review

Review of cholesterol absorption with emphasis on dietary and biliary cholesterol

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Interest in cholesterol absorption is growing as a consequence of recent information indicating a closer link between cholesterol absorption and plasma cholesterol than once thought. Kesaniemi, Ehnholm, and Miettinen (1) and Kesaniemi and Miettinen (2) have found a positive relationship between the fractional absorption of cholesterol and plasma cholesterol levels and apolipoprotein E phenotype in a random sample of Finnish men. The relative degree of importance of cholesterol absorption in determining plasma cholesterol concentrations is highlighted by the case of the "egg man," a clinically normal 88-year-old man who ate 25 eggs a day and maintained a normal plasma cholesterol, at least partly by absorbing only 18% of his dietary cholesterol (3). Studies in hyper- and hypo-responding nonhuman primates also indicate that cholesterol absorption and plasma cholesterol are positively related (4–8).

Clinicians remain interested in the metabolism of cholesterol due to its relationship to two diseases that affect millions of people: atherosclerosis and gallstones. However, until the cholesterol in the food we consume and in the bile secreted into the intestinal lumen crosses the barrier of the intestinal mucosal cell, it is not a part of the body pool of cholesterol and is unable to influence whole body cholesterol metabolism.

It is not often emphasized or appreciated in discussions of cholesterol absorption that the cholesterol present within the intestinal lumen during any one day is typically two-thirds from endogenous sources and one-third from dietary sources. This means that on a daily basis the bulk of the cholesterol absorbed from the intestine is derived from within the body, i.e., endogenous sources. While excess dietary cholesterol is known to raise plasma cholesterol in many individuals, it is the absorption of the mixture of exogenous (dietary) and endogenous cholesterol that must be considered in order to understand the relationship between cholesterol absorption and plasma cholesterol concentration. In addition to knowing the amount of cholesterol from each source, it is also important to understand the handling of both during absorption: cholesterol from different sources may exhibit different behaviors within the intestinal lumen. We will examine the evidence for and against the differential absorption of cholesterol from dietary and biliary sources.

The processes involved in the intraluminal mixing, transfer into the intestinal mucosal cell, and transfer into the lymph have been the subject of much study and discussion through the years. This paper will briefly review the major physical and enzymatic processes involved in the digestion and absorption of cholesterol, and then concentrate on the absorption of both dietary and biliary cholesterol. Readers are referred to recent reviews on the digestion and absorption of lipids for a more detailed discussion of these aspects (9, 10).

For the purposes of this discussion, "absorption of cholesterol" will be defined as the transfer of intraluminal cholesterol into intestinal or thoracic duct lymph. "Uptake of cholesterol" will refer to entry of cholesterol into enterocytes.

SOURCES OF INTRALUMINAL CHOLESTEROL

There are two major sources of the intraluminal cholesterol available for absorption: the diet and the bile. The diet of the average North American adult contains 300–500 mg of cholesterol per day in meat, eggs, and dairy products (11). A variable proportion of this cholesterol is esterified to various fatty acids. Estimates of the percent esterified vary depending on the method of extraction and analysis. For example, egg yolk cholesterol has been reported to be from 8 to 19% esterified (12, 13). The physical environment of dietary cholesterol varies as well. It may be dissolved in adipose tissue triacylglycerol

Abbreviations: UWL, unstirred water layer; VLDL, very low density lipoproteins; CEase, cholesterol esterase; ACAT, acyl-CoA:cholesterol acyltransferase.

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or it may be a structural constituent of cellular membranes. Bile provides an additional 800–1200 mg of cholesterol per day to the intraluminal pool, a significant source that is often overlooked in discussions of cholesterol absorption (11). All biliary cholesterol is nonesterified (free) and is incorporated into bile salt-phospholipid micelles (14, 15).

The turnover of intestinal mucosal epithelium provides a third source of intraluminal cholesterol. Estimates of this contribution are hard to come by, but in three patients with total bile duct obstruction, 250–400 mg of mucosal cholesterol was secreted or released into the intraluminal space daily (16). The quantitative significance of this source of intraluminal cholesterol is not known in nonpathological conditions in humans, but in some animals, particularly rats, it is estimated to contribute a significant portion of the endogenous intraluminal cholesterol (17). This uncertainty is further confounded by the fact that an undetermined proportion of this cholesterol is derived from mucosal cells in the distal portion of the intestinal tract, and therefore may not contribute to the pool of cholesterol available for absorption.

SITE OF CHOLESTEROL ABSORPTION

When cholesterol absorption was determined at different sites along the small intestine of human subjects, it was concluded that the main sites of absorption were the duodenum and proximal jejunum (18, 19). Although studies with rats have shown that the proximal half of the small intestine accumulates the most radioactivity following the administration of a test dose of radiolabeled cholesterol (20, 21), there is evidence that the entire length of the small intestine has the capability to absorb sterol from the lumen (22–24).

Cholesterol absorption occurs through the intestinal mucosal cells that cover the surface of the intestinal villi. The intestinal villus displays functional differences between the cells at the villus tip and those in the crypts. Work by Sylvén and Nordström (21) indicates that sterol uptake is greatest in mucosal cells near the tip of the villus.

