methods

Separation of micelles and vesicles within lumenal aspirates from healthy humans: solubilization of cholesterol after a meal

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Abstract Understanding the physico-chemical relationship of lumenal lipids to one another is critical when elucidating the mechanism of components known to impact cholesterol absorption. Presently, there are no studies that describe the proportion of cholesterol carried as micelles or vesicles within human lumenal contents. Part of the reason for the scarceness of data is because of the lack of appropriate methodology required for reproducible sample collection and analysis. Thus, the object of the present studies was to develop a method to measure the amount of cholesterol carried as micelles or vesicles in human lumenal samples. The method includes the collection of lumenal samples from the ligament of Trietz through a Fredrick Miller tube, separation of the aqueous subphase from the nondigested lipids, separation of micelles and vesicles on Sepharose® 4B columns within 48 h of collection using elution buffers consisting of the intermicellar bile acid composition, and finally quantitation of cholesterol eluted off of the columns. The distribution of cholesterol between micelles and vesicles obtained under different concentrations of bile acids and various lipids was comparable to results obtained from phase diagrams using the lumenal molar percentages of lipids obtained from the same samples.—Yao, L., J. E. Heubi, D. D. Buckley, H. Fierra, K. D. R. Setchell, N. A. Granholm, P. Tso, D. Y. Hui, and L. A. Woollett. **Separation of micelles and vesicles within lumenal aspirates from healthy humans: solubilization of cholesterol after a meal.** ***J. Lipid Res.*** **2002.** 43: **654–660.**

Supplementary key words bile acid • absorption • lipid

Intestinal cholesterol absorption is a major determinant for whole body cholesterol homeostasis (1, 2) and plasma LDL-cholesterol (LDL-C) concentrations (3–5) in Western populations that consume significant quantities of cholesterol. Consequently, differences in efficiency of cholesterol absorption can lead to a greater or lesser risk of development of atherosclerosis. The mechanism of cholesterol absorption is not fully understood and is dependent upon physico-chemical characteristics of lipids within the lumen, possible brush-border cholesterol transporters, and metabolism within enterocytes themselves (6, 7). Briefly, within the gut lumen, exogenous dietary cholesterol is initially emulsified with triglycerides and other undigested lipids in oily droplets. As intralumenal fat digestion occurs, a multilamellar liquid crystalline phase is generated at the surface of the emulsion particle, which in turn provides substrates for the mixed micelles (8). The partitioning of cholesterol into bile salt-containing mixed micelles is considered necessary for cholesterol absorption (9–11). The micellar cholesterol crosses the unstirred water layer followed by the movement of monomeric cholesterol across the brush border membrane either through passive diffusion or by a carrier mediated mechanism.

The effect of changing the lipid environment of cholesterol solubilization within micelles or vesicles has been examined previously using model bile. These past studies have examined the effects of type of bile acid, the amount of bile acid, and the amount and type of other lipids used in mixed micelles, such as cholesterol, phospholipids, and triglyceride digested products (TDP) (12–14). The studies have also examined the proportion of lipid moieties carried in micelles and vesicles by separating these samples by column chromatography and measuring the lipid of interest in each fraction (14). As might be expected, interconversion of micelles and vesicles can be effected by changing the bile acid hydrophobicity and/or the bile acid concentration of bile acids in the column elution buffers (12–14).

Abbreviations: Aprilapo, apolipoprotein; CA, cholic acid; CDCA, chenodeoxycholic acid; CRC, Clinical Research Center; DCA, deoxycholic acid; ELSD, evaporative light scattering detector; IMBC, intermicellar bile acid concentration; PL, phospholipid; TDP, triglyceride digestion products; UDCA, ursodeoxycholic acid.

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Even though a significant body of information exists that describes the effects of various lipids on the solubility of cholesterol in model bile, the effects that similar compounds have on the distribution of cholesterol between micelles and vesicles in the human intestinal lumen is unknown. Since micelles and vesicles are present in the human intestinal lumen after a meal, as seen by freeze fracture (8), the purpose of the present studies was to develop a method that could be used to quantitate the amount of cholesterol carried as either micelles or vesicles in the human intestinal lumen. To test the methodology, the presence of micellar or vesicular cholesterol was examined in lumenal samples with markedly variable lipid concentrations, such as those taken before and after consumption of a lipid-containing meal (15, 16). The methodology described here can be applied to various studies in which the effects of dietary or pharmacological agents on micelle formation and cholesterol absorption efficiencies are to be investigated.

