Chapter 8
Digestion and Absorption of Other Dietary Lipids

Michael D. Sitrin

1 Introduction

The small intestine absorbs a variety of important lipids present in the diet or secreted in the bile using pathways similar to those for dietary triglycerides (Chap. 7). In this chapter, the mechanisms for absorption of cholesterol, phospholipid, bile acids, and the four fat-soluble vitamins will be reviewed. Absorption of these lipids will be considered with respect to intraluminal events, mechanisms for uptake across the brush-border membrane, metabolism within the enterocyte, and intracellular transport and secretion; the emphasis will be on the unique features of each lipid compound. In addition, the hepatic uptake and metabolism of the fat-soluble vitamins will be discussed.

2 Cholesterol

2.1 Sources of Intraluminal Cholesterol

The cholesterol present in the intestinal lumen comes from both the **diet** and from **endogenous sources**. The typical North American diet contains 300–500 mg/day of cholesterol. A variable portion (typically about 15 %) of dietary cholesterol is esterified with various fatty acids. Bile provides approximately 800–1,200 mg/day of unesterified cholesterol, and the turnover of the intestinal mucosa adds 250–400 mg/day of cholesterol to the luminal contents.

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The dietary and biliary cholesterol do not appear to form a single pool in the intestinal lumen. Current data indicate that biliary cholesterol is more rapidly and perhaps more completely absorbed than is dietary cholesterol. Furthermore, while the jejunum absorbs more total cholesterol than the ileum, a greater fraction of dietary cholesterol may be absorbed in the distal small bowel than occurs with biliary cholesterol. Biliary cholesterol is secreted in micelles with bile acids and phospholipid. In contrast, cholesteryl esters require hydrolysis, and dietary cholesterol needs solubilization prior to intestinal absorption. Overall, approximately 30–60 % of the luminal cholesterol is absorbed.

2.2 Digestion of Cholesteryl Esters

Cholesteryl esters must be hydrolyzed to **free cholesterol** and **fatty acids** prior to intestinal absorption. **Pancreatic cholesterol esterase** (also known as **bile salt-activated lipase**) is the enzyme responsible for most of the cholesteryl ester hydrolysis. As previously discussed (Chap. 7), this enzyme requires bile salts (particularly glycocholate or taurocholate) for its activity; the bile salt appears to serve as a cofactor allowing for polymerization of the enzyme monomer into proteolysis-resistant polymers or for an activating conformational change.

2.3 Solubilization of Cholesterol

Cholesterol is nearly insoluble in an aqueous system, and bile salts are therefore absolutely required for cholesterol absorption. Cholesterol is only slightly soluble in a pure bile salt solution, but the **addition of other lipids**, such as phospholipid, monoglycerides, and fatty acids, **markedly increases cholesterol solubility in mixed micelles**. In the intestinal lumen, cholesterol partitions between the micellar phase and the lipid droplets.

Cholesterol in mixed micelles is in rapid equilibrium with cholesterol in monomolecular solution. The high cholesterol concentration in the micelles therefore ensures a maximal concentration of monomolecular cholesterol in the unstirred water layer lining the luminal enterocyte surface.

2.4 Brush-Border Membrane Cholesterol Transport

Traditionally cholesterol transport across the brush-border membrane was thought to occur by passive diffusion. It is now clear that several brush-border membrane proteins play key roles in regulating cholesterol movement across the apical cell membrane (Fig. 8.1). The protein **Niemann-Pick C1-Like 1 (NPC1L1)** is

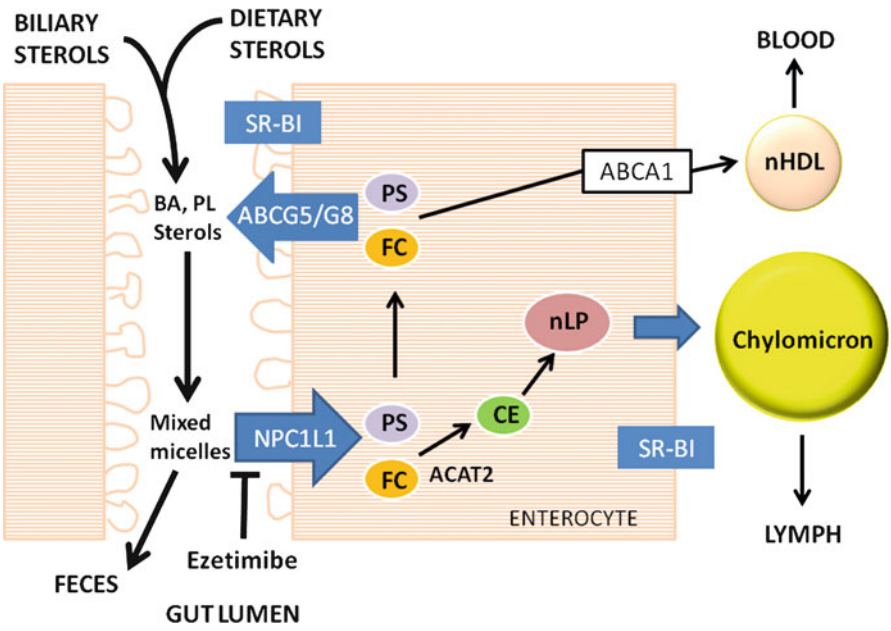


Fig. 8.1 Intestinal sterol absorption and secretion. Sterols including free cholesterol (*FC*) and free plant sterols (*PS*) from diet and bile are mixed with phospholipids (*PL*) and bile acids (*BA*) to form micelles. FC and PS solubilized in mixed micelles are transported into absorptive enterocytes via an NPC1L1-dependent and ezetimibe-inhibitable mechanism. FC is delivered to the ER for esterification by acyl-CoA: cholesterol acyltransferase-2 (*ACAT2*) to form cholesterol esters (*CE*) that is then packaged into nascent lipoprotein particles (*nLP*) and secreted as a constituent of chylomicrons. PS and FC that escapes ACAT2 esterification may be directly transported to nascent HDL (*nHDL*) through basolateral ABCA1, or back to the gut lumen via ABCG5/G8 (Adapted from Brown and Yu [2])

essential for intestinal cholesterol absorption. The gene for NPC1L1 is located on chromosome 7 and produces two alternatively spliced transcripts coding for proteins of 1332 and 724 amino acids; the significance of these two splice variants has not yet been defined. The protein has 13 transmembrane domains and sites for extensive N-glycosylation. NPC1L1 also contains a domain of about 180 amino acids that is a consensus sterol-sensing domain. Mice in which NPC1L1 has been knocked out demonstrate markedly reduced (>70 %) cholesterol absorption. NPC1L1 is the target of the **cholesterol absorption inhibitor *ezetimibe*** which is widely used in the treatment of patients with **hypercholesterolemia**. The mechanism by which NPC1L1 mediates brush-border cholesterol uptake is incompletely understood, but current evidence suggests a clathrin-mediated endocytic pathway. NPC1L1 is found in both the **apical plasma membrane** and in **intracellular compartments**, and cycling between these compartments is likely regulated by cholesterol. Cellular cholesterol depletion causes translocation of NPC1L1 to the brush-border membrane, and cholesterol repletion results in movement of NPC1L1 and cholesterol to the cell interior. Ezetimibe appears to inhibit sterol-induced internalization of

NPC1L1 via this endocytic pathway. NPC1L1 can facilitate the transport of various plant steroids, but with lower efficiency than cholesterol transport.

The regulation of NPC1L1 is incompletely understood. Some data suggest that cellular cholesterol availability influences NPC1L1 expression, and that activation of many nuclear receptors play regulatory roles. Human sequence polymorphisms in NPC1L1 have been identified that affect cholesterol absorption efficiency, plasma LDL-cholesterol levels, and sensitivity to ezetimibe treatment. These polymorphisms alter NPC1L1 subcellular localization, glycosylation, or protein stability.

A second complex plays a key role in the transport of cholesterol and plant sterols from the enterocyte across the brush-border membrane into the intestinal lumen (Fig. 8.1). **ABCG5** and **ABCG8** are genes on chromosome 2 arranged in a head-to-head orientation with less than 400 base pairs between their respective start codons. The two genes code for two distinct proteins, **sterolin-1** and **sterolin-2**, that must heterodimerize to transport sterols. Both proteins contain an ATP-binding sequence near the N-terminus and six transmembrane domains, and the complex functions as an ATP-dependent cholesterol efflux pump delivering sterols from the enterocytes into the intestinal lumen for disposal in feces. The two proteins heterodimerize in the endoplasmic reticulum, traffic through the Golgi, and subsequently move to the apical plasma membrane. ABCG5 and ABCG8 undergo N-glycosylation, and glycosylation at Asn-619 in ABCG8 is critical for proper trafficking of the complex.

The **ABCG5/ABCG8 complex** is largely responsible for the greater intestinal absorption of cholesterol compared with a variety of dietary plant sterols. **β-sitosterolemia** is a genetic disease characterized by ***increased intestinal absorption*** and ***diminished biliary secretion of cholesterol and plant sterols*** such as β-sitosterol. Affected patients have accumulation of cholesterol and plant sterols in the plasma and various tissues and often suffer from **premature atherosclerotic heart disease**. β-sitosterolemia is caused by **a mutation in either ABCG5 or ABCG8**, with the majority of mutants causing impaired transport of the heterodimer from the endoplasmic reticulum to the plasma membrane.

ABCG5 and ABCG8 both appear to be regulated primarily at the level of gene transcription. The sterol-sensing transcription factors **LXRα** and **LXRβ** up-regulate expression of both ABCG5 and ABCG8, and effects of other transcription factors have been identified.

