General introduction

Good nutrition is the cornerstone of a child’s right to live a happy and healthy life. Without adequate nutrition (including essential micronutrients that help a child’s brain to develop or body to fight disease), a child cannot achieve his or her full potential. One of these micronutrients is the vitamin A (retinol), which is key to the functioning of the immune system. Malnutrition among the vulnerable sectors of the population is one of the public health problems in most of the developing/underdeveloped countries. In early childhood, malnutrition results in serious, long-term consequences because it impedes motor, sensory, cognitive, social and emotional development. Nearly 30% of all newborns have a low birth weight, making them more vulnerable to further disease. Vitamins and mineral deficiencies also affect the survival and development of children. Anaemia affects 74% of children under the age of three, more than 90% of adolescent girls and 50% of women (NFHS-II, 2000). Iodine deficiency, which reduces learning capacity by up to 13%, is widespread because fewer than half of all households use iodized salt. One of the serious threats to the children is vitamin A deficiency (VAD), which causes blindness and increases morbidity and mortality among pre-schoolers, also remains a public health problem (Mudur, 2001). Children become vitamin A deficient for two main reasons: 1) their mothers are deficient and produce breast milk low in vitamin A and 2) they are weaned onto diets that provide too little retinol. A third contributing factor is that they spend a substantial part of childhood being sick; leading to malabsorption and increased catabolism, which further deteriorate their vitamin A status (Miller et al. 2002).

Vitamin A and its functions

Vitamin A is one of the fat-soluble vitamins and retinol is the most active form of vitamin A, found in animal foods such as liver and eggs. Chemically vitamin A refers to all isoprenoid compounds that possess the biological activity of all-trans retinol. The parent structure of most retinoids contains a substituted \( \beta \)-ionone ring (4-{2,6,6-trimethyl-2-cyclo-hexen-1-yl}-3-buten-2-one) with a side chain of three isoprenoid units linked at the 6-position of the \( \beta \)-ionone ring (Figure 1.1). The conjugated double bond system
includes the 5,6-β-ionone ring carbons and the isoprenoid side chain. Retinoids include all substances possessing vitamin A activity.

Vitamin A is required for the normal functioning of the visual system. Retinol plays an important role in the formation of rhodopsin, an important visual pigment, particularly for dim-light vision. All-trans retinol is converted to retinaldehyde, isomerized to the 11-cis form, binds to opsins to form rhodopsin. Insufficient amount of retinol affects rhodopsin synthesis, which in turn cause night blindness. This condition can, however, also be due to lack of other nutrients such as protein and zinc, which are critical to the regeneration of rhodopsin.

Vitamin A is also essential for normal growth, differentiation and proliferation of wide range of epithelial cells, bone growth, reproduction and embryonic development. It can be converted to retinal, retinoic acid and other active forms of the vitamin A family. It maintains the surface linings of eye, respiratory, urinary and intestinal tracts. When the linings break down, bacteria can enter the body and cause infection. The immune system helps to prevent or fight off infections by producing white blood cells that destroy harmful bacteria and viruses. With the exception of the essential role of 11-cis retinaldehyde in vision, retinoic acids that serve as ligands for nuclear retinoic acid receptors, namely RXR and RAR, mediate vitamin A dependent activities.

Sources of vitamin A

Human communities rely on a very wide range of plant and animal foods to meet their dietary vitamin A requirements. Vitamin A is found in food in two forms; retinol, the pre-formed vitamin and β-carotene, a ‘provitamin’ that is converted to vitamin A in the body. Provitamin A is found in plant foods and the term ‘provitamin A’ is to differentiate the carotenoid precursors of vitamin A from carotenoids without vitamin A activity. Yellow and orange fruits and vegetables such as papaya, mango, passion fruit, melons and carrots, as well as dark green leafy vegetables such as spinach, generally contain large amounts of provitamin A carotenoids (Machlin, 1984). Preformed vitamin A is found in foods of animal origin including milk, cheese, butter, egg, liver and fish (Napier, 1995). Structures of vitamin A and its related compounds possessing vitamin A activity are given in Figure 1.1.
Chapter 1. General introduction

Figure 1.1. Structures of vitamin A and its related compounds

Carotenoids

Carotenoids are a class of more than 600 naturally occurring pigments synthesized by plants, algae and photosynthetic bacteria. They are hydrophobic molecules and thus, are located in lipophilic sites of cells, such as bilayer membranes. Their hydrophobic nature is decreased with an increased number of polar substituents (mainly hydroxyl groups), thus affecting the positioning of the carotenoid molecules in biological membranes. For example, dihydroxy carotenoids such as lutein and zeaxanthin orient themselves perpendicular to the membrane surface in order to expose their hydroxyl groups to a more polar environment. In contrast, the carotenes such as β-carotene and lycopene could position themselves parallel to the membrane surface to remain in a more lipophilic environment in the inner core of the bilayer membranes (Parker, 1989; Britton, 1995). Thus, carotenoid molecules can have substantial effect on the thickness, strength and fluidity of membranes and thus affect many of their functions like transport and receptor interactions. Several epidemiological studies have reported that consumption of foods rich in carotenoids is associated with a reduced risk of certain cancers, cardiovascular disease and age-related macular degeneration (Ziegler, 1991;
Seddon et al. 1994; Van Poppel, 1996). These preventive effects of carotenoids could be related to their major function as vitamin A precursors and/or their actions as antioxidants, modulators of the immune response and inducers of gap junction communications (Olson, 1998).

**β-Carotene, a potent provitamin A carotenoid**

The term provitamin A carotenoid is to differentiate carotenoid precursors of vitamin A from carotenoids without vitamin A activity. β-Carotene is one of the most abundant hydrocarbon carotenoids found in human diet and most potent vitamin A precursor among all provitamin A carotenoids. To exhibit provitamin A activity, the carotenoid molecule must have at least one unsubstituted β-ionone ring and the correct number and position of methyl groups in the polyene chain (Wirtz et al. 2001). As a result, other provitamin A carotenoids like α-carotene, β-cryptoxanthin and γ-carotene show 30-50% of provitamin A activity (Bauernfeind, 1972; Van Vliet et al. 1996) and 9-cis and 13-cis isomers of β-carotene show <10% activity (Nagao and Olson, 1994), compared to all-trans β-carotene.

**Vitamin A activity**

The relationship of fat-soluble vitamin A to the plant pigment, carotene was demonstrated by Rosenheim and Drummond (1920) initially, but it was Moore (1957) who established the chemical relationship between vitamin A and β-carotene. In the early work with vitamin A, the only assays available were biological response tests and the unit of measure was defined as the International unit (IU). Presently, IU has been replaced by weights of the active components. Currently, retinol equivalent (RE) is defined as the amount of the substance having biological activity equivalent to that of 1 μg retinol. For calculation of RE values in foods, 100% efficiency of absorption of retinol is assumed. Considering the estimated efficiency of absorption and conversion, 6 μg of β-carotene is taken as having a biological activity equal to 1 RE. Until recently, a dietary conversion factor of 6 μg of β-carotene or 12 μg of other provitamin A carotenoids was regarded as equal to 1 μg retinol (RE) (FAO/WHO, 1988). However, some studies show that the bioavailability of carotenoids was not as efficient as it was previously thought (de Pee and West, 1996; Castenmiller and West, 1998). Therefore, the Dietary Reference
Intake Committee recently recommended that 12 $\mu$g of $\beta$-carotene or 24 $\mu$g of other provitamin A carotenoids be considered equal to 1 $\mu$g retinol (retinol activity equivalent, RAE) (Food and Nutrition Board, 2001) (Table 1.1).

<table>
<thead>
<tr>
<th>Quantity consumed</th>
<th>Quantity bioconverted to retinol</th>
<th>RAE ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 $\mu$g of dietary or supplemental vitamin A</td>
<td>1 $\mu$g of retinol*</td>
<td>1:1</td>
</tr>
<tr>
<td>2 $\mu$g of supplemental $\beta$-carotene</td>
<td>1 $\mu$g of retinol</td>
<td>2:1</td>
</tr>
<tr>
<td>12 $\mu$g of dietary $\beta$-carotene</td>
<td>1 $\mu$g of retinol</td>
<td>12:1</td>
</tr>
<tr>
<td>24 $\mu$g of dietary $\alpha$-carotene</td>
<td>1 $\mu$g of retinol</td>
<td>24:1</td>
</tr>
<tr>
<td>24 $\mu$g of dietary $\beta$-cryptoxanthin</td>
<td>1 $\mu$g of retinol</td>
<td>24:1</td>
</tr>
</tbody>
</table>

*1 IU = 0.3 $\mu$g of retinol, and 1 $\mu$g of retinol = 3.33 IU of retinol.

Earlier, the vitamin A activity of provitamin A carotenoids was expressed as retinol equivalents (RE) (FAO/WHO, 1988). Based on more recent absorption and bioconversion data, however, it is now recommended that provitamin A activity in foods be expressed as retinol activity equivalents (RAE), which are 50% of the corresponding RE (IOM, 2001). RE was defined based on the assumption that 33% of $\beta$-carotene in food is absorbed and of that 50% is converted to retinol. The definition of RAE, in contrast, is based on the assumption that 16.7% of the $\beta$-carotene is absorbed and 50% is converted to retinol.

The true biological utilization of dietary sources of retinol may be conditioned by the level of fat in the diet, with very low fat diets potentially impairing the absorption and utilization of carotenoids or retinol or both. Conversely, conversion of carotenoids may be somewhat more efficient when intakes are very low (FAO/WHO, 1967 & 1988). Other carotenoids have vitamin A activity but with lower potency than $\beta$-carotene. Some derivatives of retinol (retinal and retinoic acid) also have at least some of the biological properties of retinol, which depends on the utilization of retinol. Biological activity of some of the retinoids and carotenoids are given in the Table 1.2.
Table 1.2. Relative biological activity of retinoids and carotenoids.*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-trans retinol</td>
<td>100</td>
</tr>
<tr>
<td>All-trans retinal</td>
<td>100</td>
</tr>
<tr>
<td>Cis-retinol isomers</td>
<td>23-75</td>
</tr>
<tr>
<td>Retinyl esters</td>
<td>10-100</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>50</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>26</td>
</tr>
<tr>
<td>γ-Carotene</td>
<td>21</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>28</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>0</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0</td>
</tr>
<tr>
<td>β-Apo-8′-carotenal</td>
<td>18-36</td>
</tr>
</tbody>
</table>

*Combs (1992)

Vitamin A and carotenoids (β-carotene) metabolism

**Digestion and absorption**

Vitamin A is obtained from the diet either as long chain retinyl esters (animal source) or provitamin A carotenoids, primarily β-carotene (plant source). Both undergo metabolism during absorption, the pathways involved in the metabolism are represented in Figure 1.2. The retinyl esters are hydrolyzed to retinol in the lumen by pancreatic lipases and perhaps, by bile salt-dependent retinyl ester hydrolase, intrinsic to the brush border membrane of the intestinal villi prior to absorption (Rigtrup and Ong, 1992). The enzymes associated with the brush border membrane, involving in the digestion of dietary retinyl esters are pancreatic lipase, pancreatic carboxyl ester lipase and one or more retinyl ester hydrolases (Harrison, 1993). Approximately 80-90% of retinyl esters and 50% of carotenoids are absorbed in humans (Blomhoff et al. 1991). Retinyl esters are released from the food matrix during digestion by the action of pepsin in the stomach and proteolytic enzymes in the small intestine and partition into lipid droplets. Carotenoids are also processed in the same manner as retinyl esters and other fat-soluble compounds in the stomach.
Figure 1.2. β-Carotene and retinol metabolism—an overview.