FORM OF CHOLESTEROL ABSORBED

A variable proportion of dietary cholesterol is esterified to fatty acids (12, 13); however, only free cholesterol appears to be absorbed from the intestinal lumen. Studies have suggested that free cholesterol is absorbed to a greater extent than naturally occurring cholesteryl esters (12, 25, 26) and that synthetic cholesteryl esters, resistant to hydrolysis by pancreatic cholesterol esterase, are either poorly absorbed or not absorbed at all (27, 28). A study utilizing double-labeled cholesteryl esters showed that 65–75% of absorbed labeled cholesterol, but less than 2% of the absorbed fatty acid, appeared in the cholesteryl ester fraction of thoracic duct lymph (29), strongly suggesting that cholesteryl esters are completely hydrolyzed prior to absorption.

ROUTE OF CHOLESTEROL ABSORPTION

It was recognized early in the study of intestinal absorption of cholesterol that adding cholesterol to the diet increased the concentration of cholesterol in intestinal lymph (30). Not until the 1950s was the quantitative significance of this lymphatic pathway known. Biggs, Friedman, and Byers (31) demonstrated that following an intragastric dose of [^{3}H]cholesterol very little isotopically labeled cholesterol appeared in the plasma of rats with thoracic lymph duct cannulas. Chaikoff and coworkers (32) recovered 94–101% of absorbed labeled cholesterol in the thoracic duct lymph of rats. Similar results have been reported in lymph duct-cannulated rabbits (33) and in a human subject with chyluria (34) demonstrating that in mammals absorbed cholesterol is transported by the intestinal lymphatics and not by the portal system.

LUMINAL EVENTS

Cholesterol absorption is intimately connected to the overall process of lipid absorption which begins in the stomach when dietary constituents are mixed with lingual and gastric enzymes. The exact chemical and physical state of stomach contents is unknown, but normal gastric motility and retropulsion of antral contents are thought to bring about the formation of a crude emulsion (chyme) (35). Lingual lipases from serous glands at the back of the tongue begin the hydrolysis of triacylglycerols in the stomach (36). The stomach also functions to regulate the delivery of gastric chyme to the duodenum, delivering small portions to the duodenum where it is mixed with bile and pancreatic juice (35).

The digestion of lipid continues within the lumen of the small intestine. The pancreatic secretions contribute hydrolytic enzymes and bile contributes bile salts that solubilize the hydrolytic end products of intraluminal fat digestion.

Pancreatic enzymes

There are three lipolytic enzymes of interest secreted in pancreatic juice: pancreatic lipase, phospholipase A_2, and cholesterol esterase. All three are hydrolytic enzymes that liberate free fatty acids from ester bonds.

Pancreatic triglyceride lipase (EC 3.1.1.3) sequentially hydrolyses the two outside ester bonds of triacylglycerol, the major constituent of dietary fat, producing

2-monoacylglycerol and free fatty acids (37). This enzyme is active at the oil–water interface of emulsified lipid and is protected from the detergent actions of physiological concentrations of bile salts by a protein cofactor, colipase (38).

Phospholipids are hydrolyzed at the 2-position by phospholipase A_2 (EC 3.1.1.4) to yield lysophospholipid and free fatty acid (39). The enzyme requires bile salts for activity against natural long chain phospholipids and presumably uses micellarized phospholipid as substrate (40).

Cholesterol esterase (EC 3.1.1.13) hydrolyses the ester bond of cholesteryl esters producing free cholesterol and fatty acid. Cholic acid or its taurine or glycine conjugate is an absolute requirement for enzymatic activity of rat pancreatic cholesterol esterase; however, unlike phospholipase A_2, the requirement for bile salts is not related to their detergent effect (41). Instead, cholic acid appears to function as a cofactor, allowing the polymerization of the enzyme monomer into an active hexamer (42) or a conformational change and activation (43).

Solubilization

Some of the lipolytic products of the major pancreatic enzymes, including cholesterol, are only minimally soluble in aqueous systems and are dependent on the solubilizing properties of bile salt solutions. So great is this dependence, that the absorption of cholesterol is absolutely dependent on the presence of bile or bile salt replacement (41, 44, 45). Bile salts are biological amphipathic detergents which, when present above a critical micellar concentration, spontaneously form aggregates that are able to dissolve lipids (46). Cholesterol is only sparingly soluble in bile salt solutions, in contrast to phospholipid, monoglycerides and free fatty acids which are readily soluble (47). The addition of phospholipid or monoacylglyceride to bile salt solutions markedly increases the solubility of cholesterol (47, 48). Excess lipid not dissolved in the micellar phase will form a separate oil phase within the intestinal lumen (46), and may be maintained as a stable emulsion by bile salts, phospholipid, monoglyceride, and ionized fatty acids (49).

Intraluminal fat digestion has been characterized by Hoffman and Borgström (50, 51) and others (24, 52–55) as a two-phase system composed of an aqueous micellar phase and an oil phase. Cholesterol, nearly insoluble in a pure aqueous system (47), is described as partitioning between the micellar and oil phases (50). Ultracentrifugation of intestinal contents following a fatty meal revealed three phases: an upper oil or emulsion phase, a bile salt-rich micellar phase, and a sediment phase (19, 22, 51, 55). The lipids of the oil or emulsion phase have been identified primarily as di- and triacylglycerols (19, 51, 53). Sterols have been found in all three phases (19, 53, 55).

