine-replete individuals, those with IDD or previously exposed to iodine-deficient diets may react to sudden moderate increases in iodine intake, such as from iodized salt. Iodine-induced *thyrotoxicosis* (hyperthyroidism) and toxic modular goitre may result from excess iodine exposure in these individuals. *Hyperthyrodism* is largely confined to those over 40 years of age and symptoms are rapid heart rate, trembling, excessive sweating, lack of sleep, and loss of weight and strength. Individuals who are sensitive to iodine usually have mild skin symptoms.

Thus, the level of iodine in the body can be a vital biochemical indicator for assessing the impact of a sub-optimal iodine intake and for outlining an appropriate patient care process. Let us find out which parameters can be helpful in the field and clinical settings.

Assessment of Iodine Status

Iodide nutritional status is generally directed at population living in areas suspected to be iodine deficient. The assessment is based on both the physical examination and chemical testing of individuals. It includes:

- *Total population count*, including the number of children below 15 years of age. It is estimated that about 10 million people are exposed to the risk of IDD in India, they live in iodine deficient endemic areas.
- Incidence of *goitre,* as established by physical examination and cretinism in the population of the 150 million at risk stated above, 55 million are reported to have goitre and 22 million suffer from cretinism.
- The quantification of *urinary iodide excretion*. Urinary concentration less than 50 mcg/g of creatinine are considered at-risk. Urinary iodide less than 10 mcg/dl is considered deficient. While above 10 mcg/dl is normal.
- The quantification of iodide in the drinking water. Less than 2 mcg/L of water is indicative of iodine deficient endemic area.
- *Determination of serum (T_4) levels* in various age groups. Normal levels are 4-12 mcg/dl.
- *Determination of serum TSH*: Values less than 1 to 4 micro units/ml is considered normal. TSH is elevated in iodine deficiency disorders.
- *Determination of T4 and TSH*: Both are used in assessing the iodine status of the newborn in endemic areas. A new born infant with T_4 less than 3 mcg/dl and TSH 50 micro units / ml or higher is considered to have neonatal hypo-thyroidism.

Requirements

The minimum amount of iodide to prevent goitre is estimated between 50 and 75 mcg/day or 1 mcg/kg body weight. The 2020 ICMR recommended RDA is 150 mcg/day for adults of both sexes. Although the recommendations are the same for both males and females, iodide needs are higher during pregnancy and lactation. Therefore, the recommended intakes during pregnancy and lactation are about 250 mcg iodine per day.

Refer to Table 10.17 which presents the RDA of iodine for various physiological groups recommended by ICMR, 2020.

We shall finally review the key aspects of yet another important trace element of nutritional significance i.e., fluorine, which is very often implicated with dental health. You may also have read or heard about fluorine toxicity which arises due to the presence of fluorine in high amounts (>1ppm) in drinking water. Let us get to know more about this important aspect.

10.10 FLUORINE

Fluorine is potentially a toxic element. Its essentiality for humans is not established although the role of fluoride in providing protection from dental caries in human has been demonstrated. Fluorine (F) is a gaseous chemical element, while its ion, fluoride (F^-) is composed of fluorine bound to a metal, non-metal, or an organic compound. Examples are magnesium fluoride, hydrogen fluoride, fluro/benzene fluoride. Fluoride predominates in nature and in body, it is deposited in bones and teeth. Its incorporation into tooth enamel markedly increases the hardness and resistance to decay.

Let us next study about the food sources of fluoride.

Table 10.17 : EAR and RDA of iodine for various physiological groups

Age Group	Reference Body Weight (Kg)	Category (ICMR	ICMR 2020 (µg/day)		
			EAR	RDA	TUL**
Men	65	>18y	95	140	1100
Women (NPNL)	55	>18y	95	140	1100
Pregnant women		>18y	160	220	1100
Lactating women		>18y	200	280	1100
Infants	5.8	0-6 m	100 (AI)		ND
Infants	8.5	6-12 m	130 (AI)		ND
Children	12.9	1-3y	65	90	200
Children	18.3	4-6y	65	90	300
Children	25.3	7-9y	65	90	400
Boys	34.9	10-12y	70*	100	600
Girls	36.4	10-12y	70*	100	600
Boys	50.5	13-15y	100*	140	900
Girls	49.6	13-15y	100*	140	900
Boys	64.4	16-18y	100*	140	1100
Girls	55.7	16-18y	100*	140	1100

The values are pooled for gender; ** The TUL recommended by IOM, 2001 for adult men, adult NPNL women, pregnant women and lactating women was considered. Average adult reference body weight (men and NPNL women) of 60 kg was considered to extrapolate TUL values for children and adults based on reference body weights.