PTL- Pancreatic triglyceride lipase; ROL- Retinol; RAL- Retinal; BC- β-Carotene; RE- Retinyl esters; BOX- β-Carotene oxygenase; REH- Retinyl ester hydrolase; RBP- Retinol binding protein; CRBP II- Cellular retinol binding protein type II; LRAT- Lecithin: retinol acyltransferase.

Digestion begins in the oral cavity by mechanical action and lubrication with saliva before entering the stomach. Hydrochloric acid, pepsin and gastric lipase are secreted into the gastric lumen and mixed with the ingested foods, resulting in partial release of the carotenoids from the food matrix into the emulsified oil droplets. Once formed, the mixed micelles diffuse across the unstirred water layer and deliver carotenoids and other fat-soluble compounds to the apical surface of the mucosal epithelium. Apolar carotenoids such as β-carotene reside in the core of lipid droplets, whereas polar carotenoids are preferentially distributed at the surface (Borel et al. 1996). The lipid droplets are exposed to pancreatic enzymes and bile during transit from the stomach to the small intestine. The retinyl esters are efficiently hydrolyzed by pancreatic triacylglycerol lipase and possibly by pancreatic lipase-related proteins. Free retinol and the medium and long chain fatty acids produced by the enzymatic cleavage, as well as
residual retinyl esters, partition into mixed micelles (Borel et al. 2001; Harrison and Hussain, 2001; Breithaupt et al. 2002).

The transfer of carotenoids from micelles to the apical surface of epithelial cell, lining the small intestine, is generally assumed to occur by passive diffusion (Parker, 1996; Furr and Clark, 1997). A recent study examining lycopene absorption by humans suggested that the absorption of this carotenoid is saturable (Diwadkar-Navsariwala et al. 2003). Similarly, β-carotene uptake by intestinal cells has been reported to be saturable. These observations suggest that carotenoids, like cholesterol and fatty acids, may be transported across the brush border membrane by a facilitated process (Schaffer, 2002; Davis et al. 2004). Once taken into the enterocyte, β-carotene can be converted to retinol by the action of β-carotene cleavage enzymes (oxygenases). Retinal formed from β-carotene is reduced to retinol by the action of retinal reductase. The mechanism for this cleavage reaction remained controversial for many years, because attempts to purify the enzyme to homogeneity were unsuccessful and both retinal and apo-5-carotenals were identified as products in vitro and in vivo (Olson, 1961; Wang et al. 1991). In the mucosa, the resulting retinol is re-esterified and further incorporated into chylomicrons together with triacylglycerols. The enzymes responsible for the esterification of retinol in enterocytes are acyl coenzyme A: retinol acyltransferase (ARAT) and lecithin: retinol acyltransferase (LRAT). It has been suggested that LRAT requires the binding of cellular retinol binding protein type II (CRBP II) to retinol for the esterification reaction, whereas, ARAT esterifies free retinol (MacDonald and Ong, 1988). Therefore, it has been suggested that esterification via LRAT occurs during the absorption of a normal load of retinol, while ARAT seems to be involved in esterification when a large amount of retinol is ingested and CRBP II becomes saturated.

Transport and storage

Carotenoids released and retinyl esters synthesized after cleavage of provitamin A carotenoids are incorporated into nascent chylomicrons in the golgi of enterocytes (Parker, 1996). Conversion of chylomicrons to remnants is associated with uptake of the particles by liver, where the carotenoids may be utilized, stored or re-secreted into plasma associated with very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL). Relatively high concentrations of provitamin A carotenoids are
commonly found in tissues expressing a high density of LDL receptors such as adipose and adrenal tissues, liver, testis and ovary (Olson, 1999). Although carotenoids are also present in HDL, it is not known if these lipoprotein particles donate carotenoids to extra hepatic cells.

After esterification, retinyl esters are packaged with lipid and other fat-soluble vitamins into chylomicrons, which are transported via the thoracic lymph duct into the general circulation and to the liver for metabolism and storage. In circulation, chylomicrons undergo lipolysis, catalyzed by lipoprotein lipase, resulting in chylomicron remnants (Blaner et al. 1994). The liver takes up most of chylomicron retinyl esters, although many extra hepatic tissues, such as adipose tissue, kidney, testes, lungs and bone marrow, also can take up and store vitamin A. It is estimated that more than 90% of the body's vitamin A is stored in the liver (Basu, 1996). In liver, two different types of cells are involved in vitamin A storage and metabolism: the parenchymal cells (predominant cells of the liver) and the stellate cells (fat storing cells). Parenchymal cells, which contain 90% of all the proteins present in the liver, are directly involved in the uptake of chylomicron remnants and in the synthesis and secretion of retinol binding protein (RBP) (Blaner et al. 1994). The much smaller and less abundant stellate cells, which account for ~6-8% of all cells, are the major storage cells for retinyl esters. The uptake of the chylomicron remnants by the liver requires the participation of a cell surface receptor that recognizes the apolipoproteins (apoB and apoE) of the chylomicron remnants (Mahley et al. 1991), followed by rapid hydrolysis of the remnants by retinyl ester hydrolases (Harrison and Gad, 1989).

Chylomicron retinyl esters must first be hydrolyzed to retinol prior to being transferred to stellate cells. The resulting retinol binds to RBP and it has been reported that the relative proportion of newly absorbed vitamin A being either secreted into the circulation for delivery to the tissues or transferred to the stellate cells for storage depending on the nutritional status (retinol) of the animals (Olson, 1987). It has been shown that about 90% of the hepatic vitamin A is present in the stellate cells mainly as retinyl esters (Troen et al. 1994). Two enzymes similar to those found in the enterocytes, ARAT and LRAT have been described in the esterification of retinol in the stellate cells and it is thought that the esterification is regulated by vitamin A status (Randolph et al. 1991). The activity of LRAT appears to be predominant when retinol and fatty acyl CoA
are present at normal physiological concentrations, whereas ARAT activity is higher if the hepatic concentrations of retinol or fatty acyl CoA are markedly increased.

When required, the stored retinyl esters in the liver are first hydrolyzed and released as retinol (alcohol form). Unlike the hydrolysis of retinyl esters in chylomicron remnants, the hydrolysis of retinyl esters in stellate cells takes place with the bile salt-dependent retinyl ester hydrolases (Blaner et al. 1994). Following hydrolysis, the free retinol is subsequently conjugated with RBP and secreted as RBP-retinol complex into the circulation. The formation of a complex between retinol and RBP may solubilize the retinol in serum and protect the retinol against oxidative damage. Further, binding of RBP-retinol complex to transthyretin (TTR) serves to stabilize the association of retinol with RBP and also prevents glomerular filtration and renders catabolism of RBP (Goodman, 1984). It is estimated that ~95% of plasma retinol are present as TTR-RBP-retinol complex, 4.4% as RBP-retinol complex and only 0.1% as unbound retinol (Blomhoff et al. 1991).

Circulatory levels of retinol remain constant despite normal fluctuation in daily intake. In situations of inadequate intake of vitamin A, its hepatic stores are drawn up in order to maintain a constant relative circulatory level, until the stores are nearly exhausted. In the case of prolonged excessive intake of vitamin A, the hepatic storage capacity exceeds its limits, retinyl ester but not retinol is secreted into the circulation in association with lipoprotein (Smith et al. 1976). Retinyl esters transported by lipoprotein are available to the cell membranes, which may lead to toxicity and damage of these membranes (Goodman and Blaner, 1984).

**Cellular uptake and mechanism of β-carotene cleavage**

Several recent studies have clarified the mechanisms of β-carotene cleavage. The pathways of β-carotene cleavage are shown in Figure 1.3. Barua and Olson (2000) demonstrated that central cleavage of dietary β-carotene is the predominant mechanism for retinaldehyde formation in rats. In addition, two distinct recombinant cleavage enzymes have been purified and characterized, namely β-carotene 15,15′-monooxygenase (BCO1) and β-carotene 9′,10′-monooxygenase (BCO2) (Wyss, 2004; Von Lintig and Vogt, 2004). BCO1 is a non-heme iron enzyme located in the cytoplasm that catalyzes central cleavage at the 15,15′-double bond of β-carotene, α-carotene, β-
apo-carotenols and β-apo-carotenals (Lakshmanan et al. 1968 & 1972) to generate one or two molecules of retinol. In contrast, BCO2 catalyzes the eccentric cleavage of β-carotene to β-apo-carotenals and β-ionone. Each apo-carotenal molecule can be subsequently converted to a single molecule of retinaldehyde or the corresponding β-apo-carotenonic acid, which may serve as precursors for retinoic acid (Napoli and Race, 1988; Wang et al. 1991).

