

Review article

# Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk

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## Abstract

Triglycerides and cholesterol are important biological lipids, and their excessive intake in the diet is relevant to the development of two prevalent cardiovascular risk factors, obesity and hypercholesterolemia. Because most lipids are essentially water-insoluble molecules, their transport within and absorption from the aqueous medium of intestinal contents is rather complex. This takes place in a series of orderly and interrelated steps, including emulsification, hydrolysis by specific esterases, micellar transport, mucosal absorption, re-synthesis of parent molecules in enterocytes, and assembly with apolipoproteins and other molecules to form chylomicrons, the secretory product of intestinal cells. Many of these processes, however, are not well characterized at the molecular level. While in health the intestinal absorption of triglycerides is very efficient, the same does not apply to cholesterol absorption. Besides being generally inefficient, cholesterol absorption is highly variable, with a between-subject variability that depends in part on genetic factors and an intra-individual variability, which may be modulated by physiological and dietary conditions. All of the sequential steps in intestinal lipid absorption can be interfered with by dietary components or drugs and thus are potential therapeutic targets for inducing a controlled malabsorption of triglyceride, useful in the treatment of obesity, or for rendering cholesterol absorption even more inefficient in an attempt to lower blood cholesterol levels. Nevertheless, intestinally derived cholesterol available to the liver exerts complex feedback regulation on whole-body cholesterol homeostasis that limits the efficacy of cholesterol absorption inhibitors to lower blood cholesterol. This review focuses first on present knowledge of the physiology of intestinal fat absorption, necessary to understand the ways to manipulate it in order to obtain the desired effects on dietary triglyceride and cholesterol disposition. The second part discusses old, present and future ways, both dietary and pharmacological, of interfering with cholesterol and triglyceride absorption to reduce blood cholesterol and energy acquisition, respectively. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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## 1. Introduction

The most important function of the digestive tract is to transform the energy from food in such a way that body tissues may have access to absorbable and usable energy sources. Absorption means transport of substrates from the aqueous medium of the intestinal lumen through the barrier of the intestinal mucosal cells to the blood or the lymphatic system, and this process

is intimately linked to the digestion and physical–chemical transformation of nutrients because usually they are ingested as macromolecular aggregates (starch, triglycerides, proteins), but the usable metabolic substrates from the diet that are transported from the intestine to the tissues are monomers (glucose, amino acids) or, especially, reconstituted aggregates (chylomicrons).

Fat is an important energy source from food in the Western world, representing 30–40% of the daily caloric intake, equivalent to the daily consumption of 60–120 g in an adult. More than 95% of dietary fat is

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long-chain triglycerides; the remaining dietary fat is made up of phospholipids (2–4 g daily), such as phosphatidylcholine (lecithin) and other emulsifiers used mainly as stabilizers of industrially prepared foods, fatty acids, cholesterol (200–600 mg/day), and fat-soluble vitamins [1]. Bound for intestinal absorption are not only dietary lipids, but also phospholipids and cholesterol originating in the liver and transported in bile, where they are solubilized by way of the detergent capacity of bile acids. Biliary phospholipids, again almost exclusively formed by lecithin, contribute about 90% to the total phospholipid load absorbed daily. All lipids are made up of a relatively bulky hydrocarbon molecule, meaning that they are hydrophobic or apolar, and the problem of their insolubility in water has determined the development in higher organisms of complex mechanisms to digest and absorb them. With the exception of esterified cholesterol, biologically active lipids found in foods and in bile also possess hydrophilic parts in their molecules (Fig. 1). These polar groups both permit the interaction of lipids with the aqueous environment of intestinal contents and define their potential for absorption [2]. Because they are essentially hydrophobic molecules, but in their natural state or after hydrolysis also possess hydrophilic components, dietary lipids require a series of physicochemical changes before they are able to enter intestinal cells and can be transported in the circulation.

The first part of this paper reviews present knowledge on the mechanisms of digestion and absorption of the lipids with most pathophysiological interest, triglycerides and cholesterol. The dietary intake of fat has received considerable attention in the past few decades because of its link with an increased risk of coronary heart disease (for high intakes of saturated fatty acids and cholesterol) and obesity (for a high intake of total fat). The second part of this review deals with the growing field of dietary and pharmacological interventions designed to inhibit these same processes of intestinal triglyceride and cholesterol absorption, aiming at reducing the burden of obesity and hypercholesterolemia, principal targets of preventive medicine in the Western world at the end of the millennium. The intestine is also the main site of action of certain diet components and drugs that are meant to lower blood cholesterol levels by interference with bile acid absorption, either through binding bile acids within the intestinal lumen (classical agents are soluble dietary fiber and anion exchange resins), or by the new modality of specifically blocking their intestinal uptake, in as much as the recent identification of the ileal sodium-dependent bile acid transporter has facilitated the development of a new and promising class of cholesterol-lowering agents. These subjects are beyond the scope of this work and have been reviewed in depth recently [3–6].

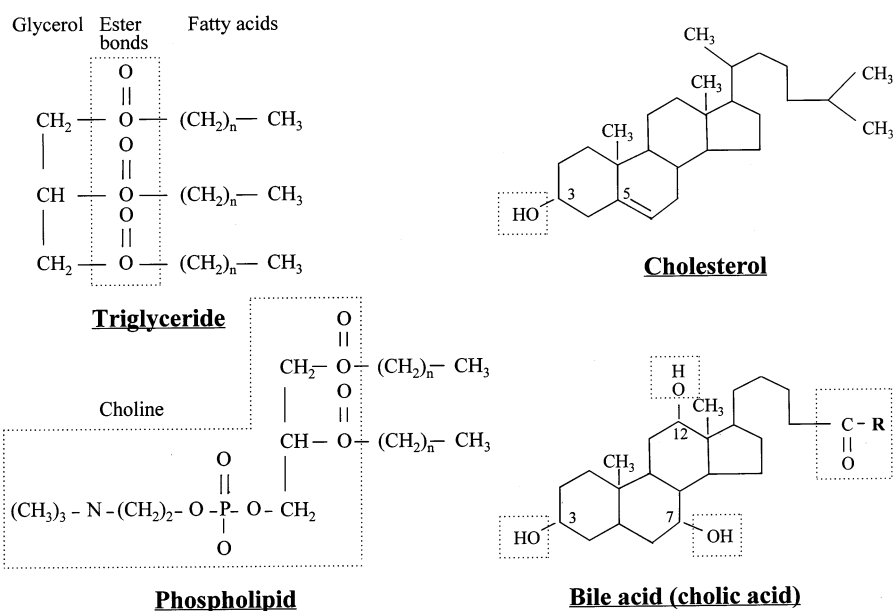


Fig. 1. Chemical structure of four lipid molecules of biological relevance, showing the variability of hydrophobic (fatty acid hydrocarbon chains and steroid nucleus) and hydrophilic domains (ester bonds, hydroxyl groups, choline; shown inside broken lines) that direct their interaction with water. Because hydrophilic groups are hidden inside the molecule, triglycerides are practically insoluble in water, a situation that changes when the ester bonds are broken and become acid or alcohol radicals. Phospholipids have a polar moiety (the choline group and ester bonds) and an apolar one (two fatty acids molecules), which make them swelling amphiphilic lipids that spread to form stable monolayers at an air–water interface and behave as liquid crystals in aqueous systems. Free cholesterol has an isolated polar group at C:3 that reacts with a fatty acid to form esterified cholesterol, a totally insoluble molecule. The transformation of cholesterol into bile acids by saturation of the double bond at C:5, acquisition of more hydroxyl groups (in C:7 and C:12 in the case of cholic acid), and shortening of the lateral chain and its acylation with an amino acid (R) originates amphipathic molecules that are water soluble and have detergent capacity.

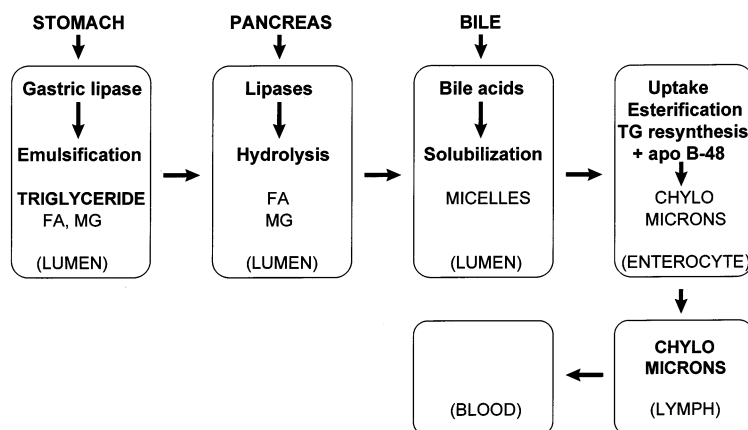


Fig. 2. Successive and coordinated steps in the process of intestinal triglyceride digestion and absorption (see text). FA, fatty acid; MG, monoglyceride; TG, triglyceride.

## 2. Intestinal absorption of triglycerides

The digestion and absorption of triglycerides is a dynamic, complex, and very efficient process that is only partially understood at the molecular level. Its physiological mechanisms were established more than four decades ago with the use, for the first time in humans, of the nonabsorbable marker ethylenglycol [7]. The principal phases in the intestinal absorption of triglycerides are emulsification, hydrolysis of fatty acid ester bonds by specific esterases, aqueous dispersion of lipolytic products in bile acid micelles, and absorption, mainly in the proximal jejunum but also in more distal parts of the small intestine. Ingested fat is an insoluble oil at body temperature, a reason why the basic process in fat absorption is the conversion of this oil into water-soluble compounds that can be efficiently absorbed. The key for this process is the pancreatic enzyme lipase, which splits triglycerides into more hydrophilic products: two molecules of fatty acid and one of 2-monoglyceride. These lipolytic products are still sparingly soluble in the aqueous environment of the intestine, and aqueous dispersion requires their incorporation into bile acid micelles. Micelles are polymolecular aggregates that act as shuttles, delivering fatty acids and monoglycerides to the intestinal microvilli, where they dissociate from micelles and diffuse inside the enterocyte. Subsequently, intestinal cells re-synthesize triglyceride molecules and incorporate them to chylomicrons that are secreted to the intestinal lymph (Fig. 2). The subject of fat digestion and absorption, and chylomicron formation has been reviewed recently [8–13].