Source: RDA and EAR Nutrient Requirements for Indians, NIN, ICMR, Hyderabad, India, 2020

Food Sources

The major source of fluoride in most diets is water, with foods providing only about 25% of total intake. These include tea and marine fish, ready-to-use infant formulas made with fluoridated water. Other foods which significantly contribute to fluoride in our diet are given in Table 10.18.

Table 10.18: Sources of fluoride

Food Group	Fluoride Content (ppm)
Dairy products	0.05 - 0.07
Meat, fish, poultry	0.22 - 0.92
Grain, cereal products	0.29 - 0.41
Potatoes	0.08 - 0.14
Green leafy vegetables	0.10 - 0.15
Legumes	0.15 - 0.39
Root vegetables	0.09 - 0.10
Other vegetables	0.06 - 0.17
Fruits	0.06 - 0.13
Fats, oils	0.13 - 0.24
Sugar	0.21 - 0.35

Source: Rao G.S. Dietary Intake and bioavailability of fluoride Ann Rev. Nutr.1984; 4:120.

Let us now see how flourine is metabolized in our body.

Metabolism

Soluble fluorides, even at high intake levels are almost completely absorbed from gastrointestinal tract. These include aqueous solutions of fluorides, sodium fluoride (NaF) used in toothpastes, and sodium fluorosilicate used in water fluoridation. However, its availability from solid foods is only about 50%-80% of that absorbed from aqueous solutions. This is because in foods, it may be bound to proteins and on hydrolysis by enzyme proteases, may still be less available for absorption. Peak plasma concentrations occur within 30-60 minutes of ingestion. Fluoride absorption occurs through diffusion.

Once absorbed, the fluoride passes into the blood for distribution chiefly to the calcified tissues. Most of the ionic fluoride enters the bone and developing teeth where the fluoride ion replaces the hdroxyl or bicarbonate in the hydroxyapatite and forms fluoroapatite. About half of the fluoride absorbed each day is deposited in the skeleton or teeth within 24 hours. Nearly 99% of the fluoride in the body is in the calcified tissues. Fluoride in the bone is in a reversible pool and can exchange for other ions such as hydroxyl ions during the process of bone remodeling. The only positive role clearly demonstrated for fluoride, however, is in the prevention of dental caries. Let us learn about this important function next.

Functions

The only beneficial role demonstrated for fluoride is in reducing the prevalence and severity of dental caries in children and adults. This is enumerated next.

Fluoride and dental caries: There are three ways in which fluoride may act to prevent tooth decay. When fluoride is incorporated into the tooth early in life at the time of tooth eruption, the enamel containing fluoroapatite becomes more resistant to dissolution by acids. Secondly, in normal course, the enamel gets demineralized by contact with food acids and demineralization occurs to ensure that enamel structure is maintained. Topical application of fluoride enhances demineralization and maintains the integrity of the enamel. Lastly, fluoride inhibits glycolysis and then reduces acid formation from sugars on the teeth, helping to prevent enamel demineralization and tooth decay. For these reasons, fluoride is considered as a beneficial element for humans, but it is not an essential element. Drinking water fluoride levels of 0.7 to 1.2 mg/L is considered safe. Levels above this can cause several health risks and should be avoided.

In this regard, let us discuss the effects on health of fluoride toxicity.

Toxicity

Fluoride is a cumulative toxin. Ingestion of fluoride 1.0-1.5 mg/L for several years may produce *dental fluorosis*, i.e. browning and pitting of teeth known as *mottling,* as you may recall studying in the Public Nutrition Course (MFN-006). Chronic high level of fluoride in the range of 2-5 mg/L can cause *skeletal fluorosis*. Crippling skeletal fluorosis can occur where drinking water containing higher than 10 mg/L is consumed over several years.