The expression of the BCO genes in many tissues has led to speculation that localized synthesis of retinoids is important, especially during periods when dietary intake of provitamin A carotenoids and retinyl esters is inadequate. While the expression of BCO2 appears to be constitutive, BCO1 activity is subject to regulation by retinol and other nutrients. Intestinal, but not hepatic, activity is increased in retinol deficient rats and decreased in response to dietary supplementation with β-carotene, retinyl acetate, apo-8'-carotenal and all-trans- and 9-cis-retinoic acid (Villard and Bates, 1986; Van Vliet et al. 1996; Parvin and Sivakumar, 2000; Bachmann et al. 2002;). Likewise, Lemke et al. (2003) found that retinol supplementation was associated with a decreased ratio of 14C-retinyl ester to 14C-β-carotene in the plasma of two human subjects; surprisingly, apparent absorption of the administered dose of 14C-β-carotene actually increased in response to supplementation. Intestinal activity of BCO1 was also decreased in rats fed diets deficient in either protein or iron (During et al. 2000; Parvin and Sivakumar, 2000). In contrast, BCO1 activity was increased in the intestines of rats fed diets rich in unsaturated fatty acids (During et al. 1998). The mechanisms responsible for such diet-mediated changes merit investigation. Oxidized products of dietary carotenoids have been identified in plasma and several tissues (Nagao, 2004). The possibility of enzymatic generation of these oxidized products are supported by reports that high intake of carotenoids increases expression of several cytochrome P450 proteins (Jewell and O’Brien, 1999). The tissue sites at which the oxidation reactions occur and the physiological activities that may be modulated by the metabolites formed in such reactions remain largely unknown.
Target cells without a concomitant uptake of either RBP or TTR, take up circulatory retinol. However, the underlying mechanism by which target cells take up retinol from plasma still remains to be elucidated. Goodman (1984) hypothesized that; the cellular uptake of retinol is mediated by a specific cell membrane receptor that recognizes the RBP but not retinol. Using cultured human retinal pigment epithelial cells (RPE), a study demonstrated that the specific binding of $[^{125}]$-RBP occurred at the apical surface of these cells and RPE cells specifically took up $[^3]$-retinol from $[^3]$-retinol-RBP (Pfeffer et al. 1986). It has also been suggested that retinol is delivered to the membrane receptor by RBP rather than RBP-TTR. TTR has been shown to inhibit the binding of the retinol-RBP complex and reduce the transfer of retinol to cells (Sivaprasadaraao et al. 1988a & 1998b). To date, RBP receptors have been identified in RPE, hepatic
parenchymal and stellate cells, testes, brain barriers, placental brush border membranes and keratinocytes. Once retinol enters a target cell, it is bound by the intracellular retinol-binding protein (CRBP), which does not cross-react immunologically with RBP (Olson, 1991). To date, many other intracellular vitamin A binding proteins, intracellular retinol-binding protein II (CRBP II), intracellular retinoic acid-binding protein (CRABP), intracellular retinaldehyde-binding protein (CRALBP) and interphotoreceptor retinoid-binding protein (IRBP), have been identified in various tissues. It is believed that they facilitate the transfer of specific forms of vitamin A to the nucleus of the cells, where they can participate in the regulation of gene expression for controlling cell differentiation and growth (Chytil and Ong, 1987).

**Biotransformation and excretion**

The absorption of carotenoids from foods is incomplete. It has been assumed that urinary losses of carotenoids are extremely low. Studies showed that human subjects excreted 25-30% of $^{14}$C from a tracer dose of orally administered $^{14}$C-β-carotene in urine within 72 hours (Bowen et al. 1993; Lemke et al. 2003). In addition, very small quantities of endogenous carotenoids are lost by exfoliation of skin and low concentrations of carotenoids have been identified in bile (Leo et al. 1995). Thus, fecal excretion represents the primary route of elimination from the body. Although several groups have reported that β-carotene is stable during *in vitro* incubation with intestinal aspirates or homogenized stools from rats or humans (Tang et al. 1996; Grolier et al. 1998), it is unknown if the lack of modification of the carotenoid was due in part to its introduction to the mixture in either water-dispersible beadlets or ethanol instead of a partially digested food matrix.

The biologically active form of vitamin A is all-trans retinol, which could be further oxidized to retinal by nonspecific retinol dehydrogenases, present in many tissues including the eye, liver and intestine (Ganguly, 1989). This reaction in the eye provides retinal for rhodopsin regeneration and in the liver; retinal could produce retinoic acid by aldehyde dehydrogenase or xanthine oxidase, through a further irreversible step. Most oxidized products are either excreted in the urine or conjugated with glucuronic acid in the liver. The newly formed glucuronides are excreted in the bile and may be partially reabsorbed in the intestinal tract while the rest is excreted in the faeces. It is estimated
that 10 to 20% of the dietary vitamin A is not absorbed. Of the remaining amount, 20% appears in the faeces through the bile, 17% is secreted in the urine, 3% is secreted as CO₂ and 40 to 50% is stored in the liver (Olson, 1994).

**Vitamin A homeostasis and regulation**

It has been demonstrated that the plasma retinol level is maintained within a normal range of concentrations as long as there is some minimal level of vitamin A in the liver. Only in the conditions of severe hypo- and hypervitaminosis A, do plasma levels of vitamin A correlate with its liver storage (Olson, 1984). When the liver's reserve is depleted (<20 µg/g liver), plasma retinol level fall rapidly and when it exceeds the limit of 300 µg/g, the homeostatic control over plasma vitamin A concentration is altered. A hypothetical relationship between plasma vitamin A level and hepatic concentrations of vitamin A (Olson, 1987) is shown in Figure 1.4.

![Figure 1.4](image)

Figure 1.4. Hypothetical relationships between mean plasma and liver vitamin A concentrations.

A number of endogenous and exogenous factors have been identified that could alter the homeostasis of vitamin A. These include dietary (vitamin A, protein, zinc and fat) and health (kidney and liver dysfunction as well as stress and hormone status) factors. Plasma vitamin A levels remain constant over a wide range of dietary intake,
except in a state of severe deficiency or toxicity. Secretion of RBP from the liver is strictly regulated by the availability of its ligand, retinol. In VAD, the secretion of RBP from the hepatocytes is specifically inhibited, leading to decreased plasma RBP levels and its accumulation in the liver (Muto et al. 1972). Administration of large doses of vitamin A results in an increased serum concentration of retinyl esters, whereas, retinol levels remains unchanged (Smith et al. 1976). Under normal conditions, the retinyl esters content is ~5-8% that of plasma retinol, while a massive overdose gives rise to more than twice as much retinyl esters as retinol (Ellis et al. 1986). Retinyl esters are thought to transport by lipoproteins that are more readily taken up by cell membranes. Thus the increased levels of retinyl esters in the plasma may lead to the damage of cell membranes and clinical signs of vitamin A toxicity.

Cumulative evidences have demonstrated the importance of zinc in the absorption and metabolism of vitamin A. Zinc deficiency in both animals and humans has been found to be associated with low plasma retinol levels. Zinc supplementation restores the level of plasma retinol to normal (Smith, 1980). Zinc is essential for enzymes, which are directly or indirectly involved in vitamin A metabolism. Kimball et al. (1995) have investigated the role of zinc on RBP and TTR synthesis in the liver of rats. A link between vitamin A and zinc has been further supported by the fact that both RBP and TTR have been found to decrease in plasma and liver during zinc deficiency (Smith et al. 1980; Bates and McClain, 1981).

Protein and lipid status are reported to affect the vitamin A homeostasis. Dietary protein is essential to maintain vitamin A homeostasis. Patients with protein-energy malnutrition (PEM) are thus associated with a decrease in plasma RBP, TTR and retinol concentrations (Goodman, 1984). This may be due to a decreased synthesis of RBP and TTR in the liver. Treatment of PEM with protein, but without dietary vitamin A supplementations, increased the concentration of RBP, TTR and retinol in plasma. These findings suggest that a low level of plasma retinol in PEM patients is largely a reflection of functional impairment in the hepatic release of retinol. Thus, RBP and TTR are sensitive markers of PEM status (Wade et al. 1988; Polberger et al. 1990).

Vitamin A is a fat-soluble vitamin. Before absorption it requires solubilization into a micellar solution. Therefore, when a diet is low in fat or an obstruction of the bile
secretion occurs, the absorption of vitamin A is impaired. When diets contain fat at levels below 5 g/day, the absorption of vitamin A is markedly reduced (Olson, 1987).

**Hypervitaminosis A and its toxicity**

Higher levels of stored vitamin A in the body leads to toxic symptoms. Toxicity can result in dry and itchy skin, headache, fatigue, hair loss, loss of appetite, vomiting, and liver damage. Signs of toxicity include dizziness, blurred vision and lack of muscular coordination. Most cases of vitamin A toxicity result from an excess intake of vitamin A as supplements. Safe upper limit of intake for vitamin A from diet and supplements combined is 8000 to 10000 IU/day. Vitamin A toxicity also can cause severe birth defects. Women of childbearing age are advised to limit their total daily intake of vitamin A from foods and supplements combined to, not more than 8000 IU/day.

**Assessment of vitamin A status**

Assessment of vitamin A status in the population groups is a prerequisite for successful prevention and control of VAD disorders. Vitamin A status is assessed by the total body content of vitamin A, which can be viewed as a continuum from deficiency to excess, with obvious health consequences at either extreme. The extremes are marked by specific indicators, for example in the case of severe depletion, by ocular signs (xerophthalmia, including night blindness) and very low serum retinol levels (<0.35 mmol/L). This is a stage referred to as subclinical depletion of body stores that defines VAD and is thought to be the beginning of an increased occurrence in the severity of infectious illness and risk of death. Monitoring the prevalence of disease severity and deaths is too non-specific to be attributable to a single nutrient and unfortunately, currently available biological indicators lack specificity and sensitivity for identifying subclinically depleted body stores. This has necessitated using prevalence values below arbitrary cut-offs specific for different vitamin A indicators. Blood retinol levels are the most commonly measured indicator of vitamin A status in surveys and using a cut-off of 0.7 mmol/L, a prevalence >10% has been set as defining a public health problem (WHO, 1996).
Chapter 1. General introduction

Vitamin A deficiency

Vitamin A is needed for the proper functioning of a number of biological processes, such as vision, reproduction, cellular growth and differentiation, embryonic development and immune response (Napoli, 1996). Humans lack the ability to synthesize vitamin A and hence, dependent on dietary intake to provide adequate level of this vitamin. VAD occurs when body stores are exhausted and supply fails to meet the body's requirements, either because there is a dietary insufficiency, requirements are increased or intestinal absorption, transport and metabolism are impaired as a result of conditions such as diarrhoea. The requirement of vitamin A to maintain the normal physiological activity is given in the Table 1.3.