Although ultracentrifugal analyses of intraluminal contents have yielded interesting qualitative data, the partitioning of the different lipid classes may be very different in vivo. Lee (52) has shown that ultracentrifugation to isolate micellar solutions may result in sedimentation of micelles, incomplete flotation of unsolubilized oil, and loss of fatty acid to the walls of cellulose nitrate ultracentrifugation tubes. Porter and Saunders (54) present data showing that heating intestinal contents to inactivate pancreatic lipase (51, 53, 55) prior to centrifugation increases free fatty acid and alters phospholipid composition. More current methods utilizing ultrafiltration (52, 54, 56) may break emulsions and perturb phase boundaries (36). To further complicate this issue, examination of triacylglycerol digestion in vitro by light microscopy indicates that there may be as many as four coexisting phases (57). Two laboratories have reported the existence of a liquid crystalline phase separate from the micellar and oil phases in duodenal contents (58–60). More recently, Staggers and coworkers (61) and Hernell, Staggers, and Carey (62) have described in detail the phase behavior of aqueous duodenal lipids. They propose that during lipolysis a liquid crystalline phase composed of multilamellar products of lipid digestion forms at the surface of an emulsion droplet. The liquid crystalline phase provides an accessible source of fatty acid, monoacylglyceride, phospholipid, and cholesterol for the generation of mixed micelles in the presence of bile salts.

UPTAKE BY MUCOSA

It is not known how cholesterol within the intestinal lumen moves from the lumen into the intestinal mucosal cells. Early evidence by Siperstein, Chaikoff, and Reinhardt (44) demonstrated the obligatory requirement of bile acids for the lymphatic appearance of exogenous cholesterol. Later, Swell et al. (41) provided evidence that bile salts are necessary for the entrance of exogenous cholesterol into the mucosa. Both in vivo (55) and in vitro (63, 64) studies indicate that micelles containing cholesterol are not taken up by the intestinal mucosa as intact aggregates. Micellar components appear to be taken up (55, 63, 64) at independent rates. As a result, the mechanism by which bile facilitates cholesterol absorption remains open to speculation (36).

Westergaard and Dietschy (45) propose that bile functions by facilitating the transport of cholesterol across the unstirred water layer (UWL), a series of water lamellae at the interface between the bulk water phase of the lumen and the mucosal cell membrane. The UWL and the cell membrane form two barriers through which a molecule in the bulk phase must pass in order to be absorbed. Westergaard and Dietschy (65) have demonstrated that the entry of lipid molecules into cell membranes is a very rapid, passive event and is not rate limiting for absorption. In contrast, diffusion through the UWL is a relatively slow process for lipids such as cholesterol which have a low

solubility in aqueous solution. They speculate that bile facilitates cholesterol absorption by decreasing the effective resistance of the UWL to the diffusion of cholesterol: mixed micelles containing solubilized cholesterol diffuse across the UWL toward the mucosal membrane and down a concentration gradient (45, 66). Micelles then act as a reservoir for cholesterol. Cholesterol in micelles is in rapid equilibrium with cholesterol in monomolecular solution, in effect, maintaining a maximal concentration within the monomolecular solution (65–68). As individual molecules of cholesterol are taken up into the cell membrane, other molecules of cholesterol move from the micelles into monomolecular solution and become available for uptake by the mucosa.

Although it is generally accepted that cholesterol absorption is energy independent, passive diffusion down a concentration gradient, several investigators have looked for a protein mediator of cholesterol absorption. The discovery of such a protein would offer the unique opportunity to therapeutically interfere with cholesterol absorption by inhibition of the protein involved. Data from Thurnhofer and coworkers (69) indicates that the absorption of cholesterol from mixed micelles by brush-border membrane vesicles may indeed be catalyzed by an intrinsic membrane protein, but confirmation of their findings awaits further purification and characterization of the responsible protein(s).

MUCOSAL CELL EVENTS

During the absorption of exogenous cholesterol, there is little increase in the cholesterol content of the small intestine (70, 71), indicating that cholesterol can be rapidly processed and exported from the mucosal cells and into the intestinal lymph. Following an intragastric dose of cholesterol mass and radioactivity, the transport of both in intestinal lymph rapidly increases and peaks after 6–8 h (33, 70, 72). Absorbed cholesterol appears partially esterified in lymph very low density lipoproteins (VLDL) and chylomicrons (33, 73, 74).

Re-esterification

Essentially all cholesterol that moves from the intestinal lumen into the intestinal mucosal cells is unesterified; however, cholesterol secreted into intestinal lymph following a cholesterol-rich meal is approximately 70–80% esterified (75). It has been suggested that the cholesterol esterifying activity of the mucosa may be an important regulator of cholesterol absorption, since re-esterification of absorbed free cholesterol within the mucosal cell would enhance the diffusion gradient for free cholesterol into the cell. Two enzymes have been implicated in the esterification of cholesterol within the intestinal mucosal cells: cholesterol esterase (EC 3.1.1.13) (76) and acyl coenzyme A:cholesterol acyltransferase (EC 2.3.1.26) (77).