### 2.1. Emulsification

The first step in the transformation of an insoluble oil into soluble and absorbable lipids is the formation

of an initial emulsion (chyme) by mastication and, foremost, by the mechanical mixing and grinding action of antral peristalsis in the stomach [14]. This emulsion is stabilized by three kinds of amphipathic molecules, which prevent the coalescence of oil droplets by the formation of a monolayer at its surface: ionized proteins, dietary phospholipids, and limited amounts of fatty acids and monoglycerides from the hydrolysis of triglycerides by lingual and gastric lipases [15] (Fig. 3a). Because it has an optimum pH of 5.4 [15], lingual lipase probably contributes little to lipolysis in the acidic gastric milieu. On the other hand, gastric lipase is stable and active at the acid pH of the stomach, and hydrolyzes one out of every four triglyceride molecules during the digestion of a meal [16,17]. However, due to this same acid pH, gastric lipolysis is limited because the fatty acids formed are insoluble in water by virtue of being protonated (i.e. unionized, because the pH is less than the  $pK_a$ ), are dispersed into the oil phase [2], and are unavailable at the emulsion surface to stabilize it and facilitate lipase action (see later).

Gastric acid hypersecretion in the Zollinger–Ellison syndrome is associated with malabsorption of fat because it interferes with the chemical phase of emulsification by maintaining a very low pH that prevents the ionization of fatty acids [18]. Bile acid precipitation at the low jejunal pH may also interfere with the micellar solubilization of fatty acids. On the other hand, the variable fat malabsorption that may follow gastric surgery is due in part to disruption of the mechanical phase of emulsification. Additional mechanisms are diminished release of gastrointestinal hormones from the vagally denervated small intestine [19] and excessive dilution of upper intestinal contents due to rapid gastric emptying [20]. Afferent loop sequestration of bile acids and pancreatic enzymes may compound the problem when a gastrojejunostomy is constructed [20].

## 2.2. Hydrolysis

Once in the duodenum, the fatty acids from the initial gastric lipolysis, and the amino acids and peptides formed by gastric digestion of dietary protein stimulate specific receptors in duodenal enterocytes to secrete the hormone cholecystokinin, which in turn stimulates gallbladder contraction, delivering bile to the duodenum, and proenzyme release from pancreatic acini, providing a pancreatic juice rich in enzymes, including lipase and colipase. At the same time, the acid pH of gastric contents emptied into the duodenum enhances the release by duodenal cells of the hormone secretin, which in turn promotes the secretion of a bicarbonate-rich fluid by epithelial cells in the biliary and pancreatic ducts [21]. Both hormones stimulate contraction of the pylorus, thus controlling gastric emptying.

Important physicochemical changes take place in the fat emulsion emptied from the stomach when duodenal alkalization (pH 6.5–7.0) ionizes the fatty acids, and their polar ends and those of phospholipids face out to the water phase at the surface of the oil droplets. This chemical emulsification induces a reduction in size of fat droplets from 2–5 to  $\leq 0.5 \mu\text{m}$ ; besides stabilizing them, the smaller size increases proportionally the lipid surface exposed to the catalytic effect of pancreatic triglyceride lipase, an enzyme that acts specifically at the oil/water interface [22].

Pancreatic lipase is the principal lipolytic enzyme. It is a 449-amino acid peptide with a high degree of structural homology with other lipases, such as lipoprotein lipase and hepatic lipase, but not with gastric lipase or hormone-sensitive lipase. All lipases share the pentapeptide Gly-X-Ser-X-Gly as catalytic site, but in the tridimensional structure of the pancreatic enzyme, this active site is hidden by a folding of the molecule on itself. The effect of colipase, secreted by the pancreas together with lipase, is to anchor lipase to the surface of emulsified lipid droplets and to unfold the molecule in order to expose the active site and permit its access to triglyceride molecules, the substrate of hydrolysis [23] (Fig. 3b). The fatty acids produced by the hydrolytic action of lipase accelerate the lipolytic reaction by increasing the affinity of colipase for its zone of interfacial union with lipase. Conversely, bile acids at physiological concentrations in the duodenum inhibit lipase activity, an effect that is reversed by colipase [24]. Colipase is secreted by the pancreas in an inactive form, which is activated by trypsin, the main proteolytic enzyme from the pancreas. Other lipolytic enzymes from the pancreas are phospholipase  $A_2$ , which hydrolyzes the ester bond at position 2 of the phospholipid molecules to produce one molecule of fatty acid and another of 1-lysophospholipid (mainly lysolecithin), and carboxyl ester lipase (also named cholesterol esterase, cholesteryl ester hydrolase, and bile

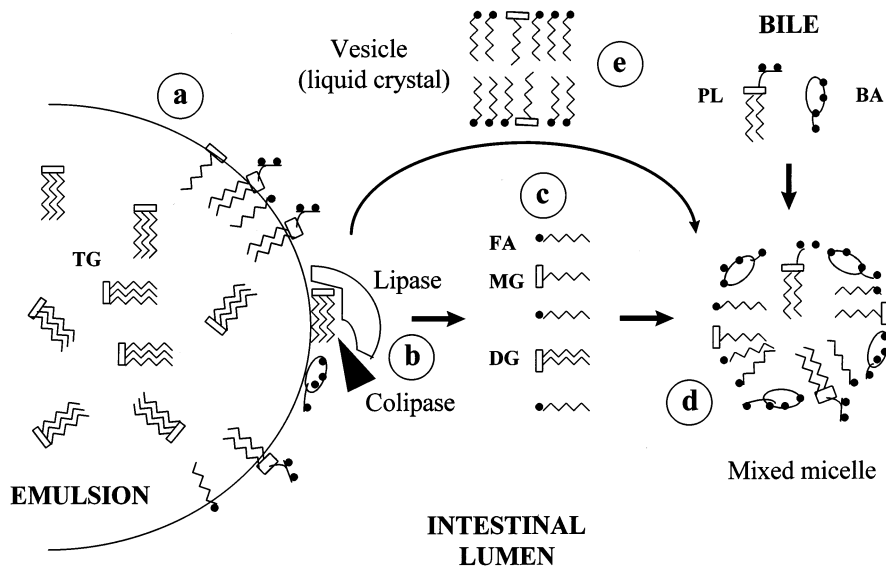


Fig. 3. Digestion of triglycerides in the duodenum and proximal jejunum. In the schematic representation of lipid molecules, black dots and small squares (glycerol) represent polar groups, which are always in close contact with water, while the other part of the molecule is hydrophobic and stays away from the water. (a) Fat emulsion of gastric chyme, with insoluble triglycerides inside oil droplets, stabilized by the products of early lipolysis by gastric lipase, fatty acids (FA) and monoglycerides (MG), and by dietary phospholipids (PL). (b) Union of bile acids (BA) coming from bile and pancreatic lipase to the water–oil interphase, and interaction with colipase to allow exposition of the enzyme's catalytic site. (c) Lipolytic products of triglyceride digestion: fatty acids, monoglycerides, and diglycerides (DG), with limited water solubility. (d) In the presence of bile, solubilization of lipolytic products in bile acid micelles, the ideal lipid transporter in the aqueous phase of intestinal contents. (e) In the absence of bile acids, triglyceride hydrolysis causes the formation of unilamellar or multilamellar vesicles (liquid crystals) of fatty acids and monoglycerides.

salt-activated lipase), 100 times less active than pancreatic lipase in triglyceride digestion but important in the hydrolysis of cholesteryl and retinyl esters, a process that, by increasing the polarity of these highly hydrophobic molecules, permits their micellar solubilization and subsequent intestinal absorption [25].

Lipase has an optimum pH of 8.0, being inactive at a pH < 6.0. The enzyme hydrolyzes emulsified triglyceride on the ester bonds at positions 1 and 3, determining the formation of two fatty acid and one 2-monglyceride molecules [8,9,11,24] (Fig. 3c). Pancreatic lipase is secreted in amounts 100–1000 times greater than those needed to complete the hydrolysis in the duodenum and upper proximal jejunum of the triglycerides contained in a normal meal, a reason why the exocrine pancreatic insufficiency characteristic of advanced chronic pancreatitis causes no fat maldigestion and subsequent steatorrhea until more than 95% of the exocrine pancreas is destroyed. The fact that the rare patients with congenital absence of pancreatic lipase [26] or isolated colipase deficiency [27] have malabsorption of up to 60% of ingested fat underlines the critical importance of this enzyme system in triglyceride digestion.