VAD is one of the various nutritional disorders as a consequence of inadequate intake of vitamin A and provitamin A containing foods with low fat content together with high prevalence of infectious diseases. VAD is receiving increasing attention in recent years, since it is the major cause of blindness in several parts of the world. The relationship of night blindness to a dietary deficiency was recognized as early as 1500 B.C. The discovery of vitamin A is generally attributed to McCollum, although Osborne and Mendel (1913) and McCollum and Davis (1913) isolated a fat soluble growth factor which subsequently proved to be vitamin A. McCollum and Davis (1913) reported that the presence of a lipid like substance in butter and egg yolk necessary for growth in rats and the substance named as fat-soluble A. McCollum related the fat-soluble A deficiency to xerophthalmia in children in the following year, providing the first indication of the diverse functionality of the vitamin. McCollum and Simmonds (1917) reported that deficiency of vitamin A caused rats to develop eye lesions known as xerophthalmia. The disease had been described in Japanese infants in 1904 and an outbreak among children in Denmark occurred in 1917. In each of the human occurrences, the disease had been attributed to a scarcity of food fats. The name, vitamin A, was first used in 1920 to signify the early discovery of the growth factor and to differentiate it from water-soluble vitamins, collectively called as vitamin B at that time. The structure of the vitamin A was determined in 1931 (Karrer et al.).
### Table 1.3. Estimated dietary vitamin A requirements. a

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Retinol equivalent (µg RE)</th>
<th>International units (IU) as retinal b</th>
<th>International units (IU) as carotene b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal requirements a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>180</td>
<td>600</td>
<td>1,800</td>
</tr>
<tr>
<td>1-6</td>
<td>200</td>
<td>650</td>
<td>2,000</td>
</tr>
<tr>
<td>Normative requirements a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>350</td>
<td>1,200</td>
<td>3,500</td>
</tr>
<tr>
<td>1-6</td>
<td>400</td>
<td>1,300</td>
<td>4,000</td>
</tr>
</tbody>
</table>

a FAO/WHO (1988); **basal requirement** is an estimate of the amount needed to prevent signs of impaired function. **Normative requirement** is judged sufficient to maintain desirable levels of tissue stores. All estimates include allowance for individual variability of requirement.

b These estimates assume that all dietary vitamin A is in the form of retinol (1 µg RE = 3.3 IU) or β-carotene (1 µg RE = 10 IU). For other carotenoids the conversion is lower (1 µg RE = 20 IU).

*Note:* The IU has been largely abandoned in favour of either RE or molar units because of confusion in interpretation of the IU and in keeping with SI rules.

VAD is characterized by changes in the tissue of the eye that ultimately result in irreversible blindness. Night blindness or impaired dark adaptation is the first functional manifestation of VAD that can be measured clinically (WHO, 1996). Primary VAD is usually caused by prolonged dietary deprivation. It is endemic in areas, such as southern and eastern Asia, where rice, devoid of carotene, is the staple food. Secondary VAD may be due to inadequate conversion of carotene to vitamin A or interference with absorption, storage, or transport of vitamin A. VAD is common in protein-energy malnutrition (marasmus or kwashiorkor), principally because the diet is deficient with vitamin A and also due to defective vitamin A storage and transport. It is a leading cause of childhood blindness in the developing world affecting over 120 million children worldwide. In countries where immunization programs are not widespread and VAD is common, millions of children die each year from complications of infectious disease such as measles.

There are 2 different stages of VAD based on its severity namely, 1) Subclinical VAD, characterized by reduced vitamin A stores with increased deficiency resulting in a
lower level of serum vitamin A and metaplasia. 2) Clinical VAD is characterized by xerophthalmia leading to blindness. Subclinical vitamin A status can be assessed based on the relative dose-response test, serum retinol, retinal-rod function, conjunctival impression cytology (CIC) and clinical VAD is assessed by night blindness, conjunctival and corneal eye signs. The term 'subclinical' implies that there is no clinical evidence of disease. Some indicators of vitamin A status span this borderline between subclinical and clinical as impairment of function and abnormality of structure increase progressively with increasing degree of deficiency (Table 1.4).

Table 1.4. Increasing impairment of retinal rod function and progressive changes in conjunctiva and cornea.

<table>
<thead>
<tr>
<th>Test</th>
<th>Abnormal appearance/ response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subclinical</strong></td>
<td></td>
</tr>
<tr>
<td>Conjunctival impression cytology (CIC)</td>
<td>In epithelial and goblet cells</td>
</tr>
<tr>
<td><strong>Test for retinal rod function</strong></td>
<td></td>
</tr>
<tr>
<td>Dark adaptometry</td>
<td>Abnormal final rod threshold</td>
</tr>
<tr>
<td>Vision restoration</td>
<td>Delayed response after bleaching</td>
</tr>
<tr>
<td>Pupillary contraction</td>
<td>Failure of contraction in low illumination</td>
</tr>
<tr>
<td>Night blindness (Clinical)</td>
<td>Subjective impairment of vision in low illumination</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
</tr>
<tr>
<td>Conjunctival xerosis (X1A)</td>
<td>Dryness</td>
</tr>
<tr>
<td>Bitot’s spot (X1B)</td>
<td>Foamy, cheesy heaping up of keratinized epithelial cells in interpalpebral fissure</td>
</tr>
<tr>
<td>Corneal xerosis (X2)</td>
<td>Hazy cornea</td>
</tr>
<tr>
<td>Keratomalacia (X3)</td>
<td>Liquefaction of part or all of cornea</td>
</tr>
</tbody>
</table>

**Xerophthalmia**

The term xerophthalmia literally means ‘dry eye’. However, dryness or xerosis, which also affects other parts of the body, is only part of the abnormal process undergone by the eye in VAD. Xerosis is confined to the epithelial structures of the eye, the conjunctiva and the cornea. In addition, the cornea undergoes other changes, known as keratomalacia.
Although many countries have not been able to assess the true level of deficiency due to technical and financial constraints, the WHO estimates that 100 to 140 million children under the age of five may be living with dangerously low vitamin A stores. More than 4 million children worldwide exhibit signs of severe deficiency. The greatest burden of deficiency is among children and women living in South Asia and Sub-Saharan Africa. VAD continues to be a major public health problem that affects more than 100 million children and as many as 7 million pregnant women residing in more than 100 countries (West, 2002; WHO, 2003).

**Prevalence of VAD**

VAD affects about 40% of the global population mainly children, pregnant and lactating women, because their demand of vitamin A is higher. VAD increases the susceptibility towards other diseases and can cause irreversible blindness (Lorch, 2005). In Nepal, pregnant women with chronic moderate-to-severe VAD were shown to be at increased risk of infection and mortality during pregnancy and up to one-year postpartum (Christian et al. 2000). It is estimated that there are 1.4 million blind children in the world, two thirds of whom live in the developing countries and that the cause of blindness vary according to region and socioeconomic development (Gilbert et al. 1999; WHO, 2000). Prevalence of VAD at various levels among the countries of the world is shown in Figure 1.5.

![Figure 1.5. Prevalence of vitamin A deficiency (WHO, 2000).](image-url)
Chapter 1. General Introduction

VAD status in India

VAD is recognized as a serious health problem in India. Though, Government of India has made a remarkable progress since 1976 in the elimination of blindness due to VAD, a report from 16 districts demonstrated prevalence of VAD (1.03% night blindness) among children aged 24-71 months (Feldon et al. 2005). Kapil and Sachdev (2001) have substantiated the recommendations of the scientists that VAD continues to be a public health problem in some geographical regions of India (Table 1.5).

Table 1.5. Vitamin A deficiency status of children in different states of India.

<table>
<thead>
<tr>
<th>State</th>
<th>District</th>
<th>No. of children assessed</th>
<th>No. (%) children with vitamin A deficiency</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maharashtra</td>
<td>Nanded (6/3)</td>
<td>368</td>
<td>4 (1.1)</td>
<td>4 Bitot’s spots (X1B)</td>
</tr>
<tr>
<td>Assam</td>
<td>Goalpara, Dhubri, Guwahati (8/3)</td>
<td>838</td>
<td>16 (1.9)</td>
<td>1 corneal xerosis (X2), 15 Bitot’s spots (X1B)</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>Farrukhabad (11/3)</td>
<td>112</td>
<td>16 (14.3)</td>
<td>16 Bitot’s spots (X1B)</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>Badaun (1/4)</td>
<td>140</td>
<td>16 (11.4)</td>
<td>13 Bitot’s spots (X1B), 3 night blindness (XN)</td>
</tr>
<tr>
<td>Karnataka</td>
<td>Bellary, Bagalkot (2/4)</td>
<td>398</td>
<td>11 (2.8)</td>
<td>11 Bitot’s spots (X1B)</td>
</tr>
<tr>
<td>Jharkhand</td>
<td>Ranchi (7/4)</td>
<td>150</td>
<td>16 (10.7)</td>
<td>16 Bitot’s spots (X1B)</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>Badaun (8/4)</td>
<td>664</td>
<td>21 (3.2)</td>
<td>1 corneal xerosis (X2), 20 Bitot’s spots (X1B)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 corneal xerosis (X2), 1 corneal xan (X8)</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>Bareilly (10/4)</td>
<td>485</td>
<td>8 (1.6)</td>
<td>8 Bitot’s spots (X1B)</td>
</tr>
<tr>
<td>Bihar</td>
<td>Khagaria (11/4)</td>
<td>235</td>
<td>17 (7.2)</td>
<td>17 Bitot’s spots (X1B)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3,390</td>
<td>125 (3.7)</td>
<td></td>
</tr>
</tbody>
</table>


Prevention and control of VAD

As a result of the worldwide prevalence of VAD among children, the WHO and the United Nations International Children's Emergency Fund (UNICEF) recommended vitamin A administration to all children diagnosed with measles. In 1994, the American Academy of Pediatrics recommended vitamin A supplementation for children of 6-24 months of age hospitalized with measles and for hospitalized children older than 6 months who are considered to be at high-risk for sub clinical VAD (Butler et al. 1993). In partnership with bilateral donors, the WHO and UNICEF have been actively promoting programs to control VAD through the encouragement of both exclusive and prolonged breastfeeding, the distribution of pharmacological vitamin A supplements to children and
postpartum mothers, the fortification of food and the implementation of programs aimed at increasing home gardening so as to increase the availability of fruits and vegetables rich in provitamin A carotenoids (WHO, 2003). The control of childhood blindness is one of the priorities of WHO’s programme “vision 2020- the right to sight”. Over the past two decades, number of countries that have formally embraced vitamin A control programs (of varying degrees of development and implementation) has risen from less than 5 to over 65. Countries with very effective and committed programs include Indonesia, Vietnam, Bangladesh, India (to varying degrees in various states), Nepal and Guatemala, amongst others.