Cholesterol esterase (CEase) activity found in the intestinal mucosa is thought to originate in the exocrine pancreas (76). An early study by Hernandez, Chaikoff, and Kiyasu (78) demonstrated that homogenates of rat duodenum showed diminishing capacity to esterify cholesterol with increasing time after pancreatectomy. This finding was later confirmed by Borja, Vahouny, and Treadwell (79) in rats with pancreatic fistulae. More recently Gallo et al. (80) used immunocytochemistry to show that monospecific antisera to pancreatic CEase forms a reaction product within mucosal cells of normal rats but not of pancreatectomized rats.

Millimolar concentrations of conjugated trihydroxy-bile salts have been described as an absolute requirement for the catalysis of cholesterol esterification by CEase (76, 81) and intestinal CEase activity is measured in vitro in the presence of 10 mM sodium taurocholate (81–84). The requirement for such a high concentration of bile salts argues against an intracellular role for CEase in cholesteryl ester synthesis; however, recent work by Kyger and coworkers (85) has described CEase synthetic activity at micromolar concentrations of taurocholate, concentrations that may indeed occur intracellularly.

The role of CEase in cholesterol absorption was recently examined in studies with Caco-2 cells by Huang and Hui (86). These authors found that there was no effect of this enzyme on the association of radiolabeled free cholesterol with these cells. Free cholesterol association with Caco-2 cells occurred at a rate that was almost three orders of magnitude higher than that of cholesteryl ester or cholesteryl ether. The importance of added CEase in cholesterol uptake by Caco-2 cells may have been to stimulate the slow uptake of intact cholesteryl esters. Although the authors concluded that CEase catalyzed cholesterol absorption, their data do not suggest an important physiologic role for this enzyme in cholesterol absorption.

Results from studies by Murthy, Mahadevan, and Ganguly (87) indicate that a CEase-like activity increases in rat intestinal mucosa with cholesterol feeding. Data from several laboratories has indicated that pancreatic juice or functioning CEase activity within the mucosa is required for normal cholesterol absorption. The appearance of radiolabeled dietary cholesterol in lymph was reduced in rats with pancreatic fistulae or pancreatectomies (79, 81, 88). In contrast, a careful study by Watt and Simmonds (89) showed little or no effect of pancreatic diversion on the initial rate of exogenous cholesterol transport in lymph duct-cannulated rats, although transport of total cholesterol mass was slightly reduced. CEase status in these rats had no effect on the proportion of exogenous cholesterol recovered as cholesteryl ester in lymph. This finding suggests that another cholesterol esterifying enzyme must be functional in intestinal mucosal cells.

Acyl CoA:cholesterol acyltransferase (ACAT) is the se-

cond enzyme that has been implicated in intestinal mucosal cell esterification reactions. Haugen and Norum (77) were the first investigators to identify ACAT activity in the intestinal mucosa of rats. The enzyme has since been described in the intestine of humans (90), guinea pigs (91), and rabbits (92). Unlike CEase, ACAT is inhibited by bile salts (90) and is dependent on the CoA "activation" of fatty acids prior to esterification (77). Intestinal ACAT activity has been shown to increase with cholesterol feeding in rabbits (93) and guinea pigs (91, 94), and to decrease with cholestyramine administration (93). Depletion of mucosal cholesterol pools by duodenal perfusion with triolein in lymph fistula rats resulted in decreased ACAT activity in intestinal homogenates (95).

Apparent effects on cholesterol absorption in vivo utilizing specific ACAT inhibitors have been variable, but most have shown a decrease in cholesterol absorption. Studies in cholesterol-fed rabbits suggest that inhibition of intestinal ACAT activity results in decreased cholesterol absorption (96). Results in chow-fed rats have been mixed. Gallo, Wadsworth, and Vahouny (97) saw no effect of ACAT inhibition on the initial rate of lymphatic [^{14}C]cholesterol transport after an intragastric dose of cholesterol in rats. In contrast, Bennett Clark and Tercyak (98) saw a decrease in the rate of [^{3}H]cholesteryl ester transport in lymph when intestinal ACAT was inhibited in chow-fed rats. Most recently in cholesterol-fed rats and hamsters, Krause et al. (99, 100) found a significant reduction in cholesterol absorption with ACAT inhibitors as did Windler et al. (101) in cholesterol-fed rats. The effectiveness of ACAT inhibitors in decreasing intestinal cholesterol absorption suggests a primary role for this enzyme in the regulation of cholesterol absorption; the role of CEase seems less well established.

The investigation of the role of ACAT in cholesterol absorption may soon become more direct with the recent description of a molecular clone for human ACAT (102). Using a somatic cell genetics approach, Chang and associates (102) have identified a human macrophage cDNA clone for the ACAT enzyme that, when transfected into AC29 cells, provides expression of ACAT activity in these cells. The open reading frame of this cDNA is 1.7 kb and codes for a protein with 550 amino acids that is apparently an integral membrane protein. This represents a breakthrough in the study of this enzyme as previously it has resisted all attempts at purification via classical protein purification methodology. With the invaluable information provided by the clone of the enzyme, direct approaches with antibodies, as well as with transfection and knockout studies, can begin to establish the physiologic role of this enzyme in cholesterol absorption and other aspects of cholesterol metabolism, both in cells in culture and in whole animals.