### 2.3. *Micellar solubilization*

The products of pancreatic lipolysis of dietary fat (fatty acids, 2-monglycerides, lysolecithin, unesterified cholesterol, and fat-soluble vitamins) are lipids that are more polar than the parent molecules, but they still have a limited solubility in the aqueous environment of intestinal contents. Bile provides the ideal detergent for the solubilization of these lipolytic products, the bile acids, molecules that have an amphipathic structure (where the hydrophobic and hydrophilic regions of the molecule are equilibrated (Fig. 1)) permitting them to form micelles, water-soluble polymolecular aggregates with a discoid configuration in which the polar groups of the lipid molecules are at the surface projecting into the aqueous medium, while the apolar hydrocarbon parts are at the core, away from the water [2,28,29] (Fig. 3d). Bile acid micelles acquire polar lipids at the surface of emulsified lipid droplets, thus allowing hydrolysis to proceed unimpeded. Nonmicellar mechanisms of lipid dispersion have also been described. When emulsion droplets shrink during lipolysis, liquid crystalline structures [30] are extruded, consisting of unilamellar and multilamellar vesicles or liposomes of fatty acids and monoglycerides (Fig. 3e), which are formed continuously but quickly dissolve into micelles at the appropriate bile acid concentrations [31–33], likely replenishing mixed micelles that have lost lipid during the process of absorption by enterocytes. It is not known whether such vesicles loaded with hydrolyzed lipid can be absorbed in the absence of bile

acids; if so, it could explain why a substantial proportion (generally, greater than 75%) of dietary fat is absorbed even in the absence of bile acids in the intestinal lumen, as in complete biliary obstruction or fistula [34].

Thus, during the digestion of a fatty meal, the duodenum and proximal jejunum contain a lipid phase of emulsified fat coexisting in dynamic equilibrium with an aqueous phase made up of polar lipid within both mixed micelles and liposomes. The recovery of intestinal contents with a duodenal tube during digestion of a fatty meal and the subsequent ultracentrifugation of the recovered material permits to separate these phases and analyze their composition in physiological studies of fat digestion [28,33,35]. Using this technique in patients with deranged bile acid metabolism, it has been established that the amount of lipid solubilized in the aqueous phase is greatly reduced and steatorrhea appears when intestinal bile acid levels fall below a critical micellar concentration of 3–5.4 mM [36–38].

### 2.4. *Absorption*

Bile acid micelles transport the products of pancreatic hydrolysis of dietary fat and biliary lecithin, together with unesterified cholesterol from bile. There are adequate data in the literature to exclude their intestinal absorption as intact structures, since the various constituent molecules in the mixed micelle are absorbed at essentially independent rates [8–11]. Lipolytic products are absorbed from the proximal jejunum, whereas bile acids are absorbed by an active transport system in the distal ileum [39]. During the process of intestinal absorption, lipids solubilized in micelles must dissociate from them, and this occurs at a thin water layer adjacent to the luminal surface of enterocytes, with a thickness of 50–500  $\mu\text{m}$ , called the unstirred water layer [40,41]. This water layer, which has no morphological basis, represents a barrier to the passage of hydrophobic molecules, in the same manner that the lipid membrane of intestinal cells is a barrier to the absorption of hydrophilic compounds. The bile acid micelle serves to overcome the resistance of the unstirred water layer and to maintain a maximum monomer concentration at the microvillous membrane [41]. The existence of an acidic microclimate in this water layer, with pH 5.3–6.0, promotes both micellar dissociation and fatty acid protonation, thus facilitating the passive diffusion of fatty acids across the cellular lipid membrane [42]. Recently, a sodium/hydrogen exchanger, which probably is responsible for the acidification of the unstirred water layer, has been described in the brush border membrane of rodents [43]. Passive diffusion of fatty acid monomers across the microvillous membrane is very rapid; a membrane fatty acid binding protein (FABP) is expressed by intestinal cells

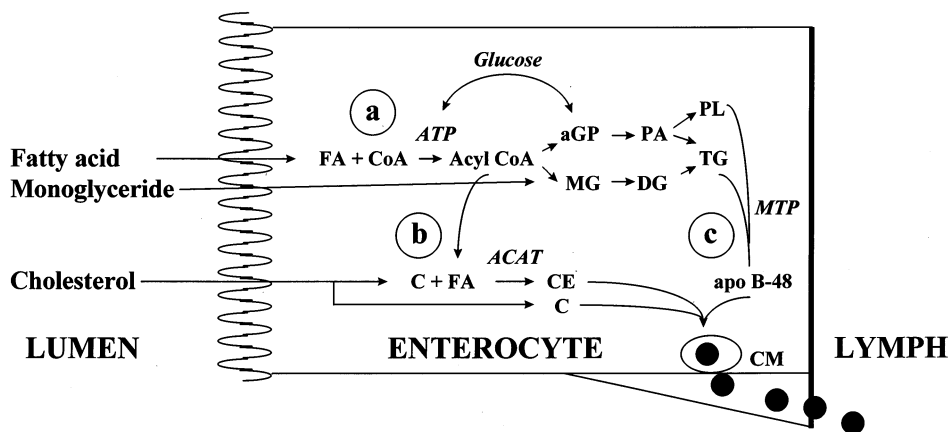


Fig. 4. Fat absorption by the enterocyte. (a) Absorbed fatty acids are ATP activated and, through the formation of  $\alpha$ -glycerophosphate (aGP) by the phosphatidic acid pathway (PA) from glucose metabolism, triglycerides are re-synthesized de novo, and absorbed lysophospholipid is esterified to phospholipid (PL). Another pathway for triglyceride re-synthesis is the re-utilization of absorbed monoglyceride (MG), with re-esterification first to diglyceride (DG) and after to triglyceride. (b) Absorbed cholesterol (C) is re-esterified with a fatty acid by the action of the enzyme acyl CoA:cholesterol acyltransferase (ACAT), originating esterified cholesterol (CE). (c) Chylomicron (CM) synthesis requires the enzyme microsomal triglyceride transfer protein (MTP) to assemble the structural protein apo B-48 to triglycerides. Mature chylomicrons also incorporate cholesterol esters to their hydrophobic core, and phospholipids, free cholesterol and apolipoproteins to their hydrophilic surface, thereby permitting the lipid transport in aqueous media (lymph and blood) that is characteristic of lipoproteins.

and facilitates transport into the cytosol, at the same time complexing with fatty acids and reducing the potential cytotoxic effect of fatty acid soaps [44,45].

In health, with good pancreatic function determining an efficient hydrolysis of dietary triglyceride and normal biliary function translating into a correct micellar solubilization of lipolytic products, the normal intestine has a great capacity to absorb a dietary fat load, in such a way that up to 95% of fatty acids and monoglycerides are absorbed and the fecal fat content is usually below 5%. Indeed, a diagnosis of fat malabsorption is considered when fecal fat is above 7 g/day on a 100 g fat diet. Even though in both experimental animal models and in Caco-2 cells (human colon carcinoma-derived cells that spontaneously differentiate into enterocytes) differences in the velocity of absorption of long chain fatty acids have been described as a function of chain length and saturation [8,11], absorption rates are essentially similar for all fatty acids of more than 14 carbon atoms [8]. Conversely, triglycerides esterified with short-chain (C:2–C:4) and medium-chain (C:6–C:12) fatty acids are more hydrophilic, and are rapidly absorbed independent of intraluminal hydrolysis, micellar solubilization, mucosal re-esterification and chylomicron formation; instead, they are transported directly to the portal vein [46]. For these reasons, medium-chain triglycerides have long been used as a useful nutritional adjunct in gastrointestinal diseases associated with impaired digestion and/or malabsorption of long-chain triglyceride, such as primary biliary cirrhosis, regional enteritis, the short bowel syndrome, and intestinal lymphangiectasia [47]. Evidence of colonic absorption of medium-chain fat in patients with

severe small bowel resection has been reported recently [48] and probably can be explained by its solubility in water.

### 2.5. Triglyceride re-synthesis and chylomicron formation

Once taken up by the apical membrane of the enterocytes and bound to FABP, fatty acids diffuse to the endoplasmic reticulum, the location of extremely active fatty acid activation and esterification enzymes, which rapidly convert them to triglycerides [49]. The process consists of the activation of fatty acids to acyl-CoA and their transesterification to appropriate acceptors: 2-monoglyceride, which contributes 70% to intestinal triglyceride synthesis, and  $\alpha$ -glycerophosphate, derived from glucose metabolism by the phosphatidic acid pathway, which makes up for the remaining 30% [50,51] (Fig. 4a). Before exiting intestinal cells to the circulation, the again apolar re-synthesized triglycerides must be incorporated into particles suitable for the transport of insoluble lipid in aqueous media, the lipoproteins chylomicrons. Lipoproteins function as pseudomicelles, transporting hydrophobic lipids such as triglycerides and cholesteryl esters in the apolar core while more polar lipids (phospholipids and unesterified cholesterol) are at the surface, acting as stabilizers, together with specific amphipathic proteins especially designed for lipid transport, the apolipoproteins (reviewed in [52]). Chylomicrons are the biggest lipoproteins (diameter > 100 nm), and their structural protein is apolipoprotein (apo) B-48. Major apolipoproteins also associated with chylomicrons are apo A-I

and apo A-IV. Another apolipoprotein secreted by the intestine in lesser amounts is apo A-II. Apo A-I and apo A-II enter the circulation in chylomicrons but are rapidly transferred to nascent high-density lipoproteins (HDL) of intestinal origin [52]. Although traces of apo E, apo CII, and apo CIII, important for the delipidation of triglyceride-rich lipoproteins in peripheral tissues, are also associated with chylomicrons, these are mostly added on the surface of the particles after interaction with other plasma lipoproteins. Apo A-IV is an apolipoprotein exclusively of intestinal origin that is associated with nascent chylomicrons and has an uncertain role in fat absorption [53]. The observations that some common genetic polymorphisms of apo A-IV modulate the response of blood lipids to changes in dietary fat and cholesterol content suggest that this apolipoprotein is involved in fat absorption [54–56]. On the other hand, it has been proposed that postprandial increases of circulating apo A-IV act as a satiety factor [57] and as a potent endogenous antioxidant [58].