**Strategies to control VAD**

Programmes to control VAD reduce the severity of childhood illnesses, enhance a child's chance of survival and contribute to the well being of children. This can be achieved through periodic administration of large dose of vitamin A among target people, fortification of foods and by improving adequate intake of vitamin A in the diet. Three major deficiency control strategies currently exist, all meant to complement ongoing public health measures for the prevention and control of infectious diseases.

**Supplementation**

Current international recommendations call for high-dose vitamin A supplementation once in every 4-6 months, targeted to all children between the ages of 6-59 months living in affected areas. Providing young children with two high-dose vitamin A capsules a year is a safe, cost-effective, efficient strategy for eliminating VAD and improving child survival. Giving vitamin A to lactating mothers helps to protect their children during the first few months of life and replenish the mother's stores of retinol, which are depleted during pregnancy and lactation.

Countries carrying out two annual rounds of vitamin A supplementation reaching at least 70% coverage among children, 6-59 months, considered ‘effective coverage’ is on track to meet international development goals. Approaches like vitamin A capsules distribution and dietary diversification led to a successful control of VAD in Thailand (Wasantwisut, 2002). West and Sommer (1987) presented an in-depth analysis of the periodic mass-dose approach. They highlighted its potential effectiveness, but warned
that the efficiency of its operation still poses a major challenge. Further, they reported that a good management programme is required for successful implementation. Unfortunately, most developing countries lack the necessary administrative sophistication. Another limitation of this approach is that, it focuses only on one sector of the population, the so-called ‘high risk years’ which corresponds to the preschool age children. In view of the challenges to rapid and large-scale implementation of food-based interventions, supplementation is currently the primary strategy adopted to control VAD.

**Food fortification**

Affordable and cost effective strategies to ensure adequate vitamin A intakes are available. The easiest and most far-reaching programme is food fortification, the addition of vitamin A to appropriate foods and beverages. Food fortification is being introduced in many countries with great hope for long-term control of VAD. Products currently serving as vehicles for fortification are sugar, oil, milk, margarine, infant foods and various types of flour. Fortification of milk has successfully reduced VAD in many countries like United States, Argentina, Venezuela, The Philippines and Malaysia. Fortification of more than one staple food increases the odds of reaching a higher percentage of the population. Thus, in addition to milk, margarine is fortified with vitamin A in the United States and it is more common in Brazil, Canada, Chile, Mexico, India, The Netherlands, Portugal, Sweden, Turkey, Malaysia, Singapore, Indonesia and Taiwan.

In most cases, fortification can take several years to initiate and longer still to reach at-risk population. Programs to deliver fortified foods to those at risk have achieved much but such programs are not sustainable and dependent on continued corporate generosity. But weak market and functional health infrastructures in many developing countries have diminished the impacts of such initiatives.

**Dietary diversification**

Dietary sources of vitamin A include the provitamin A carotenoids (plant foods) and retinyl esters (animal foods). Vitamin A fortified foods (milk) and pharmaceutical supplements containing provitamin A are readily available in affluent countries. Thus, the provitamin A carotenoids in fruits and vegetables generally account for less than one-third of the total retinol intake in nutritionally diverse diets consumed in developed
countries. Increased consumption of carotenoid-rich plant foods has generally been accepted as an effective solution to improve the vitamin A status (Rodriquez-Amaya, 1997).

Although carotenoids can be supplemented to the diet, food based approaches to enhance carotenoid bioavailability deserve attention as they are relatively easy to achieve and more affordable than fortified foods; furthermore, they can increase the intake of additional health promoting nutrients (de Pee and West, 1996; Cooper, 2004). Non-animal sources of vitamin A account for greater than 80% of intake for most individuals in the developing countries to meet the vitamin A requirement of children. Feasible control of deficiency through dietary diversification would require increased consumption of vitamin A-rich foods of animal origin, coupled with continued promotion of nutritious fruits and vegetables. Multiple interventions to this effect have been carried out; however, scale-up of these efforts is limited by a lack of well-designed assessments to attest to their efficacy and effectiveness in reducing the burden of deficiency.

An overall strategy designed to prevent and control VAD may be defined in terms of action taken (short, medium and long term). Short-term or an emergency measure includes the administration of single, large doses of vitamin A to vulnerable groups on a periodic basis. In the medium-term, the fortification of a dietary vehicle (e.g. sugar or monosodium glutamate) with vitamin A can be initiated. Increased dietary intake of vitamin A through home gardening and nutrition education programs comprises the long-term solution to this problem.

**Green leafy vegetables (GLVs) as a source of \( \beta \)-carotene to overcome VAD**

From time immemorial, plant foods containing \( \beta \)-carotene have been a major dietary source of vitamin A in developing countries. GLVs contribute to about 80% of vitamin A requirement of food in the form of provitamin A carotenoids, of which \( \beta \)-carotene is the major component where the VAD is public health concern. To persuade increased consumption of vitamin A rich food materials by the community, it is essential to know the vitamin A value of agri/ horticultural produce, which may help the rural community to select right source of provitamin A rich foods to combat VAD (Suber et al. 1995; USDA-NCC, 1998). Hence, the vitamin A activity of provitamin A carotenoids in plant foods is very much essential since it varies depending on the geographical region
where they are cultivated and the type of cultivars. We have reported the carotenoid composition and vitamin A activity of GLVs, which are commonly consumed in India (Lakshminarayana et al. 2005; Raju et al. 2007). The data on carotenoid composition may help in identifying GLVs rich in specific carotenoids for supplementation purposes and for providing information to consumers and public health workers to assess the dietary carotenoid intake and their relationship between health and diseases.

**Efficacy of β-carotene in preventing VAD**

Increased consumption of carotenoid-rich plant foods has generally been accepted as an important long-term solution to improve vitamin A status. β-Carotene is a commonly consumed nutritional precursor of vitamin A and a natural antioxidant. Within the intestine, β-carotene can be cleaved to yield two molecules of vitamin A (Olson and Hayaishi, 1965). A large percentage of consumed carotene is absorbed intact, metabolized to vitamin A and other products of uncertain vitamin A value, or excreted (Parker, 1996). However, a number of factors may affect β-carotene absorption and vitamin A value, including the type and amount of dietary fat (Dimitrov et al. 1988; Van het Hof et al. 2000), competition among co-consumed carotenoids (Kostic et al. 1995), the plant or food matrix of incorporation (Van het Hof et al. 2000), and the extent of mucosal metabolism (Lampen et al. 2000). Determining the contribution of each factor to the vitamin A value is key to understanding the individual response. The importance of β-carotene as a primary vitamin A source in less-developed regions suggests that in these individuals, status may be an important factor (Solomons and Bulux, 1993). In Filipino children of low or marginal vitamin A stores, the bioconversion of plant carotenoids to vitamin A varied inversely with vitamin A status (Ribaya-Mercado et al. 2000). These findings were strengthened by the use of isotope dilution methods for the assessment of body stores rather than the measurement of circulating retinol concentrations (Furr et al. 1989). A molecular basis for the observed relationship is suggested in rat studies that report increased activity of the enzyme β-carotene 15,15'-monooxygenase, in intestinal homogenates of retinol deficient animals (Parvin and Sivakumar, 2000; Lindqvist and Andersson, 2002). Moreover, it has been observed that large dose of β-carotene does not result in vitamin A toxicity (Mathews-Roth, 1993), suggesting homeostatic control of bioconversion or saturation of the cleavage enzyme.
Provitamin A carotenoids such as $\beta$-carotene is generally considered safe because they are not associated with specific adverse health effects. The conversion of provitamin A carotenoids from plant foods to vitamin A slows down when body has adequate stores of vitamin A, so the levels are naturally limited. A high intake of provitamin A carotenoids (from food sources) can turn your skin yellow, but this is not considered dangerous to health. Although, the evidence on benefits and adverse effects of taking $\beta$-carotene supplements is contradictory and the potential health risks are not well understood. The lung cancer trial suggested a greater incidence of lung cancer and total mortality in smokers who supplemented their diet with 20 mg of $\beta$-carotene per day (ATBC, 1994). But the Physicians' Health Study compared the effects of taking 50 mg of $\beta$-carotene every other day to a placebo (sugar pill) in 22,000 male physicians and found no adverse health effects.

The major drawback of using plant sources to meet vitamin A requirement is their poor bioavailability. For subjects dependent on provitamin A carotenoids for their vitamin A, intake and maximum absorption with maximum conversion is important. Hence, an interest in carotenoid metabolism especially the factors determining the absorption and intestinal cleavage of provitamin A carotenoids into vitamin A has increased strongly.

**Factors affecting the bioavailability of carotenoids**

Bioavailability is defined as the fraction of an ingested nutrient that becomes available to the body for utilization in physiological functions or for storage (Jackson, 1997). It depends on several dietary and non-dietary factors including the level and origin of dietary fat, the amount and type or mixture of carotenoids, the digestibility of food, the presence of antioxidants or fibers, as well as vitamin A status (Erdman et al. 1993). The factors that influence the bioavailability of carotenoids are often summarized in the mnemonic ‘SLAMENGH!, which means ‘S’pecies of carotenoid, molecular ‘L’inkage, ‘A’mount consumed in a meal, ‘M’atrix in which the carotenoid is incorporated, ‘E’ffectors of absorption and bioconversion, ‘N’utrient status of the host, ‘G’enetic factors, ‘H’ost-related factors and ‘I’nteractions (de Pee and West, 1996; Castenmiller and West, 1998; Van den Berg et al. 2000; Borel, 2003).
Chemical speciation, food matrix and processing

Absorption of dietary provitamin A carotenoids is influenced by numerous factors in addition to the amount ingested. The physicochemical properties of the carotenoid of interest, its sub-cellular location in the plant tissue that constitutes the food, the method of food preparation and the chemical composition of the meal may affect its bioavailability. Lutein was absorbed more efficiently than β-carotene when administered in oil to human subjects (Castenmiller et al. 1999; Van het Hof et al. 1999). β-Carotene bioavailability has been reported to be influenced by the food matrix, with absorption from carrots > broccoli > spinach (Micozzi et al. 1992; Castenmiller et al. 1999). These observations suggest that carotenoid bioavailability is affected by both chemical speciation and food matrix. However, interpretation is confounded by a lack of information about the extent of β-carotene conversion, potential interactions between carotenoids during digestion and uptake and transport across the mucosal epithelium and the rates of plasma clearance for individual carotenoids.