Scavenger receptor class B type 1 (SR-B1) is thought to play a role in cholesterol homeostasis mainly through its mediation of the selective uptake of HDL cholesteryl esters into the **liver**. SR-B1, however, is also abundantly expressed in the **small intestine** where it is found in both the brush-border and basolateral membranes (Fig. 8.1). SR-B1 is expressed more in the proximal than distal small bowel, paralleling the site of most cholesterol absorption. SR-B1 may be mediate some cholesterol uptake across the brush-border membrane, but may have more important roles in the intestinal uptake of circulating HDL-associated cholesterol esters and subsequent transport across the apical membrane for disposal in the feces, and/or the secretion of cholesterol-containing lipoproteins across the basolateral enterocyte membrane.

2.5 Cholesterol Esterification, Incorporation into Lipoproteins, and Secretion

Within the enterocyte, 70–90 % of the absorbed cholesterol is esterified with fatty acids. Various lipid binding proteins may be involved in directing the absorbed cholesterol to the endoplasmic reticulum. The principal enzyme responsible for cholesterol esterification is **acyl CoA:cholesterol acyl transferase 2 (ACAT2)** that is present in the endoplasmic reticulum. Sitosterol and other plant sterols are less effective substrates for ACAT2 than cholesterol. The **microsomal triglyceride transfer protein (MTTP)** transfers cholesteryl esters to the nascent chylomicron particle, and chylomicrons are the principal lipoprotein delivering cholesterol into the circulation. Within chylomicrons and other intestinal lipoproteins, cholesteryl esters are incorporated into the oily lipoprotein core, whereas free cholesterol is present on the surface of the particle.

Another route of cholesterol exit from the enterocyte may be via incorporation into HDL. A **basolateral cholesterol efflux pump ABCA1** facilitates the transfer of cholesterol to an apolipoprotein A-I acceptor molecule forming discoidal HDL particles (Fig. 8.1). The amount of cholesterol absorbed via this pathway appears to be small, but ABCA1 may be a key role in HDL production. Patients with ***Tangier disease*** with **mutations in ABCA1** have virtual **absence of plasma HDL** and **increased enterocyte cholesterol content**. ABCA1 is regulated by the **transcriptional factor LXRα**.

3 Phospholipid

Most of the phospholipid that the intestine absorbs originates from biliary secretion (10–20 g/day) rather than the diet (1–2 g/day). In bile, phospholipid is present in mixed micelles along with bile acids and cholesterol. **Phosphatidylcholine** is the major phospholipid in the diet and in bile along with small amounts of **phosphatidylethanolamine**, **phosphatidylserine**, and **phosphatidylinositol**. In the intestinal lumen, the phosphatidylcholine distributes between the lipid droplets and the micellar phase, but favors the micelles.

3.1 Digestion of Phosphatidylcholine

The major enzyme responsible for the digestion of phosphatidylcholine is **pancreatic phospholipase A_2** (Fig. 8.2). This enzyme, which catalyzes the breakdown of phosphatidylcholine to ***lysophosphatidylcholine*** and ***fatty acid***, is secreted as a **zymogen** and activated by tryptic cleavage of an N-terminal heptapeptide. Phospholipase A_2 has a molecular weight of about 14 kd and is activated by

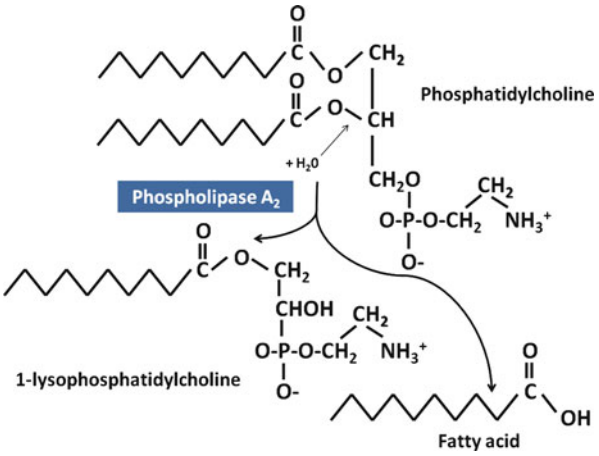


Fig. 8.2 Digestion of phosphatidylcholine by pancreatic phospholipase A_2. Phospholipids are substrates of pancreatic phospholipase A_2

calcium and by bile salts. Phospholipase A_2 in the intestinal brush-border membrane and intracellular phospholipases within the enterocyte probably also participate in phospholipid digestion.

3.2 Absorption of Lysophosphatidylcholine

Lysophosphatidylcholine can be incorporated into both **mixed micelles** and **liquid crystalline vesicles**. Studies have also demonstrated that bile salts and lysophospholipids form submicellar aggregates than can transfer lysophospholipids between membranes. The relative contributions of these macromolecular structures to intestinal lysophospholipid absorption remain to be determined. It has been traditionally thought that lysophospholipids cross the enterocyte brush-border membrane by passive diffusion, but some data suggest a protein-mediated uptake mechanism.

3.3 Phospholipid Metabolism in the Enterocyte

The absorbed lysophosphatidylcholine can be metabolized by several pathways. Most of the lysophosphatidylcholine is **reacylated** to form ***phosphatidylcholine***. Alternatively, the lysophosphatidylcholine can be **hydrolyzed** to ***fatty acid*** and ***3-phosphorylcholine***, or two molecules of lysophosphatidylcholine can react to form one molecule of ***phosphatidylcholine*** and one molecule of ***3-phosphorylcholine***. The fatty acids formed by these reactions can be used for triglyceride synthesis. In addition, the intestine is capable of synthesizing phosphatidylcholine from diglyceride (derived mainly from α-glycerophosphate) via the **Kennedy pathway**.

In the absence of absorbed lysophosphatidylcholine, there is a marked drop in the secretion of chylomicrons into lymph. The fatty acid composition of the phosphatidylcholine contained on the surface of chylomicrons is not greatly influenced by the dietary fatty acid content, but mainly reflects the fatty acid composition of biliary phosphatidylcholine. These data indicate that ***de novo*** **phosphatidylcholine synthesis by the enterocyte is inadequate to provide the phospholipid needed for optimal packaging and secretion of chylomicrons**, and that absorbed biliary phospholipid is preferentially utilized for chylomicron assembly.

4 Bile Acids

Bile acids are secreted by the liver almost exclusively as **conjugated forms**, mainly as conjugates with glycine (75 %) or taurine (25 %). A small fraction of these conjugated bile acids is absorbed in the ***proximal small intestine***, mainly the glycine-conjugated dihydroxy bile acids, chenodeoxycholic acid-glycine and deoxycholic acid-glycine. These bile acids are likely protonated in the unstirred water layer of the jejunum, which is estimated to have a pH of approximately 5, and the protonated bile acids are then passively absorbed transcellularly. **Glycine-conjugated trihydroxy bile acids** and **taurine-conjugated bile acids** would be fully ionized at the small intestinal pH and therefore would not be absorbed by this mechanism. There is also the possibility of limited paracellular absorption of ionized and nonionized conjugated bile acids in the small bowel.

Most of the conjugated bile acids, however, remain in the lumen as the intestinal contents transit through the upper intestine, where they activate important enzymes and solubilize lipids to ensure efficient absorption. In the ***ileum***, there is a high-capacity active transport mechanism for bile acids that results in the absorption of greater than 95 % of the secreted bile acids. The conserved bile acids are delivered via the portal blood to the liver, where they are taken up for secretion again into bile. This **enterohepatic circulation of bile acids**, which occurs several times during a meal, ensures an adequate delivery of bile acids into the intestine for highly efficient lipid absorption independent of the rate of hepatic bile acid synthesis from cholesterol.

4.1 Ileal Bile Acid Uptake

The active transport of bile acids across the ileal brush-border membrane is mediated by the transport protein **ASBT** (gene symbol SLC10A2) (Fig. 8.3). ASBT is a 348 amino acid membrane glycoprotein with a glycosylated extracellular amino terminus, a cytosolic carboxyl terminus, and 7 membrane spanning domains. ASBT is an **electrogenic Na^+-bile acid co-transporter**, with two sodium ions transported per molecule of bile acid. The driving force for transport is the inwardly

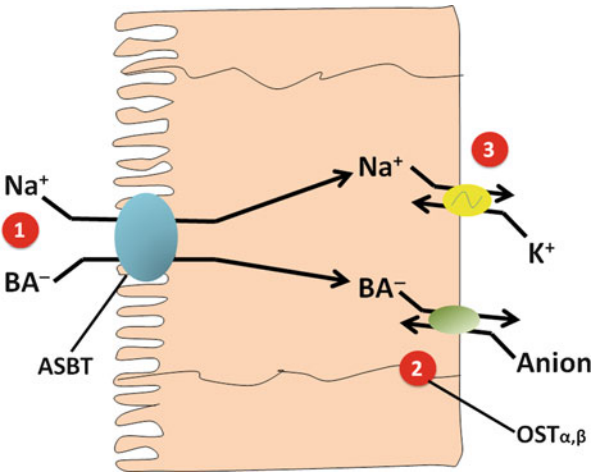


Fig. 8.3 Active transport of bile acids by the ileal enterocyte. (*1*) Apical Na^+-conjugated bile acid carrier ASBT. (*2*) Basolateral bile-acid-anion exchanger. (*3*) Basolateral Na^+/K^+-ATPase (Adapted from Hofmann [7])

directed sodium gradient. Conjugated bile acids are transported more efficiency than unconjugated forms, and the affinity for dihydroxy bile acids is higher than for trihydroxy bile acids. ASBT is expressed mainly in the ***villus cells*** of ileal enterocytes, with small amounts of ASBT in the proximal intestine and colon. The GATA4 transcription factor is essential for silencing ASBT expression in the proximal intestine.

ASBT expression is reduced by the bile acid **farnesoid X receptor (FXR)** that has chenodeoxycholate and other bile acids as ligands. The uptake of bile acids, therefore, can be altered to meet physiological requirements. ASBT is also regulated by the **PPARα receptor** and by **corticosteroids**. ASBT and Na^+-bile acid co-transport is developmentally regulated in mammals as they are absent at birth and develop in the first postnatal weeks at a variable, species-dependent rate.