The process of chylomicron formation takes place in the Golgi, and the movement of triglyceride from the endoplasmic reticulum to the Golgi appears to be the rate-limiting step in intestinal triglyceride transport [59]. The union of triglycerides with apo B-48, essential for chylomicron formation (Fig. 4c), is a complex physico-chemical process in which the recently discovered microsomal triglyceride transfer protein (MTP) plays a pivotal role (reviewed in [60]), as demonstrated by the generalized fat malabsorption occurring in a rare autosomal recessive disease, abetalipoproteinemia, characterized by the inability of hepatic and intestinal cells to secrete apo B, and caused by mutations in the MTP gene that determine the absence of the protein [61]. MTP plays a comparable role in the assembly of triglycerides with apo B-100 required for the formation and secretion of very-low density lipoproteins (VLDL) by the liver [60].

Chylomicrons transport dietary fat but also triglyceride from endogenous sources [62]. Endogenous triglycerides come in part from circulating fatty acid and chylomicron remnant uptake by enterocytes, and do not readily enter the chylomicron secretory pathway, a reason why the fatty acid composition of chylomicron triglycerides closely reflects that of dietary triglycerides [13,63]. After secretion through the basolateral membrane of enterocytes, chylomicrons enter the lymphatic capillaries of intestinal microvilli that drain into omental lymphatic channels, reaching the systemic circulation through the thoracic duct.

### 3. Intestinal cholesterol absorption

Cholesterol homeostasis is maintained by balancing intestinal cholesterol absorption and endogenous

cholesterol synthesis with biliary bile acid and cholesterol secretion (reviewed in [64]). However, because bile acids are efficiently re-absorbed and a fraction of biliary cholesterol is absorbed in the intestine, the overall whole-body cholesterol balance is kept mainly by matching cholesterol synthesis with fecal sterol losses. The latter are strictly dependent on the efficiency of the intestinal absorption of cholesterol (both dietary and biliary), which in turn relates to blood cholesterol levels [65], a reason why the regulation of cholesterol absorption has been studied extensively and is of growing interest as a target of interventions aimed at lowering blood cholesterol levels.

Because cholesterol is also a water-insoluble molecule, its intestinal absorption has a complexity similar to that of triglycerides, requiring the previously described steps of emulsification, hydrolysis of the ester bond (when esterified) by a specific pancreatic esterase, micellar solubilization, absorption in the proximal jejunum, re-esterification within the intestinal cells, and transport to the lymph in the chylomicrons. This subject has been reviewed in depth recently [66]. Unlike triglycerides, which are fatty acid esters by definition, only 10–15% of dietary cholesterol (200–600 mg daily in the usual Western diet) is present as the sterol ester [11], thus a sizable fraction of ingested cholesterol does not require pancreatic hydrolysis. Another difference with respect to the intestinal processing of triglycerides, which are exclusively of dietary origin, is that there are two important endogenous sources of intestinal cholesterol: biliary cholesterol, which is always unesterified (800–1200 g per day); and intestinal epithelial sloughing, contributing an uncertain amount that may be unabsorbable if coming from enterocytes in the more distal parts of the jejunum [66]. Finally, while triglyceride absorption is very efficient and is almost complete, there is an important limitation to cholesterol absorption, in as much as only 40–60% of dietary cholesterol is absorbed in average (although with a variability ranging from 20 to 80%), independent of the amount ingested up to 600 mg/day [67–73]. Within the normal range of dietary cholesterol, however, the absolute amount of cholesterol absorbed increases in parallel with the intake [67,70,72–75]. Thus, supposing a constant fractional absorption at 40%, a daily ingestion of 150 mg cholesterol will result in the absorption of 60 mg, whereas 200 mg will be absorbed when the cholesterol content of the diet is 500 mg. Most of the cholesterol mass escaping intestinal absorption is degraded to coprostanol through reduction of the double bond at C:5 by colonic bacteria and is excreted in feces [76].

#### 3.1. Lumenal events

Esterified cholesterol and triglycerides share the property of being virtually insoluble in water, both

existing as oils in nature. On the other hand, free cholesterol, one of the two lipolytic products of the hydrolysis of esterified cholesterol by pancreatic carboxyl ester lipase, has a water solubility much lower than that of the fatty acid liberated in the same hydrolytic process [2]. This explains the absolute dependence of cholesterol absorption on the solubilizing capacity of bile acid micelles [29,41]. The bile acid micelle acts as a carrier and a solubilizer for cholesterol, assisting in overcoming the resistance of the unstirred water layer adjacent to the brush border of enterocytes and maintaining a high concentration gradient for the passage of cholesterol into the aqueous phase, from which uptake occurs into the cellular membrane [41,77,78]. Once dissociated from mixed micelles at the aqueous phase, and by virtue of their insolubility, cholesterol monomers are taken up by intestinal cells at a slower rate than that of the more soluble protonated fatty acids, thus explaining in part the inefficiency of intestinal cholesterol absorption [66,77]. Obviously, because cholesterol is an uncharged molecule, the acidic microclimate of the unstirred water layer cannot influence its absorption. It has been pointed out that prior micellar solubilization of biliary cholesterol in the bile determines a more efficient absorption in comparison with dietary cholesterol [79]. However, mixing and exchange of dietary and biliary cholesterol in the intestine is likely to occur during the time it takes to digest a meal, so that absorptive rates would be similar [66].

### 3.2. Cellular events

Although it is generally accepted that cholesterol absorption takes place by passive diffusion down a concentration gradient, recent studies suggest that mucosal uptake of cholesterol is an active process mediated by a carrier protein on the brush border membrane of enterocytes [80–82]. Intestinal cells take up free cholesterol only, but that secreted to the lymph following a cholesterol-rich meal is 70–80% esterified. This suggests that the capacity of enterocytes to esterify cholesterol might also be an important factor regulating absorption, by creating an intracellular diffusion gradient of free cholesterol. The principal enzyme responsible for cholesterol esterification is acyl CoA:cholesterol acyltransferase (ACAT), and the reaction depends on the prior CoA activation of fatty acids [83] (Fig. 4b). Intracellular cholesterol traffic, a process in which a growing number of transport, regulatory and gene transcription activating proteins intervene [10,84], controls its own cellular metabolism in enterocytes in a manner similar to that of cells in other tissues and organs, so that increased cellular cholesterol levels stimulate ACAT activity, inhibit endogenous synthesis, and downregulate the expression

of LDL receptors (for a review, see [85]). Cholesterol in enterocytes coming from either intestinal absorption, endogenous synthesis, or lipoprotein uptake is exported to intestinal lymph in two different lipoprotein particles: chylomicrons, which is the pathway quantitatively most important, and nascent HDL [8,10–13].

### 3.3. Specificity of absorption

Cholesterol absorption appears to be very specific, in as much as structurally related sterols that differ from cholesterol only in the degree of saturation of the sterol nucleus or in the nature of the side chain at C:24 are less efficiently absorbed than cholesterol. Phytosterols like  $\beta$ -sitosterol, campesterol, and stigmasterol are constituents of many edible plants and may account for 20–25% of total dietary sterol [86]. It has been known for some time that the absorption of  $\beta$ -sitosterol, which differs from cholesterol only by the addition of an ethyl group on C:24, is less than 5% [86–88]. Other plant sterols, as well as marine sterols in shellfish, have been shown to be less efficiently absorbed than cholesterol [89–91]. The physiological impediment in the absorption of phytosterols is of clinical importance, as illustrated by patients with  $\beta$ -sitosterolemia, a rare genetic disease associated with xanthomatosis and premature atherosclerosis in relation to hyperabsorption of plant sterols by an unknown mechanism [92]. Although phytosterols are absorbed more inefficiently than cholesterol, the mechanism of absorption is similar and, being exclusively of dietary origin, the proportion phytosterol/cholesterol in plasma can be used as a reliable index of cholesterol absorption [93,94]. Phytosterols also have therapeutic interest because they inhibit the absorption of cholesterol (see later).

Why cholesterol and various plant sterols are absorbed so differently has been a matter of debate. The esterification of  $\beta$ -sitosterol by ACAT is very inefficient compared with that of cholesterol, and this was thought to account for its poor absorbability [95]. The putative protein mediator of cholesterol uptake [80–82] might also confer strict specificity to its absorption and discriminate noncholesterol sterols. On the other hand, physicochemical interactions within bile acid micelles offer a reasonable explanation for both the poor absorption of plant sterols and their effects on cholesterol absorption. As shown by Armstrong and Carey [96], noncholesterol sterols, due to their increased hydrophobicity relative to cholesterol, have a lower solubility in, but a higher affinity for, bile acid micelles than does cholesterol, thereby being less easily dissociated from micelles at the aqueous phase while restricting the micellar solubilization of cholesterol.