Food processing methods like, moderate cooking, mashing and juicing destroys plant tissue structure thereby increasing surface area and interactions of hydrolytic enzymes and emulsifiers with food particles during the gastric and small intestinal phases of digestion, which increases the carotenoid bioavailability (Edwards et al. 2002; Livny et al. 2003). Processing may also induce conversion of the all-trans isomers of some carotenoids to cis isomers.

Dietary factors

Quantity and type of fat

Dietary fat increases carotenoid bioavailability by providing a depot for hydrophobic compounds released from the food matrix, stimulating the secretion of bile salts and pancreatic lipases required for micelle formation and inducing chylomicron synthesis (Borel, 2003). Approximately 5-10 g fat in a meal is required for efficient absorption of carotenoids (Reddy and Mohanram, 1980), although a greater amount of fat is required when the dietary source is lutein ester instead of free lutein (Roodenberg et al. 2000). The type of fat may also affect carotenoid absorption. For example, absorption of carotenoids (lycopene and astaxanthin) was more efficient when administered to rats in olive oil than in corn oil (Clark et al. 2000). Similarly, the presence
of unsaturated fatty acids, particularly oleic acid, in micelles stimulated β-carotene absorption from the perfused rat intestine (Hollander and Ruble, 1978). Hu et al. (2000) reported that the efficiency of β-carotene absorption by human subjects increased when the meal was rich in sunflower oil compared with beef tallow. The type of fatty acids in mixed micelles modulated the β-carotene uptake and its cleavage into retinol in rats (Raju et al. 2006). Dietary triacylglycerols with long-chain rather than medium-chain fatty acids enhanced the absorption of β-carotene and retinyl palmitate (Borel et al. 1998b). The potential for phospholipids to affect carotenoid bioavailability is supported by the observation that lysophosphatidylcholine stimulated carotenoid absorption in mice (Baskaran et al. 2003).

**Fiber**

The water-soluble fibers such as, pectin, guar and alginate decrease the absorption of β-carotene, lycopene and lutein (Rock and Swendseid, 1992; Riedl et al. 1999). Possible mechanisms responsible for the fiber-mediated decrease in carotenoid bioavailability include decreased micellarization due to binding of bile acids and phospholipids, inhibition of lipase activity, increased viscosity and volume of luminal contents and increased rate of transit of enterocytes along the villus (Riedl et al. 1999).

**Interactions between carotenoids**

Food matrix containing mixture of carotenoids, affect their intestinal absorption depending on the type and the level of individual carotenoids. Micozzi et al. (1992) observed lowered serum response of lutein after a 6-week period of β-carotene supplementation. Kostic et al. (1995) reported that an interaction between lutein and β-carotene on intestinal absorption and serum clearance after oral administration of single equimolar doses of lutein and/or β-carotene in oil to adult human subjects. In another study with human subjects, lutein impaired β-carotene absorption, but did not affect the secretion of retinyl esters in chylomicra (Van den Berg and Van Vliet, 1998). In contrast, β-carotene absorption was not affected by lycopene in these subjects. More recently, Tyssandier et al. (2003) reported that the absorption of β-carotene, lutein and lycopene from a single vegetable was greater when the food was administered alone than when it was co-administered with either a second carotenoid-rich vegetable or the purified
carotenoid from the second vegetable. Possible sites for preabsorptive interactions between carotenoids include their competition for incorporation into micelles, uptake by intestinal cells, competitive binding to β-carotene 15,15’-monooxygenase and incorporation into chylomicrons (Van den Berg, 1999).

**Physiological factors**

_**Gut health**_

The absorption of dietary carotenoids and their bioactive products is also modulated by phenotypic characteristics of the host that affect processes associated with digestive and absorptive events. These include the composition and activity of luminal fluids and the morphological and functional integrity of the absorptive epithelium. Plasma response to a single dose of β-carotene was significantly lower in subjects administered omeprazole to increase gastric pH to the neutral range compared with the same subjects when gastric pH was acidic (Tang et al. 1996). In addition, cholestasis, pancreatic insufficiency, biliary cirrhosis, cystic fibrosis and other syndromes responsible for fat malabsorption decrease carotenoid bioavailability and can induce VAD, especially in children (Olson, 1999). Intestinal parasites can impair carotenoid metabolism. Absorption and utilization of β-carotene were enhanced after de-worming children infected with *Ascaris lumbricoides* (Jalal et al. 1998). In contrast, plasma retinol concentrations in helminth-infected preschool children in Ghana fed a stew with dark green cassava and kapok supplemented with fat and β-carotene was not further elevated by administration of antihelminthics (Takyi, 1999).

_**Nutritional status**_

Nutritional status can affect the bioavailability of provitamin A carotenoids. The plasma vitamin A response following the administration of β-carotene to protein deficient rats was decreased compared with control rats (Parvin and Sivakumar, 2000). This suppression was due to a decline in the activity of β-carotene 15,15’-monooxygenase. Because of the central role of retinoic acid in cellular differentiation, VAD compromises the integrity of epithelial barriers. Mild VAD reduced the number of duodenal goblet cells per villus and luminal mucus and decreased cellular division in the crypts of intestinal villi (McCullough et al. 1999). Gastrointestinal integrity, assessed by the dual-sugar
gastrointestinal permeability test, was markedly improved when retinol-deficient children in Gambian and India ingested β-carotene-rich mango and received vitamin A supplementation, respectively (Thurnham et al. 2000). Studies have reported decreased uptake of micellar β-carotene by brush border membrane vesicles isolated from retinol deficient Mongolian gerbils and rats compared with membrane preparations from animals fed vitamin A adequate diets (Moore et al. 1996; Boileau et al. 2000). It is unknown if the differences were due to immaturity of plasma membranes from donor cells or other biochemical alterations associated with dietary inadequacy. Decreased uptake of micellar β-carotene across the brush border membrane may offset the greater activity of β-carotene 15,15’-monooxygenase associated with VAD. Activity of this enzyme in the soluble fraction of homogenized intestinal mucosa was positively correlated with the iron content of the tissue prepared from rats fed diets with different quantities of the trace metal (During et al. 2000).

**Genotype**

Recent studies with tracer isotope techniques have confirmed earlier observations of a marked variability in the absorption of β-carotene by human subjects (Lin et al. 2000; Hickenbottom et al. 2002). Moreover, plasma β-carotene and retinol were not predictive for the absorption or conversion of β-carotene. These differences in absorption efficiency originally resulted in the classification of individuals as ‘responders’, ‘low responders’ and ‘non responders’. Explanations for the observed variation among healthy subjects tested under well-controlled conditions have included differences in the rate of cleavage of β-carotene to retinal, the efficiency of incorporation of the carotenoid into chylomicrons and the rate and extent of clearance from circulation (Borel, 2003). Lin et al. (2000) also suggested that differences in the ability to transfer the carotenoid from a complex matrix to the absorptive cell might be the basis for the reported variability, because all individuals were ‘responders’ when administered high doses of β-carotene in oil (Borel et al. 1998a).

Genetic factors are also likely to affect the efficiency of carotenoid absorption and conversion. Polymorphisms in genes whose products are required for the many reactions affecting the transfer of carotenoids from food matrix to micelles during digestion, assembly and secretion of chylomicrons and the kinetics of post absorptive
delivery of carotenoids and retinoids to tissues may contribute to the observed variations in the absorption and conversion efficiency of provitamin A carotenoids by individuals.

**Enzymes involved in β-carotene uptake and its metabolism**

**Pancreatic lipase (EC. 3.1.1.3)**

Pancreatic lipase, an enzyme (more specifically, a lipase) secreted from the pancreas that uses hydrolysis to break apart fat molecules. Bile salts secreted from the gallbladder are released into the duodenum where they coat fat droplets. Because the droplets are small, their surface area is greater, allowing the lipase to break apart the fat more effectively. The resulting monomers are then moved by way of peristalsis along the small intestine to be absorbed into the lymphatic system by a specialized vessel called a lacteal. The absorption of dietary long chain triglycerides requires their conversion to the more polar fatty acids and monoglycerides by lipolytic enzymes. The lipolytic products are dispersed into the aqueous phase of the intestinal fluid in the form of mixed micelles containing bile salts from which lipid absorption occurs.

**Phospholipases**

Phospholipases are a diverse series of enzymes designed for phospholipid catabolism. As such, they play critical roles in generating lipid-derived second messengers. They are classified according to the phospholipid ester bond hydrolyzed. Because many of these enzymes are water-soluble and their substrates are insoluble and organized in a 2-dimensional matrix, they have evolved unique strategies for carrying out and regulating catalysis at an interface.

**Phospholipase A₂ (EC. 3.1.1.4)**

Plays a crucial role in a number of diverse cellular responses, including phospholipid digestion and metabolism, host defense and signal transduction and they also provide precursors for eicosanoid generation (Six and Dennis, 2000). PLA₂ comprises a large group of enzymes that share the capacity to hydrolyze fatty acids from the sn-2 position of glycerophospholipids. It provides precursors for the generation of eicosanoids and platelet activating factor when the sn-1 position of the phosphatidylcholine contains an alkyl ether linkage and some bioactive
lysophospholipids, such as lysophosphatidic acid or lysophosphatidylcholine. Mammalian cells generally contain more than one PLA₂, each of which is regulated independently and exerts a distinct effect (Murakami et al. 1997). PLA₂ enzymes are found in all mammalian tissues including plasma and serum. The enzymes are generally classified into two large groups on the basis of their localization, namely, extracellular or secreted (sPLA₂) (Dennis et al. 1992) and intracellular or cytosolic (cPLA₂) (Six and Dennis, 2000). In mammalian extracellular fluids, several types of small (~14 kDa), calcium-dependent sPLA₂ enzymes have been characterized (Tischfield, 1997).

Studies also suggest that plasma lipoproteins could be the major source of PLA₂ in plasma or serum. The best-characterized lipoprotein-associated PLA₂ is the type VII enzyme found in plasma under normal physiological conditions, which was initially characterized as platelet-activating factor (PAF) acetylhydrolase (PAF-AH) and is also known as human plasma LDL-PLA₂ since it is associated with the LDL lipoproteins (Tjoelker et al. 1995; Tew et al. 1996). This enzyme is a single polypeptide containing a lipase consensus motif and is expressed in inflammatory cells, in particular macrophages or in tissues infiltrated with large numbers of inflammatory cells. In addition to its PAF-AH activity, this enzyme also hydrolyzes phosphatidylcholine with either oxidized or short chain fatty acids at the sn-2 position (Tew et al. 1996). An important characteristic of this enzyme is that it has no requirement for calcium.