Inactivating mutations in ASBT cause ***bile acid malabsorption***. Beginning in early infancy these children have *interruption of the enterohepatic circulation of bile acids, chronic diarrhea,* and *malabsorption of triglycerides* and *fat-soluble vitamins*.

4.2 Intracellular Transport and Export from the Enterocyte

With the enterocyte bile salts associate with a 14 kDa protein called the **ileal bile acid binding protein (IBABP)** in a 2:1 stoichiometric complex. IBABP is postulated to facilitate transport of bile acids through the cell to the basolateral membrane. IBABP may also play a role in protecting the enterocyte from the toxic effects of high intracellular bile salt concentrations. IBABP expression is increased by FXR and is also developmentally regulated.

The movement of bile acids across the basolateral membrane occurs mainly via the **OSTα/β hetereodimeric transporter** (Fig. 8.3). **OSTα** is a 340 amino

acid protein with an extracellular amino terminus, 7 transmembrane domains, and an intracellular carboxyl terminus. **OSTβ** is a 128 amino acid protein with an extracellular amino terminus and a cytoplasmic carboxyl terminus. Both subunits are required for trafficking to the plasma membrane and for bile acid transport. This transport complex appears to function by **sodium-independent facilitated diffusion**. OSTα/β can transport a number of compounds in addition to bile acids, including estrone-3-sulfate, digoxin, prostaglandin E2, and dehydroepiandstosterone-3-sulfate.

OSTα/β is also increased by FXR. **Activation of FXR by bile salts *decreases ASBT expression*** and ***increases IBABP and OSTα/β expression***, thereby preventing intracellular bile acid accumulation.

The **multidrug resistance protein 3 (MRP3)** plays a minor role in basolateral bile acid export, but may be more important for the small amounts of modified (glucuronidated or sulfated) bile acids present in the intestinal lumen. **Multidrug resistance protein 2 (MRP2)** may mediate transport of modified bile acids across the brush-border membrane into the intestinal lumen.

4.3 Absorption of Unconjugated Bile Acids

In the intestine, particularly the *colon*, bacteria modify bile acids in various ways. They **deconjugate bile acids** by enzymatic hydrolysis of the bond linking bile acids to their amino acid (glycine or taurine) conjugates. Further bacterial modifications include **7α-dehydroxylation** and **oxidation/epimerization of hydroxyl groups** at various sites. These modifications increase the hydrophobicity and pKA of the bile acids, permitting some passive absorption across the intestinal epithelium. In addition, limited amounts of unconjugated bile acids may be absorbed via the small amounts of transport proteins expressed in the colon.

Bile acids in the *distal intestine* and *colon* cause fluid and electrolyte secretion by directly altering enterocyte and colonocyte ion transport and indirectly by stimulating neuroendocrine mechanisms. **Excessive colonic bile salts** from ileal resection or disease or other processes result in ***chronic diarrhea***.

4.4 Ileal FGF19 Secretion and Regulation of Hepatic Bile Salt Synthesis

Ileal enterocytes synthesize and secrete **fibroblast growth factor 19 (FGF19)**. FGF19 is responsive to FXR. FGF19 is released into the portal circulation, and in an endocrine manner actives **fibroblast growth factor receptor 4 (FGF4)** in hepatocytes, resulting in inhibition of bile acid synthesis. This pathway works in conjunction with the local intrahepatic regulation of bile acid synthesis by bile salts.

A group of patients have been described who have low circulating FGF19 levels and chronic watery diarrhea. It appears that these patients have disordered regulation of hepatic bile acid synthesis and an expanded bile acid pool size that overwhelms a normally functioning ileal absorption mechanism. Excessive colonic bile acids cause fluid and electrolyte secretion and chronic watery diarrhea.

5 Vitamin A

5.1 Definitions

The term ***vitamin A*** denotes a family of compounds that are **structurally related to all-trans-retinol** and are required for vision, growth, cellular differentiation and proliferation, reproduction, and the integrity of the immune system. Naturally occurring compounds such as retinol, retinaldehyde (retinal), and retinoic acid and a large number of synthetic analogues with or without vitamin A biological activity are termed **retinoids**. Retinoids vary both qualitatively and quantitatively with respect to specific biological actions. For example, retinoic acid in contrast to retinol cannot effectively maintain normal vision or reproductive function.

5.2 Dietary Sources of Vitamin A

Dietary sources of vitamin A include **preformed vitamin A**, present in animal tissues largely as long-chain fatty acyl retinol esters, and certain **carotenoid pigments** present in fruits, vegetables, and some **animal fats** that are precursors of vitamin A. Of more than 500 carotenoids found naturally, only about 50 are precursors of retinol. ***All-trans-β-carotene*** is the most active on a weight basis and the most important vitamin A precursor for humans. Because the bioavailability of food carotenoids is less than that of retinol, due to poorer intestinal absorption and limited conversion of carotenoids to vitamin A, 12 μg of dietary β-carotene and 24 μg of three other dietary carotenoids are assumed to be nutritionally equivalent to 1 μg of retinol. The major sources of vitamin A or provitamin A in the American diet are liver, carrots, eggs, vegetable-based soups, whole-milk products, and fortified milk and other foods. It is estimated that on average less than one third of total vitamin A activity comes from carotenoids, although there is certainly considerable interindividual variation based on dietary habits and the efficiency of carotenoid absorption and conversion to vitamin A. The roles of β-carotene and other carotenoids in the **prevention of cancers, heart disease, and other chronic degenerative diseases** is a topic of intense current interest. Many of the physiological effects of carotenoids are not due to their function as vitamin A precursors, but reflect antioxidant and other properties of the carotenoids themselves.

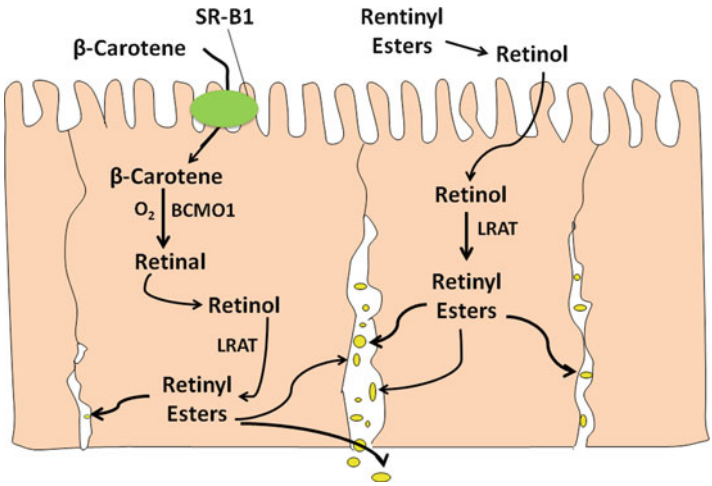


Fig. 8.4 Absorption and metabolism of β-carotene and retinyl esters (Adapted from Ong [10])

5.3 Digestion of Dietary Retinyl Esters

As with other esterified lipids, such as cholesteryl esters, retinyl esters must be hydrolyzed before intestinal absorption can occur (Fig. 8.4). Several pancreatic enzymes, including pancreatic lipase, pancreatic lipase-related protein 2, and pancreatic bile salt-activated lipase can hydrolyze retinyl esters, with pancreatic lipase appearing to be most important. Brush-border membrane phospholipase B (PLB) also participates in retinyl ester hydrolysis. The relative contributions of pancreatic and small intestinal enzymes to retinyl ester hydrolysis are currently uncertain.

5.4 Uptake of Retinol and β-Carotene by the Enterocyte

Both **retinol** (formed by hydrolysis of retinyl esters) and **β-carotene** are solubilized in mixed micelles within the luminal contents. At ***low concentrations***, retinol is taken up by a saturable, energy-independent process that is consistent with **carrier-mediated facilitated diffusion** (Fig. 8.4). A specific carrier has not yet been identified. At ***higher pharmacological retinol concentrations***, uptake is not saturable and likely occurs via **simple, passive diffusion**. Retinol uptake is greater in the jejunum than ileum and is greater in neonatal animals than adults, suggesting developmental regulation of a carrier. Uptake of β-carotene and other carotenoids across the brush-border membrane is mediated by the **scavenger receptor class B, type 1 (SR-B1)** (Fig. 8.4).

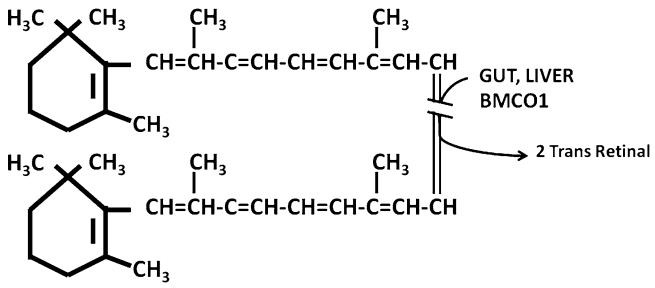


Fig. 8.5 Conversion of β-carotene to vitamin A

5.5 *Carotene Cleavage and Retinal Reduction*

Within the enterocyte, carotenes are cleaved, forming retinal. Two enzymes, **β-carotene-15,15'-monooxygenase (BCM01)** and **β-carotene-9',10'-monooxygenase (BCM02)** can cleave carotenoids (Fig. 8.5). BCM01 cleaves carotene centrally forming two molecules of retinal, whereas BCM02 cleaves asymmetrically forming **apocarotenals** that are subsequently shortened to produce retinal. Some intact β-carotene is absorbed and circulates in plasma. Liver and other tissues have the capacity to form vitamin A from carotene via BCM01. The retinal produced from carotenes is efficiently reduced to retinol in the enterocyte (Fig. 8.4). Both soluble and microsomal reductases have been identified. Within the enterocyte, most of the retinal is probably bound to a specific binding protein, **cellular retinol-binding protein, type 2** or **CRBP(II)** (see below). The **microsomal reductase** effectively utilizes retinal bound to CRBP(II) as a substrate, whereas the **soluble reductase** appears to prefer free retinal.