Table 1  
Potential genetic loci involved in the regulation of intestinal cholesterol absorption

Apolipoprotein E
Sitosterolemia
Scavenger receptor class B type I (SR-BI)
Microsomal triglyceride transfer protein (MTP)
Acyl CoA:cholesterol acyltransferase (ACAT)
Apolipoprotein B-48

### 3.4. Factors regulating absorption

Absorbed dietary and biliary cholesterol packaged into chylomicrons is transported to the liver, where it is efficiently cleared for further processing and has known regulatory effects on whole-body cholesterol homeostasis (reviewed in [64]). Thus, the delivery of increasing loads of intestinally derived cholesterol to the liver proportionally inhibits endogenous cholesterol synthesis as the more prominent compensatory effect, and also may induce a stimulation of bile acid production and/or biliary cholesterol excretion [64,74,75,97–102], in such a manner that substantial variations of cholesterol intake (or mass cholesterol absorption when measured) induce minimal fluctuations in blood cholesterol levels in humans [70,74,103]. There is, however, a great between-subject variability in the response of blood cholesterol to changes in dietary cholesterol [103,104], attributable, in part, to differences in the intestinal absorption of cholesterol or in the adaptation of the cited compensatory mechanisms [97–101]. Thus, the

Table 2  
Physiological factors and dietary components that might interfere with exogenous cholesterol absorption in the healthy small intestine

Factors	Presumed mechanism
<i>Physiological</i>	
Age?	Unknown
↑ Velocity of intestinal transit	↓ Exposure time to digestion/absorption
↑ Bile acids that are weak detergents	↓ micellar solubilization
Obesity	↑ Biliary cholesterol competing with dietary cholesterol for micellar solubilization
<i>Dietary</i>	
Low-fat meals	Impaired mixed micelle formation
↑ Dietary cholesterol <sup>a</sup>	Competition with biliary cholesterol for micellar solubilization
Plant and marine sterols	Competition with cholesterol for micellar solubilization and/or intestinal absorption
Soluble fiber	↓ Micellar solubilization (minor effect) and/or ↑ thickness of the unstirred water layer

<sup>a</sup> Decreases percent cholesterol absorption but mass absorption increases.

extreme case has been reported of a healthy octogenarian who consumed 25 eggs per day but maintained normal blood cholesterol levels by compensating for the extra cholesterol load with an absorption of 18% only and a twice-normal bile acid synthetic rate [105]. Anyhow, 15–20% of the population has an exaggerated response of the blood cholesterol concentration to an overload of dietary cholesterol [70,101,104,106]. The fact that this hyperresponse is reproducible in a given individual [106,107] suggests that genetic factors might be at play (Table 1). On the other hand, a significant intra-individual variability in the cholesterolemic response to a dietary cholesterol load has been observed when studying the same subjects in more than one occasion [108,109]. This can be attributed to physiological and dietary factors that modify intraluminal conditions such as micelle formation and the resistance of the unstirred water layer, thereby influencing cholesterol absorption by imposing or removing restrictions on the amount that can be made available for absorption in the proper physical state (Table 2).

#### 3.4.1. Genetic factors

Gender does not appear to be related to the efficiency of cholesterol absorption. Most cholesterol absorption studies involving sizable numbers of study subjects have been carried out in men [65,67,75,94,101,110], but in two studies with sufficient numbers of men and women, no gender-related differences were observed [71,111].

The differences in cholesterol homeostasis between the Tarahumara Indians of Mexico and other ethnic groups provide a good example of genetic variability in sterol absorption and feedback regulation. The Tarahumaras disclose very low blood cholesterol levels, in part because of a customary diet low in total fat and cholesterol, but also because their capacity to absorb intestinal cholesterol is low and they transform cholesterol to bile acids more efficiently in comparison with other populations [112].

The polymorphism of apo E, a ubiquitous protein of lipid transport [113], is another genetic factor influencing cholesterol absorption. There is evidence that the presence of the isoform E4 (present in  $\leq 25\%$  of subjects in Western populations) is associated with intestinal absorption of more cholesterol and with hepatic synthesis of less bile acids than the common isoform E3, while apo E2 (present in less than 10% of the population) is associated with lesser cholesterol absorption and an enhanced synthesis of bile acids [110,114]. The molecular mechanisms responsible for this variable regulation of lipid metabolism by the different apo E isoforms is only partially known. The affinity of apo E for low-density lipoprotein (LDL) receptors varies in the order  $E4 > E3 > E2$ ; hence, lipoproteins possessing apo E4 are taken up efficiently by the liver, intracellular cholesterol increases, its synthesis is inhibited, and the

expression of LDL receptors is downregulated, the final consequence being increased levels of LDL cholesterol in the circulation; the converse effects occur when apo E2 is present in lipoproteins [110,113,114]. The relationship between the apo E4 isoform and enhanced cholesterol absorption is blunted when dietary cholesterol intake is low [110]. Thus, the detrimental effects of the apo E4 polymorphism on cholesterol metabolism only may be apparent at high dietary cholesterol intake, and this could explain why in cross-sectional studies of large populations with modest overall cholesterol intakes, no major increases of cholesterol absorption were found in apo E4 bearers [71,111]. At any rate, the interaction between lipoprotein responsiveness to changes of dietary fat and cholesterol and apo E phenotype or genotype has been extensively studied, with discordant results with respect to the predicted enhanced response of individuals carrying the E4 allele and blunted response of those carrying the E2 allele, and the general finding that the apo E polymorphism is a minor determinant of dietary sensitivity. This controversial subject is beyond the scope of this work (for a review, see [115–117]). Nevertheless, the marginal variability of lipoprotein responses to dietary changes as a function of apo E polymorphism does not mean that it is not important in determining a net positive or negative overall cholesterol balance during the lifetime of apo E4 or apo E2 bearers, respectively, with obvious implications for cardiovascular risk [118]. The fact that apo E4 is associated with enhanced absorption, hepatic uptake and biliary excretion of cholesterol, together with reduced bile acid synthesis, explains why it is also a risk factor for cholesterol gallstone formation [119–121].

The pathophysiology of phytosterol absorption also points to genetic modulation of sterol absorption. Although absorbed much less efficiently than cholesterol, the absorption rates of phytosterols correlate with those of cholesterol [93,94] and, in a similar way to blood cholesterol and intestinal cholesterol absorption, circulating plant sterol levels are related to apo E phenotypes [122]. Glueck et al. [123] made the interesting observation that hyperphytosterolemia is a hereditary marker of hypercholesterolemia, hyperapobetalipoproteinemia, and premature coronary heart disease. The case of  $\beta$ -sitosterolemia and associated hyperabsorption of both cholesterol and phytosterols [92] is also pertinent because a genetic defect has been identified and ascribed to chromosome 2p21; therefore, this gene locus must be involved in cholesterol absorption [124]. However, this disease is transmitted in an autosomal recessive manner, for which reason it is difficult to conjecture that subjects with hyperphytosterolemia and hypercholesterolemia within the general population [123] could be heterozygous for this genetic defect.

As recently reviewed by Dawson and Rudel [125], there is additional evidence of a genetic basis for cholesterol absorption. Mutations in the gene encoding for the putative intestinal cholesterol carrier protein described by Thurnhofer et al. [80,81] and recently identified as a scavenger receptor class B type I (SR-BI) by Hauser et al. [82] could help explain the variability in cholesterol absorption. The SR-BI is a transmembrane protein originally described in hepatic cells and steroidogenic tissues, where it mediates the uptake of cholesterol from HDL (reviewed in [126]). Its polymorphism and that of other genes encoding for specific enzymes relevant to cholesterol absorption, such as cholesteryl ester lipase and MTP, is beginning to be investigated in humans in relation to serum lipid profiles [127–129], but no studies of cholesterol absorption have been carried out. Differences in the cholesterol-esterifying capacity of intestinal cells due to genetic variations in ACAT activity are another potential source of heterogeneity in cholesterol absorption. However, the study of this subject will be difficult due to the ubiquity of the enzyme in all cells of the body, and recent reports of its increasing diversity and genetic complexity [130–133].

The pivotal role of apo B-48 in chylomicron formation also suggests that mutant alleles of the apo B gene could be associated with the heterogeneity of cholesterol absorption. However, as recently reviewed [134,135], the truncated forms of apo B shorter than apo B-48 detected in a fraction of the uncommon heterozygotes (1:500–1:1000 in Western populations) for familial hypobetalipoproteinemia are rarely associated with fat malabsorption, indicating that a single intestinal allele for apo B-48 is sufficient for normal fat absorption. This is clearly not the case in the very rare compound heterozygotes or homozygotes, which are phenotypically similar to patients with abetalipoproteinemia. Although no formal studies of cholesterol absorption are available in subjects with hypobetalipoproteinemia, it is obvious that this relatively infrequent genetic abnormality can have no impact on the variability of cholesterol absorption at the population level. More subtle variations at the apo B gene locus also have been studied in relation to the important topic of gene-diet interactions in atherogenesis. While several polymorphic sites in the apo B gene have been related to population differences in lipoprotein levels, extent of coronary artery disease, or lipid responses to dietary modification (reviewed in [115,116]), no direct cholesterol absorption studies have been carried out. Evidence of differences in postprandial lipemia related to common genetic variation at the apo B gene locus affecting the apo B-48 region has been reported in studies of dietary fat clearance, and interpreted, among other possibilities, as an indirect proof of variable fat absorption [136,137], but the time of appearance, level

and composition of postprandial lipoproteins may be influenced by many extra-intestinal factors and does not necessarily reflect fat absorption or chylomicron synthesis. In any event, a clear role for genetic variation of apo B-48 in the heterogeneity of cholesterol absorption has not been documented to date.

#### 3.4.2. *Physiological factors*

It is unclear whether there is an association between the efficiency of cholesterol absorption and age. In two studies with significant numbers of subjects on a relatively low cholesterol diet, no age-related differences were observed [71,111], differing from the report of Gylling et al. [138] that cholesterol absorption is reduced in 75-year-old Finnish men compared with 50-year-old men from the same country. The usual Finnish diet is very high in cholesterol and, in a similar way to the blunting effect of low cholesterol intakes on the increased cholesterol absorption efficiency of apo E4 [110], an age-related difference may not be apparent at low cholesterol intakes. To settle this issue, subjects of different ages would have to be studied under conditions of varying dietary cholesterol intake.

Cholesterol absorption appears to be dependent on the velocity of intestinal transit, as it increases in association with a slow transit time and it decreases when there is a rapid transit under physiological conditions or after pharmacological stimulation of bowel motility [139–141]. Because of the intrinsic limitations in normal cholesterol absorption, the effect of an accelerated intestinal transit can probably be ascribed to reduced contact time of luminal contents with the enterocytic brush border membrane. On the other hand, a slow intestinal transit is associated with an increased rate of bacterial biotransformation of bile acids, with enhanced formation and enterohepatic recirculation of deoxycholic acid [142], a highly hydrophobic bile acid with greater detergent properties and, therefore, more cholesterol-solubilizing power in micelles than other major bile acids. Thus, bowel habit may be a relevant factor in the heterogeneity of cholesterol absorption.