The severe forms of skeletal deformity in toxic fluorosis include kyphosis (abnormal curvature of the spine), fixed spine and other joint deformities. Hyper parathyroidism secondary to high fluoride intake has been reported, which induces calcification of soft tissues. You may recall that PTH is a hormone involved in calcium homeostasis, releasing calcium from the bone into the blood when blood calcium levels tend to fall. An abnormal increase in PTH can add calcium to the soft tissues, hardening them in the process.

A form of severe skeletal flourosis known as "Genuvalgium" (knocked knees) has been reported from part of India, China and African countries. The condition is characterized by severe skeletal fluorosis and osteoporosis of the limbs. Chronic ingestion of excess fluoride coupled with low calcium and high molybdenum intakes appear to increase fluoride retention in the bone. While hyper-parathyrodism and increased levels of PTH result in calcium removal from the bone, explaining the osteoporosis of the limbs.

With this, we end our study of micro minerals. Indeed that was an exhaustive study.

Check Your Progress Exercise 6

1) Which organ of the human body constitutes maximum percentage of Iodine? Name thyroid hormones which are absorbed unaltered.

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2) What are goitrogens? Where are these found?

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3) What are the metabolic effects of thyroid hormones?

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4) Give the normal values for plasma F^- and urinary F^-.

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5) What is Fluorosis?

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10.11 LET US SUM UP

In this unit, we studied about 8 physiologically important micro minerals namely, iron, zinc, copper, selenium, chromium, manganese, iodine and fluorine. We learnt about their history, food sources along with content, their physiology of metabolism inside our body. We also focused on their vital functions, the deficiency and toxicity levels.

We also got to know about the various tests / methods used to assess their status in our body. Also, we learnt about their recommended level of intake of requirements which are essential to carryout various physiological roles.

10.12 GLOSSARY

Acrodermatitis Enteropathica : a genetic human disease related to an inability to absorb adequate zinc from the normal diet.

Alopecia : an autoimmune disease in which the immune system mistakenly attacks the hair follicle leading to hair fall on the scalp) loss of hair or baldness.

Arthopathy : any disease or disorder involving a joint.

Bradykinesia : slowness of movement.

Cardiac arrhythmias : an abnormal rate of muscle contractions in the heart.

Cardiomyopathy : a disease of the heart muscle that causes it to lose its pumping strength.

Ceruloplasmin : a Cu-containing protein.

Chelators : compounds that bind to metal ions to form a complex.

Dystonia : abnormal muscle tone of one or more muscles.

Glucose Tolerance Factor : a compound containing chromium that aids insulin in regulating blood sugar levels.

Goitrogens : substances that interfere with iodide metabolism in any way that inhibits thyroid hormogenesis.

Haematocrit : proportion of the total blood volume, that is, red blood cell; expressed as a percentage.

Haem iron : iron found in foods of plant origin.

Hypoxia : insufficient oxygen, especially as applied to cells.

Micro minerals : minerals which comprise less than 0.01% of the total body weight and are required in concentration of 1ppm or less.

Mobilferrin : an iron-binding protein.

Neoplasia : abnormal cell growth that may be pre-cancerous.

Neurtopenia : decreased number of WBCs, which greatly increase the risk of infection.

Peripheral nerves : nerves carrying impulses to and from the brain and spinal cord.

Peripheral neuropathy : a disorder of the peripheral nerves involving feet, hands and sometimes legs, arms and face. It causes pain numbness, or a tingling feeling.

Rheumatoid arthritis : a crippling form of arthritis characterized by painful and stiff joints on both sides of the body.

Total parental nutrition : a way to provide a liquid food mixture through a special tube in the chest.

Transcription factors : DNA-binding proteins.

Transferrin : a glycoprotein; a iron-binding protein.

Ulcerative colitis : a disease that causes irritation and ulcers in the lining of the large intestine and rectum.

Zinc fingers : a protein which looks like fingers and contains a series of polypeptide loops resulting from twisting and coiling of the cysteine and histidine residues.