SPECIFICITY OF ABSORPTION

The processes that control cholesterol absorption appear to be very specific. 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 carbon-24 are less efficiently absorbed than cholesterol. For example, the absorption of cholestanol, the saturated analogue of cholesterol, has been reported to be 10–22% (103, 104). The absorption of the plant sterol, β-sitosterol, which differs from cholesterol only by the addition of an ethyl group on carbon-24, is generally accepted to be less than 5% (105, 106). Other plant sterols, as well as some shellfish sterols, have been shown to be less efficiently absorbed than cholesterol (103, 107–111). In addition, many of these same noncholesterol sterols have been shown to inhibit the absorption of cholesterol (111–113) and have demonstrated hypocholesterolemic properties (114–117). The mechanisms by which both the specificity of absorption is established and cholesterol absorption is inhibited by plant sterols are unknown, but several factors may be involved.

There has been speculation that differential absorption might be due to differences in intracellular esterification of the noncholesterol sterols within enterocytes. Lymph duct cannulation studies have demonstrated limited esterification of absorbed plant sterols, whereas absorbed cholesterol is 80–90% esterified (118, 119). Both pancreatic CEase (120, 121) and intestinal ACAT (122) have been shown to esterify some plant sterols in vitro, but at rates much less than that for cholesterol. Ikeda et al. (123) have observed that the transfer of sitosterol from brush-border membranes to liposomes is significantly slower than the transfer of cholesterol. The addition of microgram amounts of SCP_2 (sterol carrier protein 2) did not significantly affect the transfer of radiolabeled cholesterol; however, the transfer of sitosterol was enhanced in a dose-dependent manner, though only moderately. Although limited intracellular esterification may play some role in the poor absorption of plant sterols, it does not explain the effects of plant sterol-feeding on cholesterol absorption. The existence of a specific protein mediator of cholesterol uptake at the enterocyte membrane might also confer specificity, but the existence of such a protein remains speculation until further studies are completed (69).

The data of Armstrong and Carey (124) suggest that many noncholesterol sterols, due to their increased hydrophobicity relative to cholesterol, have a lower solubility in, but a higher affinity for, bile salt micelles than does cholesterol. Thus a physical–chemical attribute may contribute to both the less efficient absorption of the plant sterols and their effect on cholesterol absorption by displacing cholesterol from bile acid micelles (113).

DILUTION WITH ENDOGENOUS CHOLESTEROL

Several researchers have reported that the specific activity of lymph cholesterol is only a fraction of the specific activity of labeled dietary cholesterol (33, 38, 40, 73, 125–127) indicating that exogenous cholesterol is mixed with and diluted by endogenous cholesterol during its passage from the intestinal lumen into the lymph. Part of this decline in specific activity may be explained by the dilution of dietary cholesterol with endogenous biliary cholesterol and sloughed mucosal cell cholesterol within the gut lumen. Thus Swell et al. (126) report an 11% decrease in cholesterol specific activity within the intestinal lumen of rats fed radioactive cholesterol. They report an additional 46% decrease in cholesterol specific activity between the intestinal lumen and thoracic duct lymph. There are at least three possible sources for this secondary dilution of dietary cholesterol specific activity. First, intestinal mucosal cells may contribute unlabeled cholesterol from de novo synthesis. In support of this concept, studies by Lindsey and Wilson (128) and Wilson and Reinke (129) indicate that cholesterol synthesized in the intestine appears in intestinal lymph lipoproteins. Second, transudated plasma lipoproteins present in intestinal or thoracic duct lymph will furnish unlabeled cholesterol mass which will dilute exogenous cholesterol specific activity (33, 130–132). Hepatic lymph also contributes cholesterol mass to thoracic duct lymph (49, 133) and Quintão et al. (132) state that a significant portion of lymph cholesterol mass in rats fed a cholesterol-free diet is derived from transudated plasma lipoproteins. Third, unlabeled endogenous biliary cholesterol may be preferentially absorbed from the gut and contribute to the further lowering of the specific activity of absorbed exogenous cholesterol (49).

DIFFERENTIAL ABSORPTION OF DIETARY AND BILIARY CHOLESTEROL

Dietary cholesterol, through its absorption from the gastrointestinal tract, is known to influence whole body cholesterol homeostasis by alterations in both plasma cholesterol concentration and in whole body cholesterol synthesis (134–136). However, as pointed out previously, dietary cholesterol accounts for only about one-third of the total cholesterol absorbed by North American adults. Endogenous sources provide the remaining cholesterol to the gut. Of these endogenous sources, bile provides about twice the mass of cholesterol that is ingested in the diet on a daily basis. The intestinal wall provides an undetermined amount of cholesterol in the form of desquamated intestinal epithelium and de novo synthesis. If these latter sources of cholesterol enter the gut lumen distal to sites of active cholesterol absorption, their contribution may be relatively unimportant. Once absorbed, cholesterol of exogenous and endogenous origins is presumed to be indistinguishable and should have similar potential for affecting cholesterol homeostasis.