Intestinal BCM01 mRNA expression is increased when animals are fed a retinoid-deficient diet. Polymorphisms have been identified in the human BCM01 gene that decrease the conversion of β-carotene to vitamin A, and may explain some of the inter-individual variability in the ability to absorb and convert proretinoid carotenoids to retinoids.

5.6 *Cellular Retinol-Binding Protein, Type 2*

Several retinoid-binding proteins play crucial roles in the transport, metabolism, and cellular actions of vitamin A. In the enterocyte, one of these proteins, **CRBP(II)**, is key in the regulation of vitamin A absorption and metabolism. CRBP(II) is a 133-amino-acid protein with one retinoid-binding site that constitutes approximately 1 % of the soluble protein in the jejunum. CRBP(II) belongs to a large superfamily of lipid-binding proteins that includes the fatty acid-binding proteins and another retinoid-binding protein, CRBP(I). **CRBP(I)** is found at low levels in many tissues including the small intestine, but it is not thought to play a role in

vitamin A absorption. In adults CRBP(II) is expressed only in the ***small intestinal villus epithelial cells***. CRBP(II) mRNA and protein are greater in the jejunum than ileum and increase during pregnancy and lactation. In addition to its role in the reduction of retinal derived from carotenes, CRBP(II) also appears to regulate the esterification of retinol within the enterocyte.

5.7 Retinol Esterification

Both absorbed retinol and retinol generated from carotenes are esterified in the enterocyte with long-chain fatty acids. **Palmitate, stearate, oleate,** and **linoleate** account for most of the esterified fatty acids in an approximate ratio of 8:4:2:1, and this pattern is not significantly altered by changes in dietary fatty acid composition. **Two microsomal enzymes** capable of esterifying retinol have been identified. ***Lecithin-retinol acyltransferase (LRAT)*** effectively esterifies CRBP(II)-bound retinol (Fig. 8.4). This enzyme uses phosphatidylcholine as the fatty acid donor, and the fatty acid composition of the product retinyl esters is similar to that of position 1 of intestinal lymph phosphatidylcholine. LRAT has a low Km for CRBP(II)bound retinol that would be appropriate for activity at a low dietary vitamin A intake, and LRAT accounts for about 90 % of the esterification of physiological amounts of retinol. LRAT is greater in the proximal than the distal small bowel and is developmentally regulated in a pattern similar to CRBP(II). Levels of these proteins increase prior to birth, remain high during suckling, and then decline with weaning to the adult level. ***Diacylglycerol acyltransferase 1 (DGAT1)***, a key enzyme in triglyceride absorption, also can esterify retinol, and plays an important role at high levels of retinol intake.

5.8 Export of Vitamin A and Carotenoids from the Enterocyte

Retinyl esters synthesized in the enterocyte and carotenoids that escape cleavage are incorporated into the lipid core of chylomicrons and released into the intestinal lymph. Retinyl esters and carotenoids appear to be inserted into chylomicrons by the MMTP at a late state of chylomicron production. Some retinol is released from the enterocyte into portal blood, perhaps mediated by the ABCA1 transporter in the basolateral membrane.

5.9 Hepatic Metabolism and Storage of Vitamin A

Most (65–75 %) of absorbed vitamin A stays associated with the lipid core of chylomicrons as the triglyceride in these lipoproteins is hydrolyzed, forming **chylomicron remnants**. Extrahepatic uptake of retinyl esters from chylomicrons

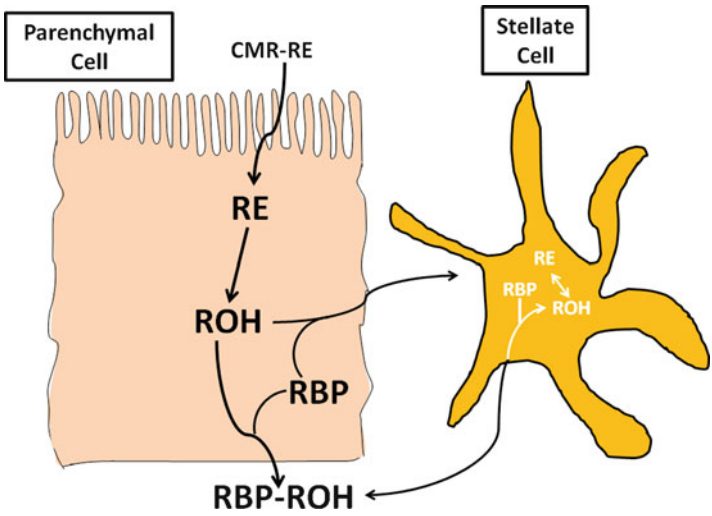


Fig. 8.6 Pathways of hepatic vitamin A metabolism and storage. *CMR RE* retinyl esters in chylomicron remnants, *RE* retinyl esters, *ROH* retinol, *RBP* serum retinal-binding protein

and chylomicron remnants in certain tissues is important in supplying vitamin A to these cells. Chylomicron remnants are taken up by hepatocytes using several receptor-mediated mechanisms. Once remnant-associated retinyl esters have been taken up by hepatocytes, rapid hydrolysis occurs; multiple hepatic enzymes can hydrolyze retinyl esters (Fig. 8.6). Retinol is transferred from hepatocytes to perisinusoidal hepatic stellate cells. **Cellular retinol binding protein 1 [CRBP1]** is important in the **transfer of retinol to hepatic stellate cells** and its subsequent esterification. Some, but not all data, suggests the involvement of the 21 kDa serum retinol binding protein (RBP) in this process. CRBP(I)-bound retinol is esterified by LRAT in the hepatic stellate cells. Normally, stellate cells contain about 90 % of liver vitamin A, and 98 % is in the form of retinyl esters. Retinyl esters are stored within the hepatic stellate cells along with other lipids in the form of lipid droplets.

In vitamin A-deficient animals, secretion of retinol and RBP from the liver is reduced and plasma concentrations are low. Repletion with small doses to vitamin A to a deficient animal does not result in accumulation of retinyl esters in stellate cells; instead, the retinol-RBP complex is rapidly secreted into the circulation. Stellate cells from vitamin A-deficient rats have been found to have reduced CRBP(I) and LRAT levels, a situation that may limit vitamin A storage and favor retinol-RBP delivery into the circulation.

Retinyl esters in hepatic cell lipid droplets are mobilized during dietary vitamin A deficiency to supply peripheral tissues. Several enzymes in stellate cells can hydrolyze retinol esters. After hydrolysis of retinyl esters, retinol is thought to be transferred back to hepatocytes where it binds to RBP. The retinol-RBP complex is

secreted from the hepatocytes into the bloodstream. This process is highly regulated by vitamin A status, as retinol-RBP secretion is reduced in vitamin A deficiency and restored with vitamin A repletion. The ability of stellate cells to control retinol storage and mobilization ensures that plasma retinol is stable at about 2 μmol/L in spite of variations in daily intake of vitamin A.

5.10 Uptake and Metabolism of Retinol-RBP

A detailed consideration of the uptake and metabolism of retinol by tissues other than the intestine and liver is beyond the scope of this chapter; however, it will be briefly considered here. In plasma, most of the retinol-RBP is reversibly complexed with another protein, **transthyretin** (molecular weight, 55 kd), and this large complex is therefore less susceptible to glomerular filtration. A transmembrane-spanning protein **STRA6** avidly binds retinol and facilitates retinol uptake into many peripheral tissues. Cells that also express LRAT take up more retinol, indicating that conversion of retinyl to retinyl ester within the cells maintains the driving force for retinol uptake. STRA6 is apparently a **bidirectional retinol transporter** and can participate in retinol efflux from cells under certain circumstances. **Mutations of STRA6** result in ***malformations*** of the *eye, heart, lungs,* and *diaphragm*; similar abnormalities are noted in the offspring of mothers with vitamin A deficiency.

As mentioned above, some retinyl esters contained within chylomicrons can be taken up by peripheral tissues. The enzyme **lipoprotein lipase** that catalyzes chylomicron triglyceride hydrolysis can also catalyze the hydrolysis of retinyl esters and facilitate retinol uptake into certain tissues Many retinol binding proteins have been identified in interstitial fluids and in the cytosol of various cell types, and they appear to play important roles in regulating cellular uptake, storage, and metabolism of retinoids. Proteins with binding specificities for retinol, retinoic acid, and retinal have been identified. **Retinoic acid** is the form of vitamin A involved in the regulation of gene transcription. Some retinoic acid is produced in the intestine and is present at low concentration in plasma, bound to albumin. Most of the retinoic acid in target tissues, however, appears to derive from the **oxidation of retinol** in these cells. Some retinoic acid may also be synthesized from **β-carotene** in certain cells. **Two families of nuclear retinoic acid receptors** have now been identified, the ***retinoic acid receptor (RAR)*** and the ***retinoid X receptor subfamilies*** (Fig. 8.7). The RAR subfamily binds all-trans-retinoic acid and 13-cis-retinoic acid; in contrast, the RXR subfamily binds 9-cis-retinoic acid. These ligand-dependent transcription factors associate with response elements in the promoters of specific genes, regulating transcription. Retinoid X receptors frequently form heterodimers with other receptors, including the RAR receptors, vitamin D receptor, and others.