10.13 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) 65% of 2-4 g iron is found in association with haemoglobin.

2) Haem iron are chicken, liver, meat, fish, egg, salmon

   Non-haem iron are dried apricots, soybeans, almonds, spinach, kidney beans, the sources of lentils.

3) There are certain dietary factors which either increase or decrease iron absorption. Factors which increase iron absorption include ascorbic acid, certain organic acids like citric, lactic and tartaric acid, animal proteins such as meat, fish, poultry, sugars such as fructose, sorbitol; physiological factors – pregnancy and growth, as well as, depleted iron status and optimum gastric acidity.

   On the other hand, factors which decrease iron absorption include increased intestinal mobility, presence of phytates, oxalates, iron-binding phenolic compounds such as ferrous pyrophosphate, ferrous citrate, calcium, phosphorus, magnesium, zinc, manganese and copper, tannic acid in coffee and tea, prolonged/excessive use of ant-acids, achlorhydria and hypochlorhydria.

4) Our body has three unique mechanisms for maintaining iron balance. These are storage of iron, reutilization of iron and regulation of iron absorption.

5) Iron deficiency anaemia can result from both a reduction in circulating haemoglobin and a reduction in iron containing enzymes and myoglobin. These include: fatigue, restlessness and impaired work performance; disturbance in thermo regulation; impairment of certain key steps in immune response; can have adverse effects on psychomotor and mental development in children; and increased mortality and morbidity of mother and infant during pregnancy.

6) Iron status cab be assessed by various methods. These include measurement of plasma ferritin concentration, serum transferrin receptors, zinc protoporphysin, transferrin saturation and haem oglobin and haematocrit levels. Mobilferrin and Transferrin; Serum ferritin, Transferrin receptors, Erythrocyte protoporphyrin, Transferrin saturation, haemoglobin and haematocrit levels.

Check Your Progress Exercise 2

1) Zinc is referred to as the most abundant intracellular trace because nearly 95% of total body zinc is present within the cells.

2) Acrodermatitis enteropathica is a genetic disease which leads to an inability to absorb adequate zinc from diet.

3) The term zinc finger is used to denote the configuration of DNA-binding proteins of which zinc is an important component. It comprises of a series of polypeptide loops resulting from twisting and coiling of polypeptide loops which gives it the impact of a finger like structure.

4) Zinc is unique among the trace elements in that it is a part of enzymes from all six enzyme commission classes. As a component of these enzymes, it either provides structural integrity to the enzyme or participates directly in the reaction at the catalytic site. Zinc is a component of over 200 metallo-enzymes and is therefore vital for many fundamental life processes.

5) Techniques of assessing Zn status are any five of the following : measurement of zinc in plasma, RBCs and neutrophils, metallothionine concentration, urinary and hair zinc levels, measurement of activity of zinc-dependent enzymes.

6) The symptoms assocated with the prolonged intakes of zinc (75-300 mg/day) have been associated with impaired copper utilization, impaired immune response and a decline of high density lipoprotein. Intakes between 25 -50 mg zinc per day have been reported to interfere with metabolism of both iron and copper.

Check Your Progress Exercise 3

1) The organs which contain a high concentration of Cu are liver, brain, heart, bone, hair and nails.

2) The components which aid in the release of Cu in the gastrointestional tract are gastric HCl, pepsin and some proteolytic enzymes

3) Dietary components exerting positive effect on Cu absorption include amino acids especially histidine, organic acids such as citric, gluconic, lactic, acetic and malic acids. While which inhibit Cu absorption include high intakes of several nutrients such as zinc, iron, molybdenum, calcium, phosphorus and excessive intake of antacids.

4) a) albumin and transcuperin

   b) ceruloplasmin

5) Refer to Table 10.11 in section 10.5 and answer the question on your own.

6) The predisposing factors of copper deficiency are prematurity, low birth weight and malnutrition, especially when combined with feeding practices such as cow's milk or total parenteral nutrition.

7) Wilson's disease: This disease affects 1 in 30,000 in most populations. It is an autosomal recessive disease of copper storage. The defect results in impaired biliary excretion of copper and accumulation of copper in the liver, brain and cornea of eye. If the disease is not treated, copper accumulation in liver and brain results in neurologic damage and cirrhosis.