Membrane bound enzymes

Every living cell has at least one membrane, which is selectively permeable to the surrounding media for maintaining a constant internal milieu that is important for life processes. Each cell membrane has an amphiphilic lipid bilayer with integrated proteins capable of moving laterally within the plane of the membrane indicating their dynamic nature. Such a motion is important for transduction of signals and maintaining the electrochemical gradient across the membrane.

Sufficient vitamin A in the diet maintains the epithelium of gastro-intestinal tract that in turn helped in maximum absorption of nutrients, resulting in improved weight gain, feed consumption and feed conversion efficiency (Raza et al. 1997). VAD causes hyperproliferation of enterocytes, a decrease in the number of goblet cells, decreased alkaline phosphatase activity and decreased expression of two brush-border enzymes
(Uni et al. 2000). Anderson et al. (1967) found that erythrocyte membranes of the rats were swollen and distorted in retinol deficiency. The absence of retinol interferes with the normal growth in chickens because it influences functionality of the small intestine by altering proliferation and maturation of cells in the small intestinal mucosa (Uni et al. 2000). Vitamin A improved appetite and growth of the birds and resulted in lower mortality (Raza et al. 1997). Retinol in vitro increased the permeability and fluidity of the natural and reconstituted membranes (Stilwell and Bryant, 1983). Mack et al. (1972) confirmed the presence of retinol in cell membranes and was essential for the normal functioning of rat kidney and plasma membranes.

Carotenoids decrease the fluidity of membrane, so their amounts control the stability of that parameter, affecting all membrane functions. The membrane reinforcing function of carotenoids is retained in mycoplasms, some fungi and animals.

**Sodium potassium activated adenosine triphosphatase (Na⁺ K⁺ ATPase, EC. 3.6.1.3)**

The Na⁺ K⁺ ATPase is a highly conserved integral membrane protein that is expressed virtually in all cells of higher organisms. The ionic transport conducted by Na pump creates both an electrical and chemical gradient across the plasma membrane. This is critical not only for that cell but, in many cases, for directional fluid and electrolyte movement across epithelial sheets. The Na⁺ K⁺ ATPase is composed of two subunits; the alpha subunit (~113 kDa) binds ATP and both Na⁺ and K⁺ ions and contains phosphorylation site and the smaller beta subunit (~35 kDa glycoprotein) is absolutely necessary for activity of the complex. It appears to be critical in facilitating the plasma membrane localization and activation of the alpha subunit. Several isoforms of both alpha and beta subunits have been identified, but aside from kinetic characterizations and tissue distribution, little is known regarding their differential physiologic importance. Cation transport occurs in a cycle of conformational changes apparently triggered by phosphorylation of the pump. As currently understood, the sequence of events can be summarized as follows: The pump, with bound ATP, binds 3 intracellular Na⁺ ions. ATP is hydrolyzed, leading to phosphorylation of a cytoplasmic loop of the pump and release of ADP. A conformational change in the pump exposes the Na⁺ ions to the outside, where they are released. The pump binds two extracellular K⁺ ions, leading somehow to
dephosphorylation of the alpha subunit. ATP binds and the pump reorients to release K+ ions inside the cell. The pump is ready to go again.

A gradient is generated when a Na⁺ K⁺ ATPase pump located in the basolateral membrane exchanges 3Na out of the enterocyte for 2K. Activation of the Na⁺, glucose transport protein allows water, electrolytes and possibly smaller molecules (including glucose and oligopeptides) to pass into the intercellular space through relaxation of the tight junctions. Ileal bile acid absorption involves Na co-transport down a gradient secured by the basolateral membrane Na⁺ K⁺ ATPase. A class of drugs, the cardiac glycoside e.g. ouabain inhibits Na⁺ K⁺ ATPase. Kaplay (1984) reported that the ouabain sensitive Na⁺ K⁺ ATPase is increased in erythrocyte membrane of children suffering from kwashiorkor and in the kidneys of protein energy malnourished rats. Growth retardation of nutritionally dwarfed patients is associated with decreased erythrocyte Na⁺ K⁺ ATPase activity, without other biochemical evidence of malnutrition (Lifshitz et al. 1991). The ubiquity of distribution and variety of functions attributed to ATPase suggests its basic importance in membrane function.

**β-Carotene 15,15'-dioxygenase (EC. 1.13.11.21)/ β-Carotene 15,15'-monooxygenase (EC. 1.14.99.36)**

Provitamin A carotenoids, when ingested in the diet, were reported to be cleaved by an intestinal 15,15'-dioxygenase to form retinal. Central cleavage of β-carotene seems to be most important metabolic pathway and enzyme activity has been detected in various tissues since its discovery in the mid 1950’s. Since then many attempts failed to purify and characterize this enzyme. Nevertheless and despite the lack of solid information regarding enzyme reaction mechanism, as well as the nature of the cofactor the enzyme was termed β-carotene 15,15'-dioxygenase (Olson and Hayashi, 1965). However, it is only in the last few years that the mammalian gene has been cloned (Redmond et al. 2001) and biochemical studies were carried out (Kiefer et al. 2002). It is now established that the central cleavage of β-carotene is, in fact, a monooxygenase type reaction. The recombinant human enzyme, called BCO, has been characterized and is highly expressed in non-digestive tissues where it may convert provitamin A carotenoids obtained from plasma into retinal (Lindquist and Andersson, 2002). Asymmetric cleavage of such carotenoids produces a range of apocarotenoids, by
cleavage of the 9,10'-double bond to yield \( \beta \)-ionone and \( \beta \)-apo-10'-carotenal, which may be a precursor of retinoic acid. Interestingly, this 9,10'-dioxygenase can also cleave acyclic carotenoids such as lycopene (Schwartz et al. 2003).

The intestinal mucosa plays a key role in the metabolism of provitamin A carotenoids such as \( \beta \)-carotene, thus greatly influences their bioavailability. Most recent evidence supports a central oxidation mechanism of cleavage of \( \beta \)-carotene to retinal in the intestinal mucosa, but the extent and site(s) of post absorptive metabolism in the human is unknown. While the human and other species clearly absorb non-provitamin A carotenoids, little is known of the extent and pathways of their metabolism and elimination. The enzyme is a cytosolic enzyme primarily localized in the duodenal mucosa although it has been found in liver. It is a 66 kDa sulfhydryl protein, requires molecular oxygen and is activated by ferrous ions. It is highly specific for 15,15' ethylenic bond of carotenoids although it has fairly broad specificity towards a number of carotenoids with at least one intact \( \beta \)-ionone ring.

Apocarotenoid oxygenases, cleaves \( \beta \)-carotene into retinoids (including retinol) in the liver and intestine and there are two pathways this enzyme being used. First, the \( \beta \)-carotene 15,15'-monooxygenase was cleaved at its 15,15'-double bond to generate two retinal molecules in order to convert into retinol. Yeum et al. (2000) confirmed that the retinal is the sole product of \( \beta \)-carotene cleavage in the presence of \( \alpha \)-tocopherol and that the observed formation of apocarotenoids occurs in the absence of an antioxidant like \( \alpha \)-tocopherol.

**Retinal reductase (EC. 1.1.1.105)**

During the intestinal absorption of dietary \( \beta \)-carotene, the carotene is largely converted to vitamin A and the newly synthesized vitamin A is then transported in the lymph mainly in the form of retinyl ester (Thompson et al. 1950; Wagner et al. 1960; Huang and Goodman DS et al. 1965; Goodman, 1966). The conversion of \( \beta \)-carotene into vitamin A involves, first, the central cleavage of \( \beta \)-carotene into two molecules of retinal (Olson and Hayaishi, 1965; Goodman et al. 1967). The newly formed retinal is next mainly reduced to retinol, which in turn is esterified with fatty acids and transported via the lymph, largely in association with lymph chylomicrons.
Chapter 1. General introduction

The reduction of retinal to retinol was examined with enzyme preparations from homogenates of rat intestinal mucosa. Retinal reduction was catalyzed by a soluble mucosal enzyme which was purified 13-fold by ammonium sulfate precipitation, column chromatography and heating (55° for 6 min). The enzyme was relatively heat-stable and had a molecular weight approximately in the range of 60,000 to 80,000 D. The partly purified reductase was unable to oxidize ethanol in the presence of NAD⁺. Retinal reduction required NADH or NADPH as cofactor (Fidge and Goodman, 1968).

Lecithin: retinol acyltransferase (EC. 2.3.1.76)

Lecithin: retinol acyltransferase (LRAT), microsomal enzyme that preferentially catalyzes the transfer of an acyl group from the sn-1 position of phosphatidylcholine to all-trans-retinol and plays an essential role in the regeneration of visual chromophore as well as in the metabolism of vitamin A (Golczak et al. 2005). This enzyme converts all-trans-retinol into all-trans-retinyl esters present mainly in the retinal pigmented epithelial cells and homogenates and microsomes prepared from liver, isolated liver cells and several peripheral tissues. In retinal pigment epithelium, LRAT plays a key role in the retinoid cycle, a two-cell recycling system that replenishes the 11-cis-retinal, chromophore of rhodopsin and cone pigments. LRAT is essential for accumulation of all-trans-retinyl esters in eye and liver. Hepatic LRAT activity is strongly influenced by vitamin A status. The liver of retinol deficient animals has essentially no hepatic LRAT activity, whereas activity is again measurable 4 to 8 hr after repletion with retinol or its active metabolite, retinoic acid. In retinol-deficient rats, dose of all-trans retinoic acid as low as 2 µg/rat produced a measurable rise in hepatic LRAT activity within a few hours (Ross et al. 1997).

Retinyl ester hydrolase (EC. 3.1.1.21)

Hydrolysis of retinyl esters plays a major role in the overall metabolism of vitamin A in the body. Retinyl ester hydrolases are likely to play a key role in retinoid metabolism to release retinol accumulated and stored as esters. A neutral, bile salt-independent retinyl ester hydrolase has been purified from a rat liver microsomal fraction. Hydrolysis of retinyl esters occurs during both the hepatic uptake of newly absorbed dietary vitamin A and during the mobilization of retinyl ester stores from the liver. Thus, hepatic
Chapter 1. General introduction

enzymes catalyzing the hydrolysis of retinyl esters are important in the body’s overall vitamin A homeostasis. Earlier studies focused on a neutral, bile salt-dependent retinyl ester hydrolase that is now understood to be the enzyme carboxyl ester lipase (Harrison, 1993). This enzyme is secreted by pancreas into the intestinal lumen, where it can presumably hydrolyze dietary retinyl esters and other lipid esters (Erlanson and Borgstrom, 1968; Lombardo and Guy, 1980; Harrison and Gad, 1989; Rigtrup and Ong, 1992).