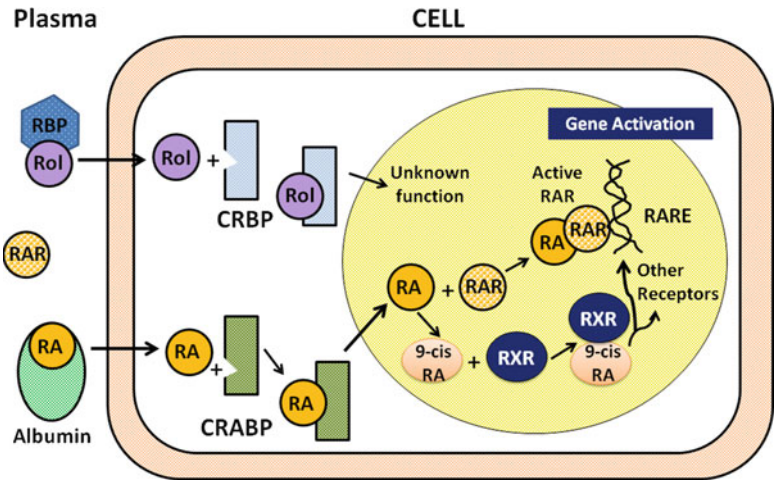


Fig. 8.7 Cellular uptake, metabolism, and mechanism of action of retinol and retinoic acid. *Rol* all-trans-retinol, *RBP* serum retinol-binding protein, *CRBP* cellular retinol-binding protein, type I, *CRABP* cellular retinoic acid-binding protein, *RAR* retinoic acid receptors, *RXR* retinoid X receptors, *9-cis-RA* 9-cis-retinoic acid, *RARE* retinoic acid response elements (Adapted from Wolf [17])

6 Vitamin D

The term vitamin D refers to a family of compounds involved primarily in the regulation of mineral and bone metabolism. **Vitamin D deficiency** results in **metabolic bone disease, *rickets*** in children and ***osteomalacia*** in adults, characterized by inadequate bone mineralization.

6.1 Sources of Vitamin D

Vitamin D_3 (cholecalciferol) is synthesized in **skin** from the precursor compound **7-dehydrocholesterol**. Ultraviolet light of wavelengths 290–320 nm opens the β ring of 7-dehydrocholesterol, forming **previtamin D_3**, which subsequently undergoes a slow, temperature-dependent, nonenzymatic isomerization to vitamin D_3. With enough sunlight exposure, sufficient vitamin D_3 can be synthesized in the skin to meet the vitamin D requirement. The amount of sunlight needed to produce adequate vitamin D_3, however, is influenced by skin pigmentation, age, season, distance from the equator, and conditions that filter out ultraviolet light such as industrial pollution. Because of these factors and customs of dress that limit sun exposure, vitamin D is considered an essential dietary nutrient.

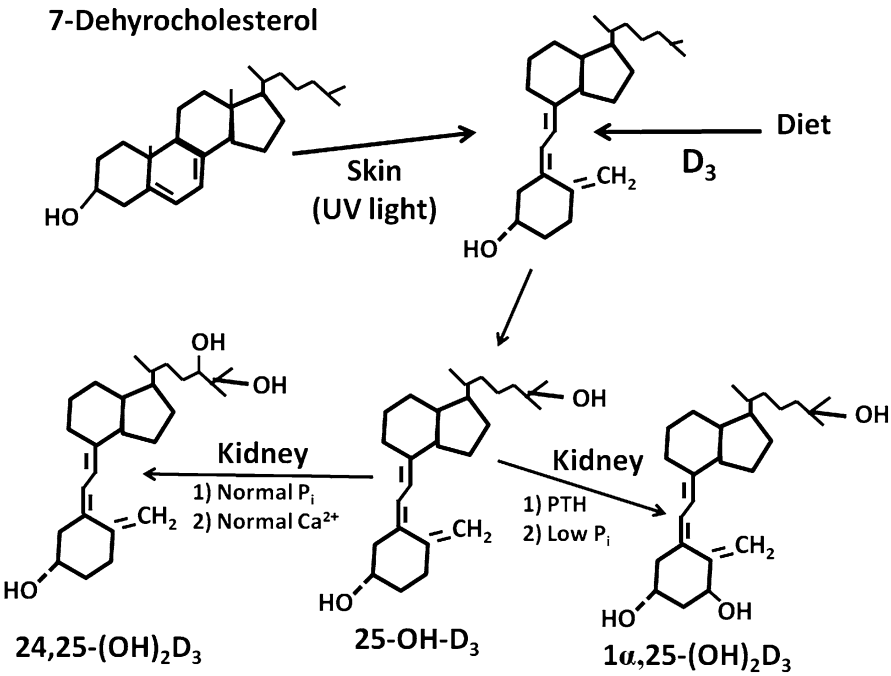


Fig. 8.8 Metabolism of vitamin D

The **major dietary forms of vitamin D** are ***vitamin D₃*** and ***vitamin D₂ (ergocalciferol)***, which is formed by ultraviolet irradiation of the plant sterol **ergosterol**. In mammals, these two forms of the vitamin have similar intestinal absorption, metabolism, and cellular effects. In the United States, foods fortified with vitamin D_2 or vitamin D_3 are the major dietary sources of the vitamin. Processed cow's milk contains 10 μg/quart of vitamin D and is the major food source of the vitamin for children. Small amounts of vitamin D are also present in liver, eggs, poultry, fortified margarines, and other foods. Human milk contains relatively little vitamin D, 0.63–1.25 μg/L.

6.2 Overview of Vitamin D Metabolism and Biological Activity

At normal serum concentrations, vitamin D is not physiologically active. Two hydroxylation reactions are required to produce the **active** form of the vitamin, **1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$)** (Fig. 8.8). The first step occurs in the ***liver*** with the formation of **25-hydroxyvitamin D_3 ($25(OH)D_3$)**, which is the major form of the vitamin circulating in plasma. The principal hepatic **25-hydroxylase** is the **cytochrome P-450 CYP2R1**. The regulation of CYP2R1 is incompletely

understood. 25 hydroxylase activity increases in vitamin D deficiency, but whether the enzyme is regulated by the substrate, product, other vitamin D metabolites, or serum calcium is unclear. The regulation of CYP2R1 is quite imprecise, as very high plasma levels of $25(OH)D_3$ are seen with excessive vitamin D intake and toxicity. $25(OH)D_3$ and all other vitamin D metabolites circulate in the plasma largely bound to a specific binding protein, the serum vitamin **D-binding protein (DBP)**. This glycoprotein is synthesized mainly in the liver and has a single high affinity binding site for vitamin D metabolites. $25(OH)D_3$ bound to DBP is taken up by the kidney and certain other tissues by receptor-mediated endocytosis.

In the ***kidney***, $25(OH)D_3$ is converted to **$1,25(OH)D_3$**, the form of the vitamin that is biologically active in the regulation of calcium and bone metabolism. The renal **$25(OH)D_3$ 1-hydroxylase** is a mitochondrial enzyme that is highly regulated. Enzyme activity is increased by parathyroid hormones secreted in response to hypocalcemia. The elevated $1,25(OH)D_3$ level then stimulates intestinal calcium absorption and together with parathyroid hormone enhances calcium mobilization from bone and renal tubular calcium resorption, thereby correcting the hypocalcemia and completing a feedback loop. $1,25(OH)_2D_3$ production is also regulated by two other feedback loops. **FGF23** is a hormone that is secreted by bone in response to an elevation in the serum phosphate level. FGF23 acting through its **receptor (FGFR)** and a **coreceptor (klotho)** inhibits CYP27B1 in the kidney, suppresses parathyroid hormone, and like parathyroid hormone causes ***phosphaturia***. $1,25(OH)_2D_3$ inhibits intestinal phosphate absorption and bone FGF23 production. $1,25(OH)_2D_3$ also directly represses CYP27B1 transcription and inhibits parathyroid hormone secretion. A variety of hormones, including insulin, estrogen, progesterone, prolactin, and growth hormone, have also been shown to raise plasma $1,25(OH)_2D_3$ levels, and these responses are likely to be important for meeting the increased mineral requirements during growth, pregnancy and lactation. Thus, vitamin D can be considered as a component of a complex endocrine system geared to the defense of serum calcium and phosphorus levels and to the provision of adequate minerals for bone formation.

A **vitamin D_3 receptor (VDR)** has been identified in many tissues, including the intestine. This receptor belongs to a large superfamily of receptor proteins involved in **transcriptional regulation**, including steroid hormone, thyroid, and retinoic acid receptors. $1,25(OH)_2D_3$ binds to the VDR, and the complex interacts with specific response elements in certain genes to either induce or repress transcription. Over 100 genes have been identified that are regulated by $1,25(OH)_2D_3$, including calcium channels, calcium-binding proteins and calcium-transporting ATPases involved in enterocyte calcium transport. The VDR is expressed in many tissues other than the classic target organs of intestine, bone, and kidney involved in mineral physiology. In addition to its effects on mineral and bone metabolism, $1,25(OH)_2D_3$ has also been demonstrated to influence cell proliferation and differentiation in many tissues, to modulate immune function, to alter insulin secretion, and to have other cellular

effects. In addition to its regulation of gene transcription, $1,25(OH)_2D_3$ can also induce rapid cellular responses that do not require new mRNA synthesis.

6.3 Intestinal Absorption of Vitamin D_3

Vitamin D is present in the diet mainly as free cholecalciferol and ergocalciferol, and thus no intraluminal digestion is required. Vitamin D_3 has limited water solubility, but does aggregate to a micellar-like form at a concentration of about 10^{-8} mM/L. At low concentrations, therefore, vitamin D_3 can be absorbed in the absence of bile salts, if the intraluminal fluid contains only small amounts of other lipids. Under conditions reflecting the typical postprandial situation, where fatty acids, monoglycerides, and other lipids are present at high concentrations, absorption of vitamin D_3 is highly bile salt-dependent. Bile salts are needed to solubilize vitamin D_3 in the mixed micellar phase and to prevent it from portioning into the lipid droplets. Patients with **intraluminal bile salt deficiency** from *cholestatic liver disease* or from *ileal disease* or *resection* causing bile salt malabsorption have **very poor absorption of dietary vitamin D_3**.