Check Your Progress Exercise 4

1) An enzyme which constitutes selenium as its integral part is Glutathione peroxidase (GPX).

2) The selenium content of food varies because of the variations in the selenium content of soil where the crops are grown. Further, factors such as pH of soil, microbial activity, rainfall etc. also determine the uptake of selenium by the plant from the soil.

3) Phytates and heavy metals, such as mercury through chelation and precipitation hinder selenium absorption. Vitamin C, A and E enhance absorption.

4) The three selenoenzymes have been identified with the following catalytic roles:

   - reduction of hydrogen peroxide and organic hydroperoxides by GPXs.
   - activation and inactivation of thyroid hormone by Iodothyronine deiodinases.

- NADPH–dependent reduction of oxidized thioredoxin regulation of cell growth and inhibition of apoptosis by the enzymes thioredoxin reductases.

Serum or plasma selenium level is the test to indicate short-term changes in dietary intake of selenium.

5) Selenium deficiency has been linked to two regional human diseases: Keshan disease and Kashin Beck's disease. Refer to sub-section on deficiency of selenium for details.

Check Your Progress Exercise 5

1) Insulin-dependent-diabetic patients absorb 2-4 times more chromium because they have an impaired ability to convert inorganic form to usable form and therefore require higher chromium.

2) The factors that enhance Cr absorption are ascorbic acid, picolinate, methionine and histidine.

3) During conditions of iron excess or iron overload such as iron storage diseases, all the metal transport sites on transferrin are occupied by iron. This may explain the high incidence of diabetes in haemochromatosis patients, which may be induced by chromium deficiency.

4) Factors which influence Mn absorption are: absorption decreases with increasing intake, percent absorption is higher among women as compared to men, increased dietary iron depresses Mn absorption whereas iron deficiency increases its absorption and high levels of dietary calcium, phosphorus and phytate impair the intestinal uptake of the element.

5) Antioxidant activity, carbohgydrate metabolism, integrity of cartilage and brain development.

6) Mn is found in most organs and tissues and preferentially accumulates in the mitochondria. There is no storage form for Mn. Bone contains substantial amount of mineral but there is no mechanism to release it and thus bone Mn is considered as passive storage. It is released only as a result of normal bone turnover or in situations of accelerating bone resorption.

7) The course and degree of Mn intoxication varies greatly but Mn toxicity has been shown to occur in the following three phases:

   - The first phase is characterized by the non-specific symptoms such as anorexia, apathy, headache, hypersomnia, irritability and spasms.
   - The next phase is characterized by expressionless face, speech disturbance, altered gait and fine tremor.
   - In the third stage, there is muscular rigidity, staggering gait and fine tremor.

Check Your Progress Exercise 6

1) Thyroid gland (70-80%) constitutes maximum percentage of iodine; T_3 and T_4 are the hormones which are absorbed unaltered.

2) Goitrogens are substances that interfere with iodide metabolism. As a result, there is augmentation in TSH release and subsequent thyroid gland enlargement. These active goitrogens are released by plant enzymes from thioglucosides or cynogenic glucosides found in cassava, kale, cabbage, broccoli, turnips, rapeseeds and mustards.

3) The mechanism of action of thyroid hormones appears to involve binding to nuclear receptors, which, in turn, alter gene expression in pituitary, liver, heart, kidney and most crucially, brain cells. Thyroid hormones stimulate synthesis of enzymes, oxygen consumption and basal metabolic rate (BMR) and thereby affect heart rate, respiratory rate, mobilization and metabolism of carbohydrates, lipogenesis and a wide variety of other physiological activities. They are necessary for the normal nervous system development and linear growth.

4) 0.01-0.02 mcg/ml is the normal value for plasma F^-

   0.02-1.1 mg/ml is the normal value for urinary F^-

5) Fluorosis is excessive intake of F^-, early signs of which are mottling and discolouration of the teeth. It is characterized by changes in bone, kidney and possibly nausea, vomiting, acidosis and cardiac arrhythmias.

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