As pointed out, another factor potentially affecting cholesterol absorption is the detergent capacity of the bile acid species predominating in the enterohepatic circulation, which determines the cholesterol-solubilizing capacity of bile acid micelles in both bile and intestinal contents [143–146]. The impact of the dissimilar detergency of bile acids on cholesterol absorption is clearly seen when most of the endogenous bile acid pool is replaced by ursodeoxycholic acid during oral bile acid treatment for gallstone dissolution (see later).

Obesity, which is characterized by an increased turnover of cholesterol, with both excessive production [147,148] and enhanced biliary excretion [149–151], was found to be negatively associated with fractional absorption of dietary cholesterol in a random Finnish

population characterized by high customary cholesterol intakes [75]. Again, no such association was observed in populations with low baseline dietary cholesterol [71,111]. An explanation for the findings of the Finnish study [75] could be precisely the hypersecretion of biliary cholesterol in obesity: competition between endogenous and exogenous cholesterol for micellar solubilization and/or membrane uptake may cause an increased luminal load of biliary cholesterol to proportionally reduce the absorption of a substantial amount of dietary cholesterol. This is supported by the observation by Kern [152] that patients with cholesterol gallstones, who also have biliary cholesterol hypersecretion, absorb less dietary cholesterol than sex-, age- and weight-matched controls. Furthermore, enhanced biliary cholesterol excretion was associated with suppression of dietary cholesterol absorption in a recently described mouse model with transgene-induced liver overexpression of SR-BI [153,154], strongly suggesting that endogenous cholesterol secreted into bile, one of the critical factors in whole-body cholesterol homeostasis and in the pathogenesis of both atherosclerosis and cholelithiasis [64], is another factor regulating cholesterol absorption.

#### 3.4.3. *Dietary factors*

Dietary components within the intestinal lumen may influence cholesterol absorption in opposite ways through enhanced or decreased efficiency of micelle formation and/or brush border membrane uptake. Because the solubility of cholesterol in bile acid solutions rises in direct proportion to added monoglyceride or fatty acid [2,29], it can be predicted that enrichment of micelles with polar lipid will favor cholesterol solubilization in the lumen, thereby facilitating absorption, when exogenous cholesterol is ingested together with a significant amount of triglyceride. Intestinal perfusion studies in humans have clearly shown the physiologic importance of available micellar fatty acid for jejunal absorption of cholesterol [155]. Furthermore, Samuel et al. [69] demonstrated that the absorption of labeled cholesterol from a fat-free meal is greatly reduced. However, although the concomitant ingestion of fat and cholesterol should favorably affect micellarization and the absorption of the particular load of cholesterol in that meal, large clinical studies relating customary fat and cholesterol intake to sterol absorption have yielded conflicting results. Studies in Finnish populations found a positive correlation between dietary fat and percent dietary cholesterol absorption [75,110,138] or mass absorption [75], whereas in American populations dietary fat related positively [71], and even negatively [72], to dietary cholesterol mass absorption but was unrelated to fractional absorption [71,72], and studies in Dutch populations found no influence of fat intake on percent dietary cholesterol absorption [111] or fecal neutral

Table 3  
Dietary supplements and pharmacological agents interfering with exogenous cholesterol absorption

Factors	Presumed mechanism
<i>Dietary supplements</i>	
Phytosterols <sup>a</sup> ( $\beta$ -sitosterol, sitostanol and stanol mixtures)	Competition with cholesterol for micellar solubilization and/or intestinal absorption
Soy lecithin	↓ Micellar solubilization
Olestra and other unabsorbable fats	Trapping in unabsorbable oil phase
<i>Pharmacological</i>	
Bile acid sequestrants	↓ Micellar solubilization (minor effect)
Clofibrate	↑ Biliary cholesterol competing with dietary cholesterol for micellar solubilization
HMG-CoA reductase inhibitors	Cholesterol depletion in enterocytes leading to ↓ ACAT activity
Ursodeoxycholic acid	↓ Micellar solubilization
Neomycin	↓ Micellar solubilization
ACAT inhibitors	↓ Re-esterification of absorbed cholesterol
MTP inhibitors	↓ Chylomicron formation
Synthetic saponins	Inhibition of cholesterol transporter?
2-Azetidinones	Inhibition of cholesterol transporter?

<sup>a</sup> Widely used as safe and effective nonsystemic cholesterol-lowering agents with the sole purpose of inhibiting cholesterol absorption.

sterol excretion, a measure of unabsorbed exogenous plus endogenous cholesterol [156]. Thus, in spite of its physiological plausibility, a positive relationship between dietary fat and cholesterol absorption has not been clearly demonstrated in clinical studies. At any rate, cholesterol is associated with saturated fatty acid-containing triglycerides in many foodstuffs that are to be avoided in a healthy diet irrespective of their interplay in cholesterol absorption.

As previously discussed, the mass of dietary cholesterol absorbed increases with the intake [67,70,72–75]. While small decreases or no changes in percent cholesterol absorption have been observed in clinical studies in which the same subjects were given diets with an increasing cholesterol content [69,72,101], Ostlund et al. [73] recently provided clear evidence that the fractional absorption of cholesterol in a given meal is negatively related to the amount of cholesterol in that meal over a physiological range. The plausible explanation again is overt competition between too many sterol molecules for limited micellar solubilization.

If excess cholesterol can acutely depress the efficiency of cholesterol absorption, then it is understandable that other sterol molecules in meals might have a similar effect, as has been shown for phytosterols in common vegetable foods [89] and for marine sterols in shellfish

[90]. The competition of poorly absorbable vegetable sterols with cholesterol for micellar solubilization [96] and ensuing restriction on the absorption of cholesterol underlies the time-honored cholesterol-lowering effect of  $\beta$ -sitosterol and related compounds when given as dietary supplements, as discussed later.

Another diet-related factor that might influence cholesterol absorption due to physical interactions within the intestinal lumen is dietary fiber. Viscous or gel-forming soluble fiber, like pectin and guar gum, nonspecifically binds bile acids in the intestine, promoting their fecal elimination and thereby stimulating the hepatic catabolism of cholesterol to bile acids in a manner similar to anion exchange resins [3,157,158]. The resulting reduction in the cholesterol content of liver cells with ensuing upregulation of LDL receptors and increased clearance of LDL cholesterol is the main mechanism invoked for the small hypocholesterolemic effect of soluble dietary fiber within the practical range of intake [4]. The bile acid-binding properties of soluble fiber would be expected to interfere with intestinal micelle formation, but even with substantial quantities of fiber, the effect is too weak to decrease the amount of cholesterol solubilized [157]. Soluble fiber could also hamper cholesterol absorption via its effect of increasing the thickness of the unstirred water layer overlying the intestinal mucosa [159,160]. Nevertheless, neither of these two effects appears to be biologically relevant, as judged by the lack of statistically independent relation between dietary fiber intake and percent cholesterol absorption observed in two cross-sectional studies [71,75] and an interventional study [161].

#### 4. Interference with cholesterol absorption in the treatment of hypercholesterolemia

In as much as some constituents of the normal diet have the capacity to render cholesterol absorption even more inefficient and, consequently, have a potential cholesterol-lowering effect, their administration in commercial preparations as dietary supplements constitutes a nonpharmacological therapy to lower blood cholesterol levels (Table 3). Furthermore, the knowledge of the molecular mechanisms of cholesterol absorption permits the design of new drugs targeted to interfere with these processes (Table 3). Because of the sizable cholesterol load provided daily to the intestine by biliary secretion, the efficacy of these therapies does not necessarily depend on the level of dietary cholesterol. The following section examines the therapeutic modalities to inhibit cholesterol absorption that have been used, are in current use or are likely to be used in the future.

## 4.1. Dietary supplements

### 4.1.1. Plant sterols

It has been known since the early 1950s that the ingestion of naturally occurring phytosterols, especially abundant in legume seeds and oils derived from them, results in favorable modification of lipid profiles (reviewed in [162–164]). Daily consumption of plant sterols has been estimated to be around 150 mg in Western diets, where the main sources are vegetable fats and oils, but is over 400 mg/day in more primitive populations consuming high amounts of beans and corn [162]. As discussed, these compounds inhibit cholesterol absorption by competition with cholesterol for micellar solubilization and because, due to their hydrophobicity and poor absorbability, they remain in intestinal micelles and continuously interfere with the micellar solubility of cholesterol [96,165]. The maximal hypocholesterolemic activity of plant sterol mixtures or of  $\beta$ -sitosterol, the most abundant in nature and most commonly used up to the 1980s, occurs at doses of 3 g/day, with reductions of total cholesterol and LDL cholesterol levels by close to 10% [166].

As a therapeutic agent,  $\beta$ -sitosterol has the inconvenience of an unpleasant taste and texture, so that the high doses needed to obtain a cholesterol-lowering effect are not well tolerated. The saturation of the sterol ring of sitosterol produces sitostanol, a more hydrophobic molecule that is virtually unabsorbable while notably enhancing the hypocholesterolemic efficacy of the parent molecule, with a 50% reduction of cholesterol absorption at low doses and up to a 85% decrease at high doses [167]. Several studies have shown that 2.0–3.0 g sitostanol given daily for up to 12 months in sitostanol ester-containing margarines or dressings significantly reduces total and LDL cholesterol concentrations by 5–15% in hypercholesterolemic subjects with no limitation of dietary cholesterol [168–172]. Recently, a similar cholesterol-lowering effect of sitostanol-containing phytosterol blends has been observed in hyperlipidemic patients following low cholesterol diets [173,174], indicating that sitostanol inhibits not only the absorption of dietary cholesterol, but that of biliary cholesterol as well. Both free sitostanol and fat-soluble sitostanol esters have been shown to be particularly effective as nonsystemic cholesterol-lowering agents in children with familial hypercholesterolemia [175–177]. As reviewed by Miettinen and Gylling [164], dietary phytosterol treatment combined with standard hypolipidemic drugs has an additive cholesterol-lowering effect.