MATERIALS AND METHODS

Study design, duodenal intubations, and sample collection

Healthy subjects (two males and four females, aged 24–34 years) were enrolled in the study. Enrollment criteria included *1*) good health with no medical conditions affecting the gastrointestinal tract or liver and no medication usage known to affect cholesterol or bile acid metabolism, *2*) plasma total and LDL-C concentrations less than 200 and 120 mg/dl, respectively, and *3*) apoE-3/3 and apoAIV-1/1 genotypes to ensure that differences were not due to genotypes known to affect cholesterol absorption (17–19). All protocols used were approved by the Institutional Review Boards of the University of Cincinnati and Children's Hospital Medical Center. All subjects gave informed consent, and understanding was confirmed using a standardized test including questions about the study; subjects were required to achieve a score of 70% as a prerequisite to participation.

Prior to initiation of the study, persons were weighed, and their daily caloric, or energy, requirements were calculated taking into account individual physical activities, previous caloric consumption based on a 3 day diet diary, and the sex of the subject. Diets were made in the Clinical Research Center (CRC) of Children's Hospital Medical Center on a 3 day rotating schedule. Diets were made such that subjects would consume 30% of the daily calories as fat (15% monounsaturated; P/S ratio of 0.5) and 220 mg cholesterol per day. Subjects picked up prepared meals from the CRC every 2–3 days. Any unconsumed food was returned when the new food was obtained. Subjects were weighed every other day and calories adjusted to maintain a constant weight. Compliance was verified by daily check sheets and the return of any uneaten food. After 14 days, the subjects came to the CRC after an overnight fast for nasoduodenal intubations. At their option, subjects either received sedation with intravenous midazolam or no sedation for the procedure. After placing topical anesthetic in the nares and obtaining a suitable level of sedation (if sedation was administered), a nasoduodenal tube (8 Frederick Miller feeding catheter, Cook, Inc., Bloomington, IN) that had an additional 10 side ports made by the radiologist over the distal 6 cm of the tube was placed under fluoroscopic guidance with the tip at the ligament of Trietz. Tube placement was verified by injection of water soluble contrast. A 15–20 ml sample of lumenal contents was collected either by siphonage or with gentle suction using a syringe. Once this baseline sample was collected, subjects drank 240 ml of a liquid meal consisting of olive oil, 1 raw pasteurized egg, sucrose, vanilla extract, salt, and water (8); the composition of the diet was 33.6 g fat, 122.9 mg cholesterol, 3.7 g protein, and 13.6 g carbohydrates. Six samples (15–20 ml) were collected at 15 min intervals from −15 to 90 min and one final sample was collected from 90 to 120 min after consumption of the meal.

Duodenal aspirates

Immediately after the collection of each sample, lipase and bacterial inhibitors, including diisopropylfluorophosphate, diethyl (p-nitrophenyl) phosphate, acetophenone, phenylboronic acid, sodium azide, and chloramphenicol, were added to the lumenal contents as described (8), and samples were aliquoted into several fractions. Fractions were stored for analysis of lipids at a later time (see below), or were separated into the oil, subphase (aqueous), and pelleted phases by ultracentrifugation at 39,000 rpm for 60 min in a SW40 rotor (Beckman Coulter, Inc., Fullerton, CA) at 37°C (8). After aspiration from the middle of the tube with a 20 gauge needle, the subphase was aliquoted into five different fractions. One fraction of the subphase was maintained at 37°C to be separated into micelles and vesicles; three fractions were stored for future lipid analyses; and the final fraction was spun in a Centricon® YM-30 concentrator tube (Amicon, Inc., Beverly, MA) to obtain a filtrate from which the monomeric or intermicellar bile acid concentration (IMBC) could be determined (see below for bile acid concentration determination) (12). The Centricon® concentrator YM-30 tubes were used in these studies, instead of the Centricon® concentrator YM-10 tubes as previously described for model bile (12), because of the extreme viscosity of the samples 30 min after consumption of the liquid meal and, consequently, the inability to obtain filtrate to measure the IMBC. Using a buffer consisting of the concentrations of bile acids in the subphase IMBC, the sample that had been maintained at 37°C was resolved into micelles and vesicles on a Sepharose® 4B column (Amersham Pharmacia Biotech, Piscataway, NJ) (14). Thus, each individual sample had its own specific IMBC used during the separations. Fractions from the column were collected, and cholesterol content in each fraction measured enzymatically (Roche Diagnostics Corp., Indianapolis, IN). Concentrations in various samples were verified by gas-liquid chromatography (GLC). In additional studies, lumenal subphase samples were maintained at 37°C for 0, 24, 48, and 144 h, and the distribution of cholesterol between micelles and vesicles was determined. Also, subphase samples were eluted with the correct IMBC or with 0.9% saline.