Although it is generally assumed that endogenous and exogenous sources of cholesterol mix within the gut lumen to form a single homogeneous pool from which absorption occurs, there is some evidence that biliary cholesterol may actually be more efficiently absorbed than dietary cholesterol. This potential difference in the efficiency of absorption may be mediated by the physicochemical state in which biliary and dietary cholesterol enter the gastrointestinal tract. Biliary cholesterol is delivered to the gut lumen in a bile salt/phospholipid micelle, and is presumably immediately available for absorption. On the other hand, dietary cholesterol must first be transferred into the micellar phase from an oil phase (dietary triacylglycerol) or from a phospholipid/cholesterol lamellar phase (tissue membrane) before it can be absorbed. If mixing of these two sources of cholesterol does not occur immediately after introduction into the gut lumen, then biliary cholesterol may be initially absorbed with greater efficiency than is dietary cholesterol.

The earliest evidence supporting the possibility of differential absorption of dietary and biliary cholesterol comes from the intestinal perfusion studies of Simmonds, Hofmann, and Theodor (55). These investigators infused a micellar solution of radiolabeled cholesterol, mixed bile salts, and monoacylglyceride into the upper jejunum of human volunteers. They found that the specific activity of cholesterol in samples of intestinal contents decreased between proximal and distal sampling sites, suggesting that the micellarized radiolabeled exogenous cholesterol was more efficiently absorbed by the test segment than the mass of chemically determined cholesterol within the gut lumen. Similar changes in the specific activity of intestinal contents were reported by Grundy and Mok (137) who used exogenous cholesterol that had been solubilized in the detergent, triglycerol monoleate. Although preferential absorption of micellarized exogenous cholesterol is consistent with the difference that these investigators saw, there are at least two other plausible explanations for these results. Simmonds et al. (55) hypothesized that the decrease in specific activity along the absorptive segment was due to "the continuous addition of nonradioactive endogenous cholesterol by the test segment." Grundy and Mok (137) favored "isotope exchange" between radiolabeled cholesterol in the lumen and unlabeled cholesterol in the intestinal mucosa as the most likely explanation of their findings.

Results from other laboratories using rats (138–140) and chickens (141) also support the concept of differential absorption of endogenous and exogenous sources of cholesterol, but these studies, like the studies in human subjects, were unable to distinguish differential absorp-

tion from isotope exchange or mucosal secretion of cholesterol. They also looked only at changes in specific activity of luminal contents.

The studies of Samuel and coworkers (142, 143) strongly suggest that micellarized sources of cholesterol are preferentially absorbed. In these studies the absorption of endogenous cholesterol ([^{3}H]cholesterol given intravenously 6 weeks prior to the studies), exogenous cholesterol ([^{14}C]cholesterol incorporated into liquid formula), and total cholesterol mass were determined simultaneously during the intraduodenal infusion of a liquid formula in six patients. In preliminary studies during the infusion of a cholesterol-free formula, intravenously administered [^{3}H]cholesterol was shown to be a valid marker for endogenous cholesterol absorption. Measurements of endogenous cholesterol mass absorption were the same whether calculated based on the disappearance of [^{3}H]cholesterol or cholesterol mass; therefore, the exchangeable pools of body cholesterol were uniformly labeled, and the contribution of unlabeled newly synthesized mucosal cholesterol to the gut lumen by isotope exchange or direct secretion appeared to be minimal. Subsequent experiments demonstrated that the calculated absorption of exogenous cholesterol administered as an emulsion in liquid formula was significantly lower than that of endogenous cholesterol in six of nine experiments. Overall, exogenous [^{14}C]cholesterol absorption was 34 ± 8% (mean ± SD), while endogenous [^{3}H]cholesterol absorption was 46 ± 15%. In contrast, when exogenous [^{14}C]cholesterol was dispersed in a micellar solution of the detergent triglycerol monoleate, its calculated absorption was significantly higher than that of the endogenous [^{3}H]cholesterol in four out of seven experiments. The mean absorption of the exogenous and endogenous radiolabels was 33 ± 9% and 30 ± 14%, respectively. In both cases, cholesterol mass absorption was intermediate between these values. These findings suggest that the initial rate of cholesterol absorption is dependent on the physicochemical state of the source cholesterol.

These experiments still leave us with the question of whether absorption rates for endogenous and exogenous cholesterol are different over the entire length of the small intestine. Samuel and McNamara (142) studied one patient in which endogenous and exogenous cholesterol absorption was measured over both 1-meter and 2-meter segments of intestine. When the absorptive segment was increased from 1 meter to 2 meters, the calculated percent absorption of exogenous cholesterol more than doubled, increasing from 16% to 41%. The absorption of endogenous cholesterol did not increase to nearly this extent (64% versus 74%), suggesting that the two sources of cholesterol were approaching an equilibrium as they traveled down the absorptive gut.