Sitostanol-containing fats such as those described are good examples of functional foods or nutraceuticals, naturally-occurring or processed dietary products that have been transformed in such a way as to provide a benefit beyond flavor, taste, texture or nutritional value, and affect physiology in a measurable way in

terms of disease prevention or health promotion (reviewed in [178]). Presently, these design foods are principal targets of research by the food industry that slowly but relentlessly appear in the shelves of supermarkets around the world. Other functional foods designed to influence favorably the lipid profile and/or cardiovascular risk [179] include soy or linseed flour bakery products, milk with quantitative or qualitative modifications of its fat content, folic acid-enriched cereals, and fat substitutes such as sucrose polyester or structured triglycerides (see later).

### 4.1.2. Soy lecithin

Soy lecithin products are generally consumed with the belief that they have a favorable influence on blood cholesterol levels. Lecithin ingestion probably inhibits cholesterol absorption by displacement of cholesterol from micelles by the principal hydrolytic product of lecithin, lysolecithin [180]. However, in spite of an antiatherogenic effect when given to laboratory animals, lecithin administration in humans has no clear effects on the lipid profile beyond those attributable to its high linoleic acid content or to a secondary reduction in dietary fat intake to compensate for the energy provided by lecithin itself [181].

### 4.1.3. Sucrose polyester (*olestra*)

This compound, recently approved in the US for its exclusive use as a fat substitute in some commercial snacks, is a mixture of transesterified sugars with the physical properties of fat that is unabsorbable by the intestinal cells, thus lacking caloric value [182]. In experimental studies in volunteers following a high-cholesterol diet, the substitution of *olestra* for a significant proportion of dietary fat reduced cholesterol absorption in a dose-dependent manner up to 50% [183,184]. This effect can be imputed to the dispersion of a fraction of intestinal cholesterol into the oil phase formed by the ‘false oil’, which traverses the digestive tract as an inert emulsion and is excreted unmodified in feces. The effects of this compound on fat absorption are discussed in Section 5.

## 4.2. Pharmacological agents

### 4.2.1. Bile acid sequestrants

The cholesterol-lowering effect of anion-exchange resins such as cholestyramine and colestipol comes from their capacity to irreversibly bind bile acids in the intestinal lumen, thereby promoting their fecal elimination. The ensuing increase in the conversion of cholesterol to bile acids in the liver to re-establish the enterohepatic circulation of bile acids depletes liver cells of cholesterol, which causes upregulation of LDL receptor, thereby reducing blood levels of total and LDL cholesterol [5,39,64]. The intraluminal sequestration of

bile acids by cholestyramine reduces marginally the micellar solubilization of dietary fat [158], but a 40% reduction in the absorption of cholesterol ingested just prior to drug intake has been reported [185]. Evidently, this effect may contribute in part to the cholesterol-lowering efficacy of bile acid sequestrants.

#### 4.2.2. *Fibrates and HMG-CoA reductase inhibitors*

A small inhibitory effect of cholesterol absorption also has been reported for two commonly used systemic hypolipidemic drugs, fibrates and HMG-CoA reductase inhibitors. The fibric acid derivative clofibrate marginally decreases cholesterol absorption in humans [185,186]. Because fibrates enhance biliary cholesterol excretion [187], an inhibitory effect on dietary cholesterol absorption might be expected given its inverse relationship with biliary cholesterol content, as previously discussed. Miettinen [188] reported that cholesterol absorption was reduced by HMG-CoA reductase inhibitors, and suggested that this effect might be due to cholesterol synthesis inhibition lowering the cholesterol content of enterocytes and subsequently reducing ACAT activity. However, it is unlikely that these intestinal effects substantially contribute to the potent systemic hypolipidemic activity of these two classes of lipid-regulating agents.

#### 4.2.3. *Ursodeoxycholic acid*

The dihydroxy bile acid ursodeoxycholic is of great medical interest because its oral administration induces dissolution of cholesterol gallstones and also improves biochemical markers of cholestasis and inflammation in cholestatic liver diseases [189]. By virtue of being very hydrophilic, ursodeoxycholic acid has by far the lowest micellar cholesterol-solubilizing ability of all common bile acids [190], therefore its enrichment of the endogenous bile acid pool during prolonged oral treatment reduces both biliary cholesterol secretion and intestinal cholesterol absorption [191]. Although ursodeoxycholic acid has other effects on hepatic cholesterol homeostasis [191], inefficient cholesterol absorption may explain in part why its administration can slightly lower blood cholesterol levels [192,193].

#### 4.2.4. *Neomycin*

Neomycin is a nonabsorbable aminoglycoside antibiotic with a cholesterol-lowering effect that was discovered fortuitously in the 1950s. Its effect on lipid metabolism is due to interference with the micellar solubilization of cholesterol in the digestive tract, with a near 50% reduction in cholesterol absorption and an increase in fecal neutral sterol excretion [194]. Neomycin was used during the 1960s and 1970s for treatment of hypercholesterolemia at doses of 2 g/day, singly or in combination with cholestyramine or clofibrate [195], but the potential renal toxicity of even the

minimal amounts absorbed, and the discovery of safer and more potent lipid-regulating drugs such as the statins fortunately prompted its abandonment as a cholesterol-lowering agent.

#### 4.2.5. *ACAT inhibitors*

While many active drugs have been developed and commercialized without a clear comprehension of their mechanisms of action, the search for new therapeutic agents is actually centered precisely on the desired action, the target being frequently a stimulatory or inhibitory effect on a regulatory protein in a well-known metabolic pathway. In this sense, the discovery of ACAT as the enzyme responsible for re-esterification of absorbed cholesterol within the enterocytes [83] led to the design of nonabsorbable molecules inhibitory of its activity. Studies in various animal models showing that ACAT inhibitors concomitantly reduced absorption and blood levels of cholesterol (reviewed in [196]) made this class of drugs look very promising as cholesterol-lowering agents, but human experiments with ACAT inhibitors have been disappointing [197,198]. Because ACAT esterifies cholesterol in all cells of the body, including macrophages, there is an active investigation in search of new, soluble ACAT inhibitors. The potential antiatherogenic effect of ACAT inhibitors by the effect of preventing cholesteryl ester storage in arterial wall macrophages has been demonstrated in animal models of atherosclerosis (reviewed in [199]). However, ACAT knockout rats absorb cholesterol at rates similar to wild animals, suggesting that there might be several species of ACAT enzymes in mammals [130]. This has been confirmed by the recent identification of a second ACAT [131,132]; therefore, the search for specific inhibitors promises to be rather complex.

#### 4.2.6. *MTP inhibitors*

The knowledge of the obligatory role of MTP in the formation of VLDL in the liver and chylomicrons in the intestine [60] provided an obvious therapeutic target to reduce blood lipid levels through its inhibition. Effective inhibition of MTP activity would reduce not only the transport of exogenous lipid by impairing chylomicron formation, but also that of endogenous lipid by interfering with hepatic VLDL secretion, thus having the potential of profoundly influencing lipid metabolism. In cell culture studies, pharmacological inhibition of MTP reduces both VLDL output in liver cell lines [200] and chylomicron secretion in intestinal cell lines [201]. A marked cholesterol-lowering effect also has been demonstrated in Watanabe heritable hyperlipidemia rabbits, an animal model of familial hypercholesterolemia [202]. These drugs have gone beyond the preclinical stage and human studies are just beginning, although there is a concern about the fate of unexported lipid in target tissues should profound MTP inhibition occur [203].

#### 4.2.7. Other drugs

There is a long list of nonabsorbable agents that have the capacity to inhibit cholesterol absorption and are at different stages of development (reviewed in [5]). A conceptually interesting drug is an inhibitor of another enzyme of intestinal cholesterol metabolism (in this case, cholesteryl ester hydrolase) still in the preclinical stage [204]. One class of naturally occurring cholesterol absorption inhibitors is the saponins, steroidal glycosides found in a variety of edible plants that form insoluble stoichiometric complexes with cholesterol *in vitro* and interact with bile acid micelles excluding cholesterol from them, thereby inhibiting sterol absorption and lowering serum cholesterol levels in a variety of species including humans (for review, see [205]). Synthetic saponins such as pamaqueside and tiqueside, recently shown to impair cholesterol absorption by a nonstoichiometric mechanism suggesting a potential interaction with a cholesterol transporter [206], are already in the clinical trial stage [207] and may find a place in the treatment of hypercholesterolemia. Another promising class of drugs is the 2-azetidinones [208,209], high potency cholesterol absorption inhibitors with an efficacy at small doses that also suggests inactivation of a putative cholesterol transport molecule in the intestine. Therefore, in addition to their pharmacological potential as nonsystemic cholesterol-lowering agents, synthetic saponins and 2-azetidinones are conceptually interesting because they are helping to unravel poorly understood molecular steps in intestinal cholesterol absorption [125].

### 5. Reduction of fat absorption in the treatment of obesity

Due to its direct association with various chronic diseases, including coronary heart disease, diabetes, colon carcinoma and gallstones, and because of its epidemic nature in developed societies, obesity is a very important public health problem [210]. Societies and individuals consume an extraordinary amount of resources in an attempt to prevent and treat obesity, usually with little success. The only reasonable treatment of overweight or obesity is the voluntary limitation of energy intake in the form of high-calorie foodstuffs associated with an increased energy expenditure by physical exercise, but this is more easily said than done. Due to its high energy content, dietary fat is frequently a source of undesirable calories, and a life-long adherence to a relatively low-fat, high-complex carbohydrate diet provides the best opportunity for prevention and treatment of obesity (reviewed in [211]). However, one of the identity traits of Western culture is the abundance of appetizing foodstuffs, their fat content being usually a critical feature regarding palatabil-

ity and satiating power, whereas low-fat foods are less tasty and their ingestion clashes with sensorial preference. In front of the difficulty in voluntarily omitting fat from the diet, the food industry has, in past decades, developed the technology to substitute it, producing processed foodstuffs that are low in fat but preserve the flavor and texture of high-fat ones (for review, see [212]).