Chemical analyses of duodenal aspirates

Samples of the total lumenal contents and/or the subphase were collected at each time point after the meal and stored in Folch for a total lipid extraction, in ethanol for bile acid extraction, and in alcoholic base for cholesterol extraction. Four different assays were performed: *1*) Phospholipids in the subphase were measured using a chemical assay (20). *2*) Triglyceride digestion products in the subphase were separated into the different lipid classes by TLC (21). The separated lipids were stained with phosphomolybdate and visualized after charring at 80°C for 40 min. The charred plates were scanned and the densities determined using NIH Scion Image Software. The triglycerides, diglycerides, monoglycerides, and FFAs were quantitated by running a standard curve (0–50 μg) of each TDP on each plate. *3*) Total and subphase lumenal samples that were stored in alcoholic base were saponified, and cholesterol was extracted with petroleum ether. Concentrations were measured by GLC using stigmastanol as an internal standard (22). *4*) Subphase bile acids

were extracted in ethanol and reconstituted in methanol using 5β-cholanic acid-7α, 12α-diol as an internal standard. A portion of the sample plus internal standard was injected onto a 5 μm ODS HYPERSIL (C_{18}) 4.6 × 250 mm analytical column (Keystone Scientific, Inc., Bellefonte, PA). A linear gradient mobile phase system was used to elute the bile acids from the C_{18} column. Mobile phase A included 15% iso-propanol, 15% acetonitrile, and 70% 20 mM acetic acid (pH = 4.45). Mobile phase B included 40% iso-propanol, 20% acetonitrile, and 40% 20 mM acetic acid (pH = 3.5). Bile acids were detected using an evaporative light scattering detector (ELSD, Alltech Associates, Deerfield, IL) (23). Bile acids were identified based on the retention time of known standards and quantified based on the amount of standard added to the sample using Shimadzu Class-VP software.

RESULTS

Figure 1 represents the average concentration of lipids that have partitioned into the aqueous phase, or subphase, before and after a liquid lipid-containing meal in the intestine of adults. All lipids follow the same pattern in that concentrations are low prior to the meal and then increase after the meal. Most lipids decrease 45–60 min after the meal because of absorption or movement through the intestine. The increase in the concentrations is caused by the emptying of the stomach into the small intestine and/or the contraction of the gallbladder in response to the meal. Cholesterol and bile acid concentrations (Fig. 1A, D) increased from 0.7 and 6.1 mM, respectively, and were greatest at 15–30 min post-meal (3.0 and 73.3 mM for cholesterol and bile acid concentrations, respectively). Phospholipid concentrations (Fig. 1B) increased from 1.1 mM to a peak concentration of 8.3 mM by 15–30 min of meal consumption. The triglyceride and diglyceride concentrations (Fig. 1C) increased only slightly in the aqueous fraction of the lumenal sample; triglyceride concentration increased from 0.7 to 0.8 mM, and diglyceride concentrations increased from 0.8 to 3.7 mM during the sample collection. Concentrations of these lipids were much greater in the total lumenal sample (data not shown). The concentrations of monoglycerides and FFAs increased from 1.1 and 1.5 mM to 16.9 and 21.5 mM, respectively, by 15–30 min after the meal and remained above baseline throughout the 2 h study possibly because of a continuation in lipolysis of triglycerides, diglycerides and phospholipids, and/or a slower rate of absorption of monoglycerides (24).