The previously cited studies have suggested that radiolabeled cholesterol of endogenous and exogenous origins disappears from the intestinal lumen at different rates (55, 137–140, 142, 143). The inference from most of these studies is that cholesterol that is dispersed in a micellar solution is preferentially absorbed (or exchanged). However, cholesterol absorption can be defined not only by the disappearance of cholesterol from the gut lumen, but also by the subsequent transfer through the mucosal cell into chylomicrons which appear in intestinal and thoracic duct lymph. The first report that provides this type of data for both biliary and dietary cholesterol was published in 1983. Reisser, Boutillon, and Clement (144) collected thoracic duct lymph following the simultaneous short-term (5 h) intraduodenal infusion of labeled donor bile and liquid diet into rats with bile fistulae. Radiolabeled bile for the infusions was collected from donor rats labeled intravenously with [^{3}H]cholesterol. The liquid diet contained [^{14}C]cholesterol solubilized in a stable suspension (triolein, phosphatidyl choline, casein hydrolysate, and sucrose). The recovery of biliary [^{3}H]cholesterol in 48-h lymph collections was markedly higher than the recovery of [^{14}C]cholesterol (33% versus 18%, n = 6) suggesting that biliary cholesterol was preferentially absorbed. Although the results from this study agree with results from other laboratories, they are in direct conflict with an earlier report from the same group which suggested that dietary cholesterol, not biliary cholesterol, was preferentially absorbed (139).

It is likely that much of Reisser's data reflects differential isotope exchange during a period of abnormal lipid absorption and not preferential absorption. Dulery and Reisser (139) used surgically prepared rats in both of their studies. Diet and bile infusions (identical for both studies) were begun immediately postoperatively after establishing bile fistulae with or without lymph duct cannulation. Early investigation in lymph duct-cannulated rats indicated that surgical trauma adversely affects lymphatic transport (i.e., absorption) of dietary lipid, particularly in rats also receiving bile fistulae (145). If the mechanisms by which cholesterol is absorbed and transferred into the lymphatic system are suppressed during the immediate postoperative period, it is likely that the distribution of radiolabeled cholesterol in intestinal mucosa reflected differential isotope exchange between luminal contents and mucosal cholesterol (139).

Data from our laboratory provide additional information about the possible differential absorption of dietary and biliary cholesterol (146, 147). We found that in lymph duct-cannulated rats with hepatic bile fistulae, there was little difference in the percent absorption of radiolabeled cholesterol infused intraduodenally in a stable suspension (casein, dextrin-maltose, safflower oil, and vitamins and minerals) or in a clear micellar solution (bile salts and phospholipid).

Twenty-six Sprague-Dawley rats each received a thoracic lymph duct cannula for quantitative intestinal

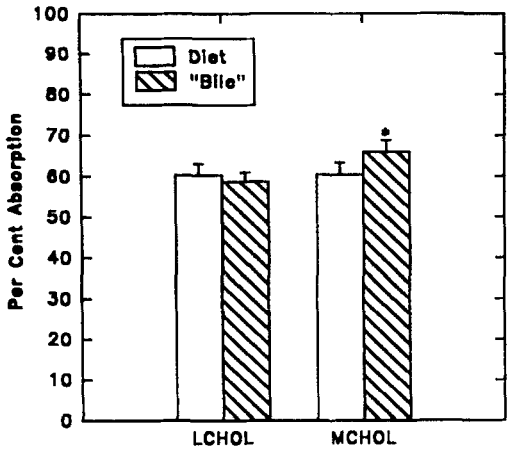

Fig. 1. Percentage of dietary [^{14}C]cholesterol and micellar [^{3}H]cholesterol appearing in thoracic duct lymph during the constant intraduodenal infusion of two liquid diets (LCHOL and MCHOL) and micellar bile salt replacement in rats with biliary fistulae. The data are the mean ± SEM, n ' 12 for each diet group. Asterisk (*) indicates significant difference from diet cholesterol absorption by two-tailed paired *t*-test, $P < 0.01$.

lymph collection, a common bile duct cannula for total biliary diversion, a double-lumen duodenal cannula for the simultaneous infusion of a liquid diet and a bile replacement, and a jugular vein catheter for hydration. After completion of the surgery, animals were placed in restraint and allowed a 24-h post-operative recovery period during which they received a constant intraduodenal infusion of 5% dextrose in 0.9% saline. At the end of the recovery period, if lymph and bile flow were judged acceptable, animals continued in the experimental protocol.

Three liquid diets differing only in cholesterol content were used in the infusion experiments. The cholesterol content of the low (LCHOL), moderate (MCHOL), and high (HCHOL) cholesterol diets was 0.15, 0.75, and 1.50 mg/kcal, respectively. Diets contained 35% of calories as fat, 47% as carbohydrate, and 18% as protein. Crystalline cholesterol was dissolved in the oil of the diet prior to mixing. Diets were infused into the duodenum at 3.0 kcal/3.0 ml per hr.

Three clear micellar solutions differing only in cholesterol content and saturation[2] of cholesterol were used in the infusion experiments: unsaturated, 0.37 mM cholesterol or 58% saturated; saturated, 0.87 mM cholesterol or 131% saturated; and supersaturated, 1.74 mM cholesterol or 250% saturated. Micellar solutions were infused into the duodenum at 1.8 ml/h. Throughout a continuous period of 26 h, each rat received duodenal infusions of one of the three diets and one of the micellar bile salt replacements. Diets were labeled with [^{14}C]cholesterol; bile replacements were labeled with [^{3}H]cholesterol. Sequential lymph samples of 2–4 h duration were collected into graduated vessels maintained at 4°C throughout the infusion period. The percent absorption of each source of radiolabeled cholesterol was calculated during the final 12 h of infusion during the steady-state appearance of cholesterol in thoracic duct lymph.