Recent studies have shown that some of the transport proteins involved in the absorption of cholesterol and other lipids, such as SR-B1, CD-36, and NPC1L1, facilitate intestinal vitamin D_3 uptake. At high concentrations, vitamin D is likely absorbed by **passive diffusion**. Most of the absorbed vitamin D_3 is incorporated into chylomicron particles and secreted into the intestinal lymph. Within the lymph, there is transfer of the vitamin from chylomicrons to unoccupied DBP, a process that continues in the bloodstream. Some of the absorbed vitamin D_3 is released from the enterocyte by a **nonchylomicrondependent pathway** directly into the portal venous circulation, where it associates with DBP. In the absence of luminal bile salts, most of the absorbed vitamin D_3 appears in portal blood, whereas bile salts favor incorporation into chylomicrons.

6.4 Absorption of Vitamin D Metabolites

The hydroxyvitamin D metabolites **$25(OH)_2D_3$** and **$1,25(OH)_2D_3$** are not significant dietary sources, but are important as pharmacologic agents for the treatment of metabolic bone diseases and disorders of mineral metabolism. Studies in the rat have shown that these forms of the vitamin are more efficiently absorbed than vitamin D_3 itself and are less dependent on intraluminal bile salts and chylomicron production and secretion. These differences likely reflect the somewhat **greater water solubility** of these compounds. Clinical studies have also demonstrated that $25(OH)D_3$ is better absorbed than vitamin D_3 in normal humans and particularly in patients with digestive diseases causing intraluminal bile salt deficiency and steatorrhea.

6.5 Catabolism and Excretion of Vitamin D Metabolites

Renal mitochondria also contain a **24-hydroxylase enzyme (CYP24)** that produces **24,25-dihydroxyvitarnin D_3 [24,25$(OH)_2D_3$]**. Factors that stimulate the 1-hydroxylase (parathyroid hormone and low 1,25$(OH)_2D_3$) depress the 24-hydroxylase, whereas agents that inhibit the 1-hydroxylase (1,25$(OH)_2D_3$ and low parathyroid hormone) activate the 24-hydroxylase. This reciprocal relationship has led to the conclusion that the **formation of 24,25$(OH)D_3$ represents the initial step** in a pathway for degradation of 25$(OH)D_3$ and for regulation of the circulating 1,25$(OH)_2D_3$ level. 24,25$(OH)_2D_3$ is further metabolized in the kidney by a side-chain oxidation pathway that proceeds through several oxo- and keto-intermediates and culminates in oxidation and cleavage of the side chain. When radioactive vitamin D_3, 25$(OH)D_3$, 24,25$(OH)_2D_3$, or 1,25$(OH)_2D_3$ are injected intravenously, most of the radioactivity is recovered in bile, with lesser amounts in urine. In bile, the radioactivity is present in an array of lipid- and water-soluble products, probably generated mainly by the side-chain oxidation pathway described above, including conjugates with glucuronic acid, sulfate, and amino acids.

Many extra-renal tissues, including the intestine, also express CYP27B1 and CYP24. These enzymes in extra-renal tissues are not influenced by factors such as parathyroid hormone that act in the kidney to control the plasma 1,25$(OH)_2D_3$ concentration, but instead are involved in the regulation of 1,25$(OH)_2D_3$ at the local tissue level.

7 Vitamin E

7.1 Structure and Biological Activity

Vitamin E is a major lipid-soluble antioxidant present in plasma and cellular membranes. Vitamin E does not prevent the formation of carbon-centered radicals, but because it reacts more rapidly with peroxyl radicals than do polyunsaturated fatty acids, it traps peroxyl radicals and breaks the chain reaction of lipid peroxidation. **α-tocopherol**, the most active form of vitamin E, reacts with peroxyl radical to form an α-tocopheroxyl radical that is resonance stabilized and breaks the chain reaction. Subsequently, α-tocopherol is regenerated by ascorbic acid and potentially by other agents such as glutathione or uric acid. Alternatively, two α-tocopheroxyl radicals can react together, forming a dimer, or the radical can be completely oxidized to tocopherol quinone. Vitamin E also regulates gene expression and alters cell signaling and proliferation.

Vitamin E occurs in **eight forms: α-, β-, γ-,** and **δ-tocopherols** (which have a chromanol ring and a phytyl tail and differ in the number and position of methyl groups on the ring), and **α-, β-, γ-,** and **δ-tocotrienols** (which have unsaturated tails) (Fig. 8.9). The eight forms of vitamin E differ considerably in their biological

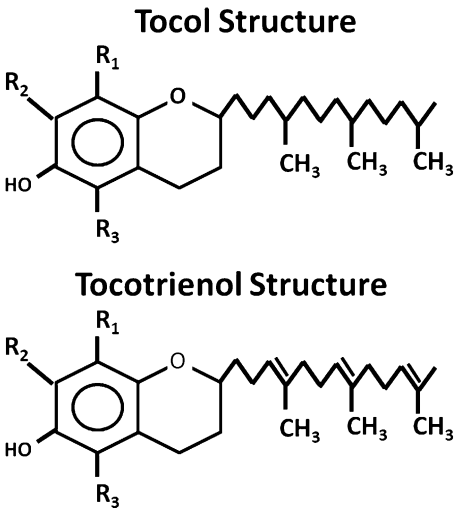


Fig. 8.9 Structures of tocol and tocotrienol forms of vitamin E

activity. This is due both to differences in their intrinsic antioxidant activities and to important differences in bioavailability. α-tocopherol is the most important form of the vitamin for human nutrition, accounting for about 90 % of the vitamin E in tissues.

In experimental animals, vitamin E deficiency causes many different types of physiological impairment and tissue damage, depending on the species studied and on dietary and other factors producing oxidant stresses. **Severe vitamin E deficiency** in humans occurs mainly in premature infants and in patients with diseases leading to impaired fat absorption. In patients with malabsorption syndromes, vitamin E deficiency causes a ***reduced red blood cell life span, neurologic dysfunction,*** and ***myopathies***. In premature infants, deficiency has been linked to ***anemia, intraventricular hemorrhage*** in the brain, ***lung disease (bronchopulmonary dysplasia),*** and ***blindness (retrolental fibroplasia)***. Vitamin E nutritional status has also been associated with development of certain *cancers, heart disease,* and *impaired immune responses*.

7.2 Dietary Sources of Vitamin E

The richest sources of vitamin E in the American diet are **vegetable oils** (soybean, corn, cottonseed, and safflower) and products made from these oils, such as margarine and shortening. Wheat germ, nuts, and green leafy vegetables also contain appreciable amounts of this nutrient. Data from studies in experimental animals and to some extent in humans indicate that the requirement for vitamin E increases as dietary polyunsaturated fatty acid intake increases.

7.3 Intestinal Absorption of Vitamin E

The fractional intestinal absorption of vitamin E has varied from 20 % to 80 % in different studies. The absorptive efficiency declines with increasing dose and is influenced by ingestion of other dietary lipids. There do not appear to be major differences in the extent or rate of absorption of the different forms of vitamin E. Bile salts are required for solubilization of the vitamin in mixed micelles. Pancreatic secretions also facilitate vitamin E absorption, but this is likely due to effects on the formation of mixed micelles rather than a specific action on dietary vitamin E digestion or uptake. For pharmaceutical purposes, vitamin E is often given as **α-tocopheryl acetate** because of its greater stability. α-tocopheryl acetate is hydrolyzed prior to intestinal absorption, and only α-tocopherol appears in the circulation. Pancreatic and enterocyte enzymes are responsible for the hydrolysis of α-tocopheryl acetate.

Some of the same transport proteins that mediate cholesterol absorption are involved in vitamin E absorption. SR-B1 has been shown to mediate both the uptake and efflux of vitamin E across the brush-border membrane. NPC1L1 is involved in the apical membrane uptake of both α- and γ-tocopherol. Several intracellular vitamin E binding proteins have been identified in different tissues, but their role in intracellular vitamin E transport in the enterocyte has not been established.

Most of the absorbed vitamin E is secreted from the intestine in chylomicrons, but some is transported via the basolateral ABCA1 transporter and incorporated into HDL particles. Some absorption of vitamin E directly into the portal venous circulation has been demonstrated in laboratory animals.

7.4 Vitamin E Transport in Plasma, Tissue Uptake, and Catabolism

As chylomicrons are metabolized in muscle, adipose tissue, and other organs, some vitamin E is taken up by these tissues. **Lipoprotein lipase** plays a role in the transfer of vitamin E. There is also movement of vitamin E from chylomicrons to circulating **high-density lipoproteins (HDL)**. In turn, HDL particles can transfer the newly acquired vitamin E to all of the other plasma lipoproteins. The vitamin E remaining in chylomicron remnants is taken up into the liver and is re-secreted in **very low-density lipoproteins (VLDL)**. There is preferential incorporation of the stereoisomer RRR-α-tocopherol into nascent VLDL for secretion into the plasma, whereas other forms of vitamin E are largely metabolized in the liver and targeted for excretion in bile or urine. For example, γ-tocopherol is abundant in the human diet and is absorbed as efficiently as α-tocopherol, but relatively little is retained in tissues. In the hepatocyte, **α-tocopherol-transfer protein (α-TTP)** has binding specificity for α-tocopherol, and selectively directs that form of vitamin E for incorporation into VLDL and secretion from the liver. **Mutations in the gene encoding α-TTP** cause ***vitamin E deficiency*** and ***neurological abnormalities*** such

as *ataxia*. Vitamin E absorption is normal in these patients, but incorporation of vitamin E into VLDL and hepatic secretion is impaired.