In addition, dissolution of released carotenoids in bulk lipid droplets in the stomach or intestine is followed by the formation of multilamellar lipid vesicles from lipid droplets consequent to the action of bile salts and pancreatic lipases. Hernell et al. (1990) studied the physicochemical nature of lipid particles in the human duodenal lumen during lipid digestion. These and other studies indicate that the ultimate structure produced during lipid digestion is a discoidal mixed lipid micelle composed largely of bile salts, free fatty acids, monoglycerides and phospholipids, with a diameter of about 80Å.

Micelles are water soluble, spherical aggregates of bile salts, monoacylglycerides, phospholipids, cholesterol and other fat-soluble compounds in the small intestine. Canfield et al. (1990) demonstrated the incorporation of β-carotene using model micelles, although concentrations of carotenoids in either model or duodenal bile salt micelles per se (moles carotenoid per mole bile acid) have not been reported. Micellar capacity for carotenoid inclusion may differ with carotenoid structure or micellar lipid composition. Micellar incorporation may represent one factor limiting human absorption of carotenoids at higher levels of intake (>20 mg), but it is probably less important at intakes representative of typical intake from foods (<10 mg), where the limiting factor is probably their release from food particles and dissolution in bulk lipid.

Phospholipids

Phospholipids are a class of lipids formed from four components: fatty acids, a negatively charged phosphate group, nitrogen containing alcohol and a backbone. They are key components of the membrane structure, modulate membrane enzyme activity and participate in signaling pathway. They are natural surfactants and emulsifiers consisting of an alcohol such as glycerol, one or two molecules of fatty acid and a phosphoric acid compound. They are found in all plants and animals and include such
substances as lecithin, cephalin and sphingomyelin. Lecithin is a significant constituent of brain and nervous tissue consisting of a mixture of diglycerides of stearic, palmitic and oleic acids, linked to the choline ester of phosphoric acid. Cell walls and other biological membranes consist of two layers of phospholipids where the fatty acid tails of the phospholipids are oriented towards each other and the phosphate groups form the outer surfaces of the membrane. These bilipid layers are semi permeable, allowing some molecules to pass freely through the membrane while blocking others. The most common phospholipids are lecithin, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol.

**Phosphatidylcholine (lecithin, PC)**

PC is usually the most abundant phospholipid in animals and plants, amounting to almost 50% of the total and as such, it is obviously the key building block of membrane bilayers. In particular, it makes up a very high proportion of the outer leaflet of the plasma membrane. PC is also the principal phospholipid circulating in plasma, where it is an integral component of the lipoproteins, especially the HDL. On the other hand, it is less often found in bacterial membranes, perhaps 10% of species. It is a neutral or zwitterionic phospholipid over a pH range from strongly acidic to strongly alkaline. In animal tissues, some of its membrane functions appear to be shared with the structurally related sphingolipid- sphingomyelin.

**Lysophosphatidylcholine (lysolecithin, LPC)**

LPC found in small amounts in most tissues, with one mole of fatty acid per mole of lipid in the sn-1 position. It is formed by hydrolysis of membrane phosphatidylcholine by the enzyme PLA$_2$, as part of the de-acylation/ re-acylation cycle that controls its overall molecular species composition. It can also be formed inadvertently during extraction of lipids from tissues. Also, there is a phospholipase A$_1$ (PLA$_1$), which is able to cleave the sn-1 ester bond.
Fatty acids

Chemically, fatty acids are aliphatic carboxylic acids, having the general formula, ‘R-COOH’. Where, -COOH (carboxylic group) represents the functional group. Depending on the ‘R’ group (the hydrocarbon chain), the fatty acids may vary. The fatty acids are derived from natural fats and oils. Most of the natural fatty acids have an even number of carbon atoms, because their biosynthesis involves acetyl CoA, a coenzyme carrying a two-carbon atom group. Saturated fatty acids have all the hydrogen that the carbon atoms can hold and therefore, have no double bonds between the carbons. Monounsaturated fatty acids (MUFA) have only one double bond and polyunsaturated fatty acids (PUFA) have more than one double bond.

The main components of all fats are the fatty acids which might be saturated, monounsaturated or polyunsaturated. Fats containing a high proportion of saturated fatty acids are solid at room temperature. These are commonly known as saturated fats and are usually derived from animal sources (lard and butter). Most plant fats are high in either polyunsaturated or monounsaturated fats except palm and coconut fat which is highly saturated. Saturated and monounsaturated fats are not necessary in the diet as they can be made in the human body. Two PUFA that cannot be made in the body are linoleic and α-linolenic acids. They must be provided through diet and are known as essential fatty acids. Within the body both can be converted to other PUFA such as arachidonic acid or eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). PUFA (EPA and DHA) are important for maintaining the membranes of all cells; for making prostaglandins, which regulate many body processes like inflammation and blood clotting. Another requirement for fat in the diet is to enable the fat-soluble vitamins A, D, E and K to be absorbed from food and for regulating body cholesterol metabolism.
Oleic acid [OA, 18:1] is a monounsaturated omega-9 fatty acid found in various animal and vegetable sources. Systemic chemical name is (9Z)-octadec-9-enoic acid. The saturated form of this acid is stearic acid. Olive oil comprises 55-80% of oleic acid. Molecular formula: C_{18}H_{34}O_{2} or CH_{3}(CH_{2})_{7}CH=CH(CH_{2})_{7}COOH.

Linoleic acid [LA, 18:2 (n-6)] is an polyunsaturated omega-6 fatty acid containing 18-carbon chain with two cis double bonds; the first double bond is located at the sixth carbon from the omega end. Systemic chemical name: cis, cis-9, 12-octadecadienoic acid. Molecular formula: C_{18}H_{32}O_{2} or CH_{3}-(CH_{2})_{4}-(CH=CH-CH_{2})_{2}-(CH_{2})_{6}-COOH.

Eicosapentaenoic acid [EPA, 20:5 (n-3)] is an omega-3 fatty acid, with a 20-carbon chain and five cis double bonds; the first double bond is located at the third carbon from the omega end. Systematic chemical name: all-cis-5,8,11,14,17-icosapentaenoic acid and trivial name is timnodonic acid. Molecular formula: C_{20}H_{32}O_{2}.
Aim and scope of the study

Vitamin A is essential for the important physiological processes such as vision, reproduction, immune functions, growth and development. Vitamin A must be obtained from the diet either as preformed vitamin A (retinoids) in foods of animal origin or as provitamin A carotenoids in fruits and vegetables. Provitamin A carotenoids in particular \( \beta \)-carotene rich fruits and vegetables are the major sources of vitamin A for a large proportion of the world’s population. Vitamin A deficiency (VAD) is a serious public health problem in most of the developing countries including India. The main cause of the VAD is, low or inadequate intake of vitamin A rich foods. Food based strategies using dietary sources of vitamin A when adequately implemented, can be effective in the control of VAD. Green leafy vegetables (GLVs) rich in provitamin A carotenoids are considered to be good sources of vitamin A. However, studies have shown poor efficiency of these vegetables as vitamin A source demonstrating lower bioavailability of \( \beta \)-carotene from GLVs than synthetic \( \beta \)-carotene. This may be due to lower bioavailability of provitamin A carotenoids such as rate of absorption, their conversion into vitamin A, transport, chemical nature and fat content of the diet. Bioconversion of provitamin A carotenoids into vitamin A varies inversely with vitamin A status. Provitamin A carotenoid content of plant foods, the food matrix in which they are present, the extent of their release and incorporation into mixed micelles in the intestinal lumen determines their bioavailability. The contribution of plant foods rich in provitamin A carotenoids is substantial only when their consumption, provitamin A content and their bioefficacy is high. With respect to provitamin A carotenoids, the term bioefficacy is defined as the product of the fraction of the ingested amount that is absorbed (bioavailability) and the fraction of that is converted to retinol (bioconversion). Provitamin A content of the plant foods (GLVs) is very much essential to determine their bioefficacy.

The present study was undertaken to assess the vitamin A value of GLVs of familiar and less familiar agri/ horticultural produce by screening the provitamin A carotenoids content to explore them as \( \beta \)-carotene source. Results indicate that less familiar GLVs were found to contain higher level of provitamin A carotenoids and hence, they could be exploited as a good source of vitamin A to manage vitamin A malnutrition.
To study the effect of dietary components (phospholipids and fatty acids) on the bioavailability and bioconversion provitamin A carotenoids into vitamin A in normal and under VAD condition in growing rats. It also aims to study the role of dietary components on the activity of certain carotenoid and retinoid metabolizing enzymes and biochemical molecules (lipid profile) that regulate the processes of carotenoid metabolism in target tissues.

Physicochemical properties of mixed micelles containing provitamin A carotenoids either with phospholipids and fatty acids were studied to assess the process of formation of mixed micelles and incorporation of β-carotene into micelles. The particle size, structure and the intensity of β-carotene in micelles were determined using particle size analyzer, phase contrast microscopy and image processing techniques. Other properties like, pH, viscosity of the mixed micelles was determined to know the mechanism of intestinal uptake of β-carotene in vitro.

Dietary factors such as specific phospholipids (phosphatidylcholine and lysophosphatidylcholine) and fatty acids (oleic, linoleic and eicosapentaenoic acid) are used as a fat source to study the efficiency of intestinal β-carotene uptake and its conversion into vitamin A by employing single dose and repeated dose of micellar β-carotene containing the above dietary components in normal and VAD rats.

Dietary studies have been carried out using specific fatty acid-rich vegetable oils, phospholipids (soy PC) and β-carotene rich GLV as vitamin A source to assess their effect on recovering vitamin A status in VAD rats. The activity of β-carotene cleavage enzyme, β-carotene 15,15'-dioxygenase, retinoid metabolizing enzymes like retinal reductase, retinyl ester hydrolase, lecithin: retinol acyltransferase, membrane bound enzyme, Na⁺K⁺ ATPase in VAD rats and other lipid metabolizing enzymes like pancreatic lipase and PLA₂ on micelles containing fatty acids and phospholipids. Fatty acid composition, triacylglycerides, phospholipids and cholesterol in plasma and other tissues were determined to assess the possible role of these dietary factors on β-carotene metabolism to overcome the problem of VAD.