The degree of saturation of the three bile replacements had no effect on the efficiency of absorption of either "dietary" or "biliary" cholesterol (data not shown). There was no difference in the efficiency of absorption of "dietary" and "biliary" cholesterol in the LCHOL diet group, 60.3 ± 4.0% versus 58.7 ± 2.3% (mean ± SEM, n = 12), and only a small difference in the MCHOL diet group, 60.4 ± 3.0% versus 66.0 ± 2.8% (mean ± SEM, n = 12, $P < 0.01$) (**Fig. 1**). Only in the HCHOL diet group, where solubility of the cholesterol in the dietary triacylglycerol was greatly exceeded, was there a large difference in the absorption of "dietary" and "biliary" cholesterol (46.2% versus 64%, n = 2). Our results suggest that when one considers the entire absorptive length of the gut, micellarized and nonmicellarized cholesterol tend to mix quickly so that there is little opportunity for preferential absorption of premicellarized cholesterol.

These experiments were designed to measure cholesterol absorption during the steady-state appearance of radioisotopic cholesterol in thoracic duct lymph. Since exogenously supplied radioisotopic free cholesterol introduced into the gut exchanges with many other available sources of free cholesterol during the absorptive process (148), cholesterol absorption was calculated only after lymph cholesterol specific activity became constant relative to the infused sources after 12 h of infusion (**Fig. 2**). The attainment of a constant lymph cholesterol specific activity suggests that isotope exchange between infused radiolabeled free cholesterol and exchangeable pools of free cholesterol along the metabolic pathway of cholesterol absorption has reached an equilibrium. During this period the effects of isotope exchange are minimal

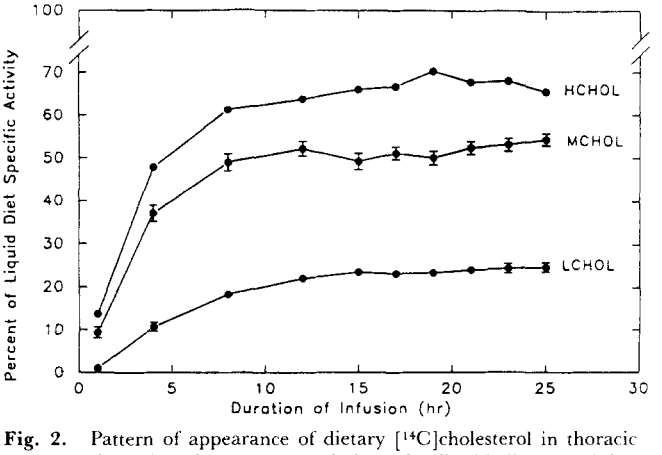

Fig. 2. Pattern of appearance of dietary [^{14}C]cholesterol in thoracic duct lymph during the constant infusion of a liquid diet containing [^{14}C]cholesterol, normalized to the diet cholesterol specific activity (100%). The data are the mean ± SEM, n = 12 for the LCHOL and MCHOL diet groups and n = 2 for the HCHOL group.

[2]Percent saturation was determined by the molar ratio method of M.C. Carey (*J. Lipid Res.* 1978. **19:** 945–955).

and net transport of radiolabeled cholesterol in thoracic duct lymph most closely reflects the net transfer of cholesterol mass from the radiolabeled intraluminal source.

The time required for exchangeable pools to come to isotopic equilibrium with luminal cholesterol during the constant infusion of radiolabeled cholesterol was 8–12 h in rats (Fig. 2) and 18–24 h in monkeys (131). These results suggest that prior to isotopic equilibrium, estimates of the absorption of radiolabeled cholesterol will be high if based on the specific activity of intraluminal contents and low if based on lymphatic transport of isotopic cholesterol. The most accurate estimates of the net absorption of dietary and biliary cholesterol will be obtained after isotopic equilibrium has been reached, and this occurs much later in the time course of cholesterol absorption than almost all other experiments have covered (55, 137–144).

In this article we have reviewed factors that regulate cholesterol absorption and have examined the evidence surrounding the efficiency of endogenous and exogenous cholesterol absorption. Our conclusion is that while the efficiency of absorption of micellar cholesterol in bile is potentially greater, the time that it takes to get the fat and cholesterol from a meal digested, emulsified, and into micelles and then absorbed is long enough that mixing of cholesterol from endogenous and dietary sources is essentially complete. As a result, the percentage absorption of cholesterol from both sources is equal.

The relative degree of importance of cholesterol absorption in the determination of plasma cholesterol concentrations is still not well understood. However, in several cases a statistically significant correlation between the percentage cholesterol absorption and plasma cholesterol concentration has been seen (2, 4, 5, 6, 7, 8, 149). Thus, the potential for cholesterol absorption efficiency to be a partial determinant of the degree of hypercholesterolemia is established and needs to be considered further.

These findings imply that maneuvers designed to decrease cholesterol absorption as a mechanism to lower plasma cholesterol concentrations must consider both endogenous and exogenous cholesterol as the target. Since the endogenous cholesterol pool is typically significantly larger than the exogenous cholesterol load, the total effective pool size must include both sources of cholesterol.

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