The ratio of cholesterol concentration in the subphase to the total sample was calculated next, with the ratio of subphase to total cholesterol concentrations representing the solubility, or partitioning, of cholesterol into the aqueous fraction of the lumenal contents. As seen in **Fig. 2**, the percentage of cholesterol in the aqueous fraction was greatest in the samples before (115%) and just after the meal (73–104%). The solubility of cholesterol decreased within 2 h of the meal, however, to ≈50%, possibly because of the lack of other lipids in the lumen.

Because the purpose of these studies was to establish a method by which to determine how the cholesterol is carried in the aqueous phase, we separated the subphase into micelles and vesicles and measured the cholesterol concentration in each fraction. We then verified our results by plotting the data presented in Fig. 1 on a phase diagram previously designed for human lumenal samples (15) and comparing the plotted results to those obtained from the gel exclusion chromatography. Prior to the determination of the presence of micelles and vesicles in the lumenal

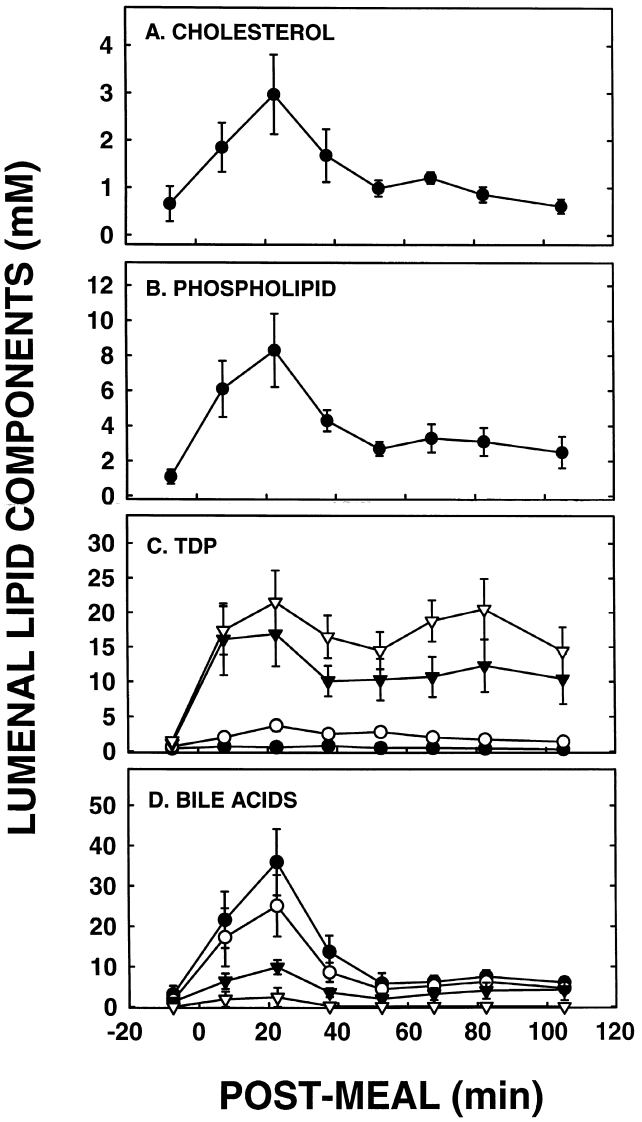

Fig. 1. Absolute concentrations of cholesterol, phospholipid, triglyceride digested products, and bile acids in the subphase of lumenal samples taken before and after a liquid meal. Subjects were maintained on a diet consisting of 30% fat (cal) and 220 mg cholesterol/day. Samples were collected from a nasogastric tube prior to consumption of a liquid meal and every 15 to 30 min after the meal. Cholesterol (A); phospholipid (B); triglyceride digested product (TDP) including, TG (closed circle), diglyceride (DG) (open circle), MG (closed triangle), and free fatty acids (FFA) (open triangle) (C); and bile acids, including cholic acid (CA) (closed circle), chenodeoxycholic acid (CDCA) (open circle), deoxycholic acid (DCA) (closed triangle), and ursodeoxycholic acid (UDCA) (open triangle) (D). Concentrations were measured as described in the Materials and Methods. Data are presented as mean ± SEM for six subjects.