The α-tocopherol secreted in VLDL can have several metabolic fates. Some will be taken up by peripheral tissues or transferred to HDL during lipolysis of the VLDL triglyceride, as was described previously for chylomicrons. Some α-tocopherol will remain associated with VLDL during its metabolism and return to the liver with VLDL remnants or become part of low-density lipoproteins (LDL). Cells containing the LDL receptor will efficiently take up LDL α-tocopherol by a receptor-mediated process. Vitamin E is also incorporated into HDL secreted by the liver into the circulation using the ABCA1 transporter, and HDL likely can deliver some α-tocopherol to peripheral tissues. It should be appreciated therefore that multiple pathways exist for supplying peripheral tissues with α-tocopherol, using chylomicrons, VLDL, LDL, and HDL as transport vehicles. It is not surprising therefore that **in situations where one of these mechanisms is defective, normal tissue α-tocopherol levels are *still* attainable**. For example, patients with homozygous familial hypercholeserolemia, who have defective LDL-receptor activity, do not have clinical or biochemical vitamin E deficiency.

The **liver** is the primary site of α-tocopherol catabolism and excretion. The primary hepatic oxidation product is **α-tocopherol quinone,** which is further reduced to the **hydroquinone**. This compound is then conjugated with glucuronic acid and excreted in bile or degraded in the kidney to **α-tocopheronic acid**, followed by conjugation and elimination in the urine. Several transporters including SR-B1, NPC1L1, and the multidrug resistance 3 protein appear to mediate vitamin E transport across the canalicular membrane.

8 Vitamin K

8.1 Structure and Biological Activity

Vitamin K refers to a group of compounds that *all contain* a **2-methyl-l, 4-naphthoquinone-ring structure**, but *differ* in the structure of the **side chain** at the **carbon 3 position** (Fig. 8.10). The form found in plants, **phylloquinone** (also called **phytonadione, vitamin K_1**) contains a 20-carbon phytyl group at carbon-3. Bacteria synthesize a family of compounds known as **menaquinones (vitamin K_2)** that contain polyisoprenyl side chains 4–13 isoprenyl units long. Animal tissues contain small amounts of both phylloquinone and menaquinones. **Menadione (vitamin K_3)** is a synthetic compound that has no side chain, but its water-soluble derivatives are alkylated in the liver to biologically active menaquinones. Menadione, however, can combine with sulfhydryl groups in membranes and cause ***hemolytic anemia, hyperbilirubinemia,*** and ***kernicterus*** in infants and, therefore, should not be used as a therapeutic form of vitamin K.

The biological function of vitamin K is to serve as an essential **cofactor for a specific post-translational modification** of certain proteins in which selected

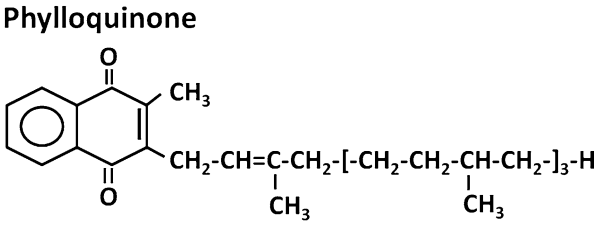
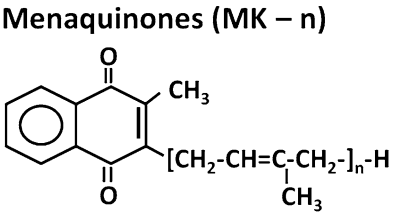


Fig. 8.10 Structures of phylloquinone and menaquinone forms of vitamin K



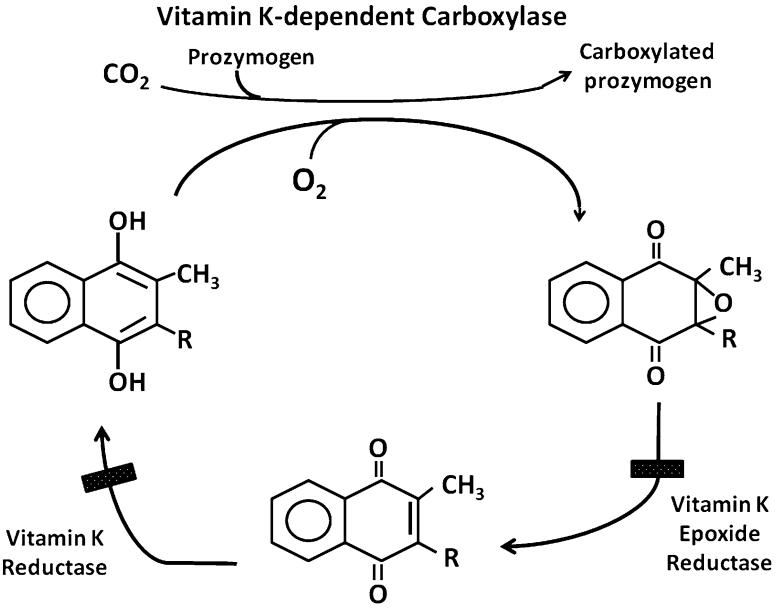


Fig. 8.11 Vitamin K cycle. Post-translational modification of prothrombin by vitamin K. *Shaded bars*, sites of inhibition by warfarin anticoagulants (Adapted from American Gastroenterological Association Teaching Slide Collection 13B ©, Bethesda, Maryland, slide 68; Used with permission)

glutamic acid residues are transformed to γ-carboxyglutamic acid (Gla) residues (Fig. 8.11). **Four clotting factors** synthesized in liver, ***factors II (prothrombin), VII, IX,*** and ***X,*** undergo this post-translational modification, which is required for these factors to bind calcium and interact with phospholipid in cellular membranes. **Vitamin K deficiency** is manifested clinically by ***defective blood coagulation*** and

excessive bleeding; the serum of vitamin K-deficient patients contains inactive clotting factors lacking the Gla residues. Additional proteins in liver and in other tissues also undergo **vitamin K-dependent γ-carboxylation**, including proteins C and S, which are inhibitors of coagulation, and the bone proteins osteocalcin and matrix Gla-protein, which play a role in calcification of bone.

8.2 Dietary Sources of Vitamin K

Phylloquinone is the major dietary form of vitamin K. **Green leafy vegetables** are the best source, providing about 100–500 μg/100 g of food. Smaller amounts of vitamin K are present in milk and other dairy products, meats, eggs, cereals, other vegetables, and fruits, oils, and margarine. Human milk is relatively low in vitamin K, containing about 2 μg/L.

8.3 Intestinal Absorption of Phylloquinone

Phylloquinone is **highly insoluble in water**, and bile salts are therefore required for solubilization in mixed micelles. **Severe vitamin K malabsorption** occurs in patients with ***cholestatic liver diseases***, and vitamin K deficiency is more common in this group than in other gastrointestinal diseases. Pancreatic secretions also facilitate phylloquine absorption, probably by generating fatty acids and β-monoglycerides that are components of mixed micelles.

Studies of phylloquinone absorption in the rat demonstrated that intestinal uptake was saturable and dependent on metabolic energy. These features suggest the involvement of a plasma membrane transporter or intracellular binding proteins, but these components have not been definitively identified to date. Other lipids such as fatty acids, monoglycerides, and phospholipids have been found to influence phylloquinone absorption in experimental animal models.

Most of the absorbed phylloquinone is incorporated into chylomicrons and transported via intestinal lymph to the circulation. Phylloquinone is taken up into the liver as a component of chylomicron remnants, and subsequently some is secreted from the liver in VLDL and HDL and distributed to other tissues. A portion of the absorbed phylloquinone is released from the intestine into the portal venous circulation.

8.4 Absorption and Utilization of Menaquinones

The extent to which menaquinones produced by bacteria in the GI tract are absorbed and utilized in the vitamin K dependent γ-carboxylase reaction is very controversial. Measurements of the vitamin K content of human liver have shown that 75–90 % of hepatic vitamin K is in the form of various menaquinones, with menaquinones-7, -9,

and -11 being the predominant forms. Hepatic menaquinones could represent absorption of the small amount of menaquinones present in the diet or absorption of menaquinones derived from gut bacterial production, with accumulation in the liver due to a relatively slow turnover rate. Studies using various experimental preparations of rat small and large intestine have demonstrated limited uptake of menaquinones into all intestinal segments. **Menaquinone-4**, a minor bacterial form, was slowly absorbed from the jejunum and appeared in both mesenteric lymph and portal blood, whereas absorption from the colon occurred mainly into the portal circulation. In contrast, **menaquinone-9**, a typical bacterial form, was slowly absorbed from the jejunum and transferred mainly to lymph, whereas no transfer to lymph or to the portal circulation was seen when menaquinone-9 was administered in the colon. These data suggest therefore that menaquinones ingested in the diet, produced by small bowel bacteria, or synthesized by colonic bacteria can, at least to some extent, be absorbed and delivered via the mesenteric lymph and portal blood to the circulation. In the colon, however, there is only limited absorption of menaquinones into the portal circulation, and the absorption rate markedly declines with an increase in the number of isoprenoid units in the side chain. These differences likely reflect the low concentration of bile salts and the lack of formation of chylomicrons in the colon.

Studies in rats and in humans have demonstrated that consuming a vitamin K-deficient diet rapidly causes a fall in hepatic and plasma phylloquinone, with little change in liver menaquinones, and induces biochemical changes of vitamin K deficiency. In some studies, however, relatively mild vitamin K deficiency was produced, and in others the biochemical changes reverted toward normal in spite of continuing the vitamin K-deficient diet. These data can, therefore, be interpreted as indicating that **gut bacterial vitamin K production is not sufficient to ensure completely normal vitamin K nutritional status, but may contribute sufficiently to prevent severe deficiency**. Human studies and clinical experience indicate that clinically apparent vitamin K deficiency and bleeding rarely occurs due to dietary lack, but is regularly seen in patients with the combination of poor dietary intake and use of broad-spectrum antibiotics. Interpretation of this observation is complicated as some antibiotics have direct effects on coagulation independent of alteration of gut bacterial flora.