Although each source of dietary fat is composed of hundreds of different complex triglycerides, every one of them tends to have a stereospecific structure (individual fatty acids are located in particular positions of the three available ones in the glycerol molecule) that might affect metabolic disposition in a variable manner, from intraluminal hydrolysis to adipose tissue deposition (reviewed in [213]). The transformation of naturally occurring triglycerides into molecules structured in such a way as to limit their intestinal absorption (reviewed in [214]) is another fertile field of alimentary research, with objectives similar to those of fat replacers.

There also are natural products with the ability to interfere with triglyceride absorption, such as the chitosans, polyaminosaccharide molecules derived from crustacean and fungal chitins, already approved for use as food additives or supplements in several countries (for a review, see [215]). Chitosans possess both cationic groups that can bind fatty acids and bile acids through ionic bonds, and hydrophobic domains that can trap neutral fats such as triglycerides and cholesterol, with the effect of promoting fecal fat excretion. There are preliminary evidences that chitosans may potentiate the weight loss induced by a hypocaloric diet in obese subjects [215].

In the pharmacological field, there is the potential to reduce the intestinal disposition of dietary fat by interference with one or more of the metabolic processes implicated in fat digestion and absorption (for a review, see [216]). Pancreatic lipase inhibition is the only therapeutic modality that has been tried in humans and has demonstrated a reasonable efficacy to promote weight loss by way of an induced fat malabsorption [217]. Beyond the scope of this review is another important area in the pharmacological treatment of obesity, that of appetite suppressants and satiety factors (reviewed in [218]).

#### 5.1. Dietary agents: fat substitutes

Fat substitutes, also called fat replacers, are components of processed foods, considered technically as intentional additives, which imitate the functions of fat in the processes of manufacturing, tasting, and/or cooking, but lack or have a reduced caloric value and permit the consumer to ingest them in substitution of similar foods with higher caloric value, thereby reducing energy intake. Thus, the alimentary products that contain

them are functional foods [178]. To date, most fat substitutes available to food manufacturers are based on modified carbohydrates. They are an integral part of a variety of processed foods, ranging from baked goods and pastries to ice-creams, dairies, dressings, sauces, and chewing gum, and include carbohydrate polymers, hydrocolloids, fiber gels and polyols with familiar names in food labels like maltodextrin, modified corn starch, guar gum, cellulose gel, sorbitol, polydextrose, etc. [212]. Although less developed, some fat substitutes are protein-based, like microparticulated egg white and whey protein concentrate. The effects on overall energy balance of foods containing fat substitutes are difficult to measure because their ingestion only reduces marginally the caloric intake of the consumer. The situation is different with respect to fat-based fat replacers.

The fat substitute with most supportive scientific information is the aforementioned sucrose polyester (generic name, olestra), a transesterified sugar that is neither hydrolyzed by pancreatic lipase [219] nor absorbed by enterocytes [182]. Furthermore, olestra has no effect on duodenal cholecystokinin release [220], gallbladder emptying [221], or absorption of other lipophilic molecules, with the exception of cholesterol [183,184], as previously discussed, and, logically, of fat-soluble vitamins (reviewed in [222]). In any case, this substance can be considered as a false fat, practically inert to all effects, therefore lacking energetic value. In short-term studies where olestra was substituted for a substantial proportion of dietary fat, a reduced fat intake was observed, but the overall energy intake did not change because the study subjects compensated for the calories omitted from fat by increasing their carbohydrate intake [222]. In long-term studies, however, not all energy omitted from fat was compensated with other energy sources, and this was associated with the corresponding weight loss [223–225].

At any rate, it should be pointed out that the average intake of olestra by consumers of snacks made with this false fat is  $\approx 3$  g/day [226], equivalent to omitting 27 kcal from fat, a minor contribution to weight control. Obviously, lacking fat substitutes that can replace significant amounts of dietary fat without side-effects, one cannot expect much success in the prevention and treatment of obesity from the isolated use of current fat replacers.

### 5.2. *Pharmacological agents: tetrahydrolipstatin*

The aqueous insolubility of triglycerides and their absolute requirement for hydrolysis by gastrointestinal lipases prior to absorption makes lipase inhibition an optimal target to obtain a controlled fat malabsorption, equivalent to a reduction of energy intake useful in the treatment of obesity. The drug tetrahydrolipstatin (generic name, orlistat) selectively inhibits the activities

of gastric and pancreatic lipases, thus preventing dietary triglyceride hydrolysis in the intestinal lumen [217]. Orlistat is a highly lipophilic lactone with amphipathic properties that, when interacting with fat emulsion particles, is located in the surface layer for ideal interaction with enzymes acting at the oil–water interface such as pancreatic lipase [227]. The orlistat molecule is recognized by lipase as a substrate for its action and specifically reacts with the active residue of the lipase molecule by tight covalent binding. While the bond in the interaction triglyceride–lipase is easily broken, liberating fatty acid monomers and lipase molecules that can continue the reaction with fresh substrate, the orlistat–lipase product is stable (the bond is practically irreversible) and undergoes fecal excretion without degradation [228]. Lipase inhibition is associated with increased triglyceride in the lipid phase of intestinal contents, subsequent fat malabsorption, and development of steatorrhea. Orlistat also inhibits carboxyl ester lipase; therefore, the potential exists for the malabsorption of cholesterol and fat-soluble vitamins [228]. Dose–response studies in humans have determined that maximal inhibition of fat absorption ( $\approx 30\%$  of dietary fat) occurs at orlistat doses of 120 mg three times a day with meals [229]. The intestinal absorption of orlistat is negligible, and no drug interactions have been described. When the drug is ingested with a fat-rich meal, the ensuing steatorrhea can be associated with abdominal pain, urgency to defecate, increased flatus, diarrhea and, in some cases, fecal incontinence. In this sense, orlistat treatment may act as an aversive by dissuading the recipient from ingesting fatty foods.

Recently, the results of three large multicenter studies of 1-year duration with orlistat treatment in conjunction with a moderately hypocaloric diet in obese subjects have been published [230–232]. The data reported in these trials is remarkably consistent. About one-third more patients treated with 120 mg orlistat three times daily lost  $\geq 5\%$  of their initial body weight than did those treated with placebo, while about twice as many patients given orlistat lost  $\geq 10\%$  of their initial body weight than did those treated with placebo. Patients with type 2 diabetes in one trial [230] had more difficulty losing weight than those without diabetes in the other two studies [231,232]. Patients treated with orlistat also showed improvement in obesity-related cardiovascular risk factors, including blood lipid and glucose concentrations, and blood pressure. Furthermore, orlistat had an additional beneficial effect on plasma lipids that was independent of weight loss alone [230–232]. This effect was probably related to the mechanism of action of the drug on the absorption of dietary fat, assuming that much of the dietary fat consumed was saturated. Treatment in association with an eucaloric weight maintenance diet for one further

year in two trials [231,232], and in a fourth study during attempted weight maintenance after weight loss by diet alone [233], showed that patients treated with orlistat regained less weight and maintained more the beneficial effects on risk factors than did those treated with placebo. In these trials, safety issues were the gastrointestinal side effects and reduction in blood levels of fat-soluble vitamins, which rose to normal with appropriate supplementation.

Orlistat, which has been released recently in several countries, is a new and conceptually interesting therapy for obesity, but in no way substitutes the prescription of voluntarily limiting calorie intake while increasing energy expenditure through exercise.

## 6. Conclusion

The physicochemical events that facilitate the solubilization and transport of biologically important hydrophobic lipid molecules such as triglyceride and cholesterol in the aqueous media of intestinal contents are well characterized, but basic steps in the mucosal absorptive process have remained relatively poorly understood at the molecular level. Knowledge of the rate-limiting steps in dietary lipid digestion have permitted the development of novel nonsystemic approaches to limit energy acquisition, and the refinement of old ones to reduce cholesterol absorption in the treatment of obesity and hypercholesterolemia, respectively. Thus, the absolute requirement of triglyceride for hydrolysis prior to absorption has made lipase inhibition an optimal target to obtain a controlled fat malabsorption. On the other hand, micellar solubilization, an indispensable event for cholesterol absorption, is easily disturbed by a number of xenobiotics, including naturally derived compounds such as the stanols, for which much information has been collected in the past two decades that has led to its incorporation into low-cost nutraceuticals suitable for mass intervention of mild hypercholesterolemia. The ongoing search for nonsystemic alternatives to the commonly used HMG-CoA reductase inhibitors and for complementary mechanisms of action suited for combination therapy with them or other hypolipidemic agents has led to the design of agents that are capable of blocking cholesterol absorption by mechanisms other than competition for micellar solubility, and unraveling their modes of action may further the knowledge of molecular mechanisms of intestinal cholesterol absorption. However, drugs acting to inhibit cholesterol absorption are not of outstanding efficacy as cholesterol-lowering agents, possibly because there is always the potential for a compensatory increase in hepatic cholesterol synthesis.

Finally, the inefficiency of cholesterol absorption and its large inter-individual variation suggesting multiple

genetic regulation are of great scientific and clinical interest, in as much as the efficiency of cholesterol absorption is an important variable in specific studies of the effects of diets on the serum lipid profile and in the broader field of diet–gene interactions affecting atherosclerosis.

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