8.5 Hepatic Vitamin K Metabolism

The forms of vitamin K absorbed from the GI tract have a stable quinone structure. The **vitamin K-dependent carboxylase** that catalyzes the conversion of glutamate residues to γ-carboxyglutamate residues uses the quinol form of the vitamin (Fig. 8.12). This carboxylase also requires molecular oxygen, carbon dioxide, and the precursor of the vitamin K-dependent protein as a substrate. **Vitamin K-dependent clotting factors** contain a **carboxylation recognition site** in the proprotein region that designates an adjacent glutamic acid-rich domain for

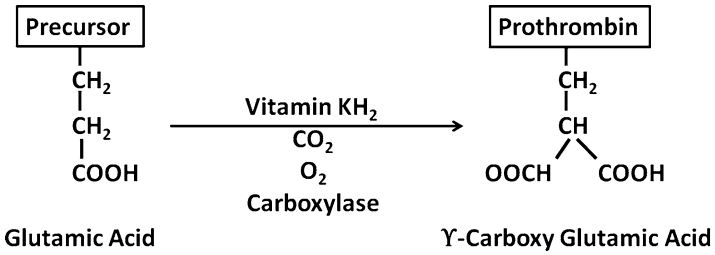


Fig. 8.12 Vitamin K-dependent post-translational modification of prothrombin to the ϒ-carboxylated form (Adapted from American Gastroenterological Association Teaching Slide Collection 13B ©, Bethesda, Maryland, slide 69; Used with permission)

γ-carboxylation. This γ-carboxylation recognition site probably directly binds to the vitamin K-dependent carboxylase, which is an integral endoplasmic reticulum membrane protein. Following γ-carboxylation, the substrate protein is released and transported to the Golgi apparatus for further processing and secretion. During **γ-glutamyl carboxylation**, the vitamin K quinol is simultaneously converted to the **vitamin K 2,3-epoxide** (Fig. 8.12). Evidence indicates that the same enzyme contains both the carboxylase and epoxidase activities. The epoxide is then recycled by two membrane-bound enzyme activities. First, the epoxide is reduced to vitamin K quinone by a **dithiol-dependent reductase**; and second, the quinone is reduced back to the active quinol form. Again, these two reactions may be catalyzed by the same enzyme. The **warfarin-type anticoagulants** are potent ***inhibitors*** of the activities of dithiol-dependent vitamin K-epoxide reductase and vitamin K-reductase. Vitamin K is therefore trapped in the epoxide form, resulting in deficiency of the active quinol, reduced γ-glutamyl carboxylation, and diminished production of active clotting factors. A second **NADPH-dependent pathway** that is relatively ***insensitive*** to warfarin exists for reduction of vitamin K to the quinol form. In the **treatment of warfarin overdose** with **large doses of vitamin K**, vitamin K is converted to the quinol using this alternative pathway, reversing the anticoagulant effect.

Under normal physiological conditions, 30–40 % of absorbed vitamin K is excreted into bile as partially degraded, conjugated, water-soluble metabolites, whereas 15 % is excreted as water-soluble forms in the urine.

Clinical Correlations

Case Study 1

A 42-year-old woman with a history of Crohn's disease for many years had a resection of 3 ft of terminal ileum 5 years ago. Since then, she has taken cholestyramine, a bile salt-binding resin, for chronic diarrhea. She now complains of diffuse bone pain, most severe in the lower back and upper legs, and is noted to be hypocalcemic. Her serum 25-hydroxyvitamin D_3 level is 5 ng/mL (normal: 10–50 ng/mL).

Questions

1. **Why would a patient with an ileal resection develop vitamin D deficiency?**
   Answer: Patients with a resection of 3 ft of ileum have **bile salt malabsorption**, diminished enterohepatic circulation, and a reduced bile salt pool. Because of intraluminal bile salt deficiency, they have **malabsorption of dietary lipids**, including **vitamin D**.
2. **How might the use of cholestyramine contribute to her vitamin D depletion?**
   Answer: Malabsorbed bile salts cause colonic fluid and electrolyte secretion and diarrhea. A **bile salt binding resin** can therefore be *useful* in the ***treatment of watery diarrhea*** in patients with **small ileal resections** (less than 3 ft), where increased hepatic bile synthesis maintains the bile salt pool size. In patients with **larger ileal resections**, increased bile salt synthesis cannot maintain a normal pool size, cholestyramine treatment will further reduce the enterohepatic circulation and the intraluminal bile salt concentration and will *worsen* lipid malabsorption.
3. **Why might treatment with 25(OH)D_3 orally be preferable to supplementation with vitamin D_3?**
   Answer: 25(OH)D_3 has been shown to be **more efficiently absorbed** than vitamin D_3 in patients with ileal resections. Absorption of 25(OH)D_3 is less dependent on intraluminal bile salts, probably because it has somewhat greater solubility in an aqueous system than does vitamin D_3.

Case Study 2

A 14-year-old boy with cystic fibrosis has pancreatic insufficiency and is being treated with enzyme replacement. Because of worsening pulmonary symptoms, he is admitted to the hospital for intensive respiratory therapy and broad spectrum antibiotics. At the time of admission, he has a normal prothrombin time, a serum alkaline phosphatase that is five times normal, and serum transaminases that are twice normal. After 1 week of treatment in the hospital, he complains of a severe nosebleed and is now noted to have a prothrombin time of 21 s (normal, 10–13 s).

Questions

1. **What factors are responsible for the development of an abnormal prothrombin time during his hospitalization?**
   Answer: The patient would likely have some degree of **vitamin K malabsorption** due to his pancreatic insufficiency. Cystic fibrosis can also cause cholestatic liver disease. An **elevated prothrombin time**, however, occurs when the ***level of active γ-carboxylated prothrombin falls to less than 30 % of normal***, a deficit that correlates with a clinically significant bleeding risk. During the hospitalization, he may have been eating more poorly than usual because of his worse pulmonary symptoms. In addition, the **broad-spectrum antibiotics** would alter his intestinal flora and decrease bacterial menaquinone synthesis. Although the contribution of these menaquinones to vitamin K nutritional status is still controversial, most data suggest that they are absorbed to some extent and may

provide enough vitamin K to prevent severe deficiency. Some antibiotics directly interfere with coagulation independent of effects on gut flora.

2. **How does pancreatic insufficiency cause vitamin K malabsorption?**
   Answer: Dietary vitamin K does not require intraluminal digestion. The vitamin K malabsorption seen in pancreatic insufficiency is likely due to **abnormal formation of mixed micelles** and to the **trapping of vitamin K within lipid droplets** composed of undigested triglyceride and other lipids.
3. **How might the patient's abnormal liver chemistries relate to his vitamin K depletion?**
   Answer: Some patients with cystic fibrosis develop ***cholestasis*** and ***biliary cirrhosis*** due to inspissation of bile in the biliary tract. Cholestasis leads to **severe vitamin K malabsorption**, as bile salts are required for solubilization of vitamin K in mixed micelles.

Case Study 3

A 70-year-old man has extensive diverticulosis of the small intestine. He complains of chronic diarrhea, bloating, and a 20-lb weight loss. On laboratory testing, he is noted to have 30 *g*/day of steatorrhea while ingesting a 100 g/day-fat diet. A bile acid breath test is performed, in which cholyl-^{14}C-glycine is given by mouth and breath samples are collected for measurement of $^{14}CO_2$. The patient is found to have a markedly elevated breath $^{14}CO_2$ excretion.

Questions

1. **How do you explain the results of the bile acid breath test?**
   Answer: In a normal individual, the administered cholyl-^{14}C-glycine is efficiently absorbed by the **ileal Na^+-bile salt cotransporter** and enters the enterohepatic circulation. In this patient with small bowel diverticuli, bacteria proliferate in the stagnant environment of the diverticuli. These bacteria deconjugate the ^{14}C-glycine and the ^{14}C-glycine is absorbed and metabolized, forming $^{14}CO_2$ that appears in the breath.
2. **Why would bile salt deconjugation result in fat malabsorption?**
   Answer: Unconjugated bile acids are, to some extent, passively absorbed by the small bowel. Deconjugation of bile acids by bacteria in the proximal intestine will, therefore, result in **absorption of the unconjugated bile acids in the proximal jejunum** and cause a decrease in the intraluminal bile salt concentration in the remaining small bowel. In addition, unconjugated bile acids have **limited solubility** in an aqueous system and will tend to precipitate in the luminal contents. These two factors can result in intraluminal bile salt deficiency and fat malabsorption.
3. **What other disease process would give a similar bile acid breath test?**
   Answer: Ileal dysfunction causes bile salt malabsorption. The bile salts are then deconjugated in the colon, and the $^{14}CO_2$ is excreted in breath. Thus, an abnormal bile acid breath test is also characteristic of patients with **ileal disease** or **resection**. Although on average, the $^{14}CO_2$ appears in the breath earlier in patients with small bowel bacterial overgrowth than in those with

ileal dysfunction, intestinal transit time is so variable that this cannot be used to reliably distinguish between these two causes of an abnormal bile salt breath test.

Case Study 4

A 55-year-old woman has advanced primary biliary cirrhosis. She is jaundiced with a serum bilirubin of 11.0 g/dl. Laboratory testing shows that she has a very low serum retinol level.

Questions

1. **Why would this woman have vitamin A malabsorption?**
   Answer: Patients with advanced primary biliary cirrhosis have cholestasis and intraluminal bile salt deficiency. Bile salts are required for solubilization of retinol and carotenes in mixed micelles, permitting efficient intestinal absorption. Pancreatic bile salt-activated lipase is also one of the enzymes that hydrolyze retinyl esters prior to intestinal absorption.
2. **What other factor would contribute to the very low serum retinol level in this patient?**
   Answer 2: Retinol is secreted from the liver into the circulation bound to serum retinol binding protein (RBP). Hepatic synthesis and secretion of RBP would also be impaired in advanced liver disease, and both serum RBP and serum retinol levels would be low in this patient.
3. **What symptoms might this patient experience?**
   Answer 3: The patient might have visual problems, particularly poor vision when in dim light (night blindness). She could also have a skin rash, damage to the conjunctivae leading to eye infections and eventually blindness, immune dysfunction, and bone disease.

Further Reading

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