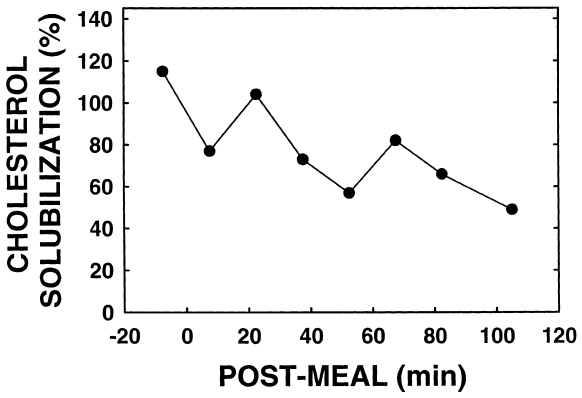

Fig. 2. Cholesterol solubilization, or partitioning, between the aqueous and lipid phases of intestinal contents after a liquid meal. The average cholesterol concentration within the aqueous fraction presented in Fig. 1 was divided by the average cholesterol concentration in the total fraction. Data are presented as percentages.

samples by gel exclusion chromatography, however, we needed to test the importance of the bile acid concentration in the elution buffer and the stability of the particles over time.

In model bile, it has been demonstrated that a change in the bile acid concentration in the elution buffer can convert micelles to vesicles and vice versa (12–14). To test if the bile acid concentration is also critical in the separation of particles within lumenal contents, a sample obtained from the lumen was separated into micelles and vesicles using an elution buffer with the subphase IMBC, the environment of the micelles and vesicles in vivo, or with saline. As seen in **Fig. 3A**, this test lumenal sample had solely micelles when the IMBC was used as the elution buffer (7.3 mM bile acids). When the same sample was separated using the unphysiological saline as the elution buffer, all the micelles were converted to vesicles. Thus, as with model bile (12, 13), the bile acid concentration in the elution buffer has a major role in the determination of the distribution of micelles and vesicles in lumenal samples.

To test the stability of the micelles and vesicles, a single lumenal sample was collected and particles separated on the Sepharose® 4B column within 3 h of collection using the IMBC as the elution buffer. The same sample was also run 24, 48, and 144 h later using the identical elution buffer. As seen in Fig. 3B, the distribution of cholesterol between micelles and vesicles remained constant from 0–48 h. By 144 h, micelles and vesicles became dispersed within samples (data not shown).

Using the information obtained from these preliminary studies, subphases isolated from samples collected before and after a meal were separated into micelles and vesicles by gel exclusion chromatography. Because of the requirement to run samples within 48 h of collection, only three samples from each subject were analyzed, the pre-meal samples and samples taken 15–30 and 45–60 min after the meal. The average concentrations of IMBC were 1.6 ± 0.9, 5.3 ± 3.8, and 0.9 ± 0.3 mM for samples collected before the meal and 15–30 and 45–60 min post-meal, respectively. An example of the distribution of micelles and vesicles in a representative subject is shown in **Fig. 4**. The IMBCs used for separation of these samples were 4.4, 2.4, and 2.1 mM for samples collected before the meal and 15–30 and 45–60 min post-meal, respectively. As seen, most of the cholesterol was carried as micelles with a small proportion as vesicles prior to the meal. By 15–30 min after the meal, the distribution of cholesterol was more evenly distributed between the two different-sized particles. By contrast, mostly vesicles were present in the samples collected 45–60 min after the meal. It should be noted that the difference in the peak fractions containing cholesterol is most likely not due to a change in particle size, but buffer viscosity. Using the percentage of cholesterol carried in either the micelle or vesicle peak for each subject, an average percentage of cholesterol carried in micelles or vesicles for all of the subjects was obtained (**Table 1**). Lumenal samples taken any time before or after the meal contained both micelles and vesicles. In the samples collected just prior to the meal or 15–30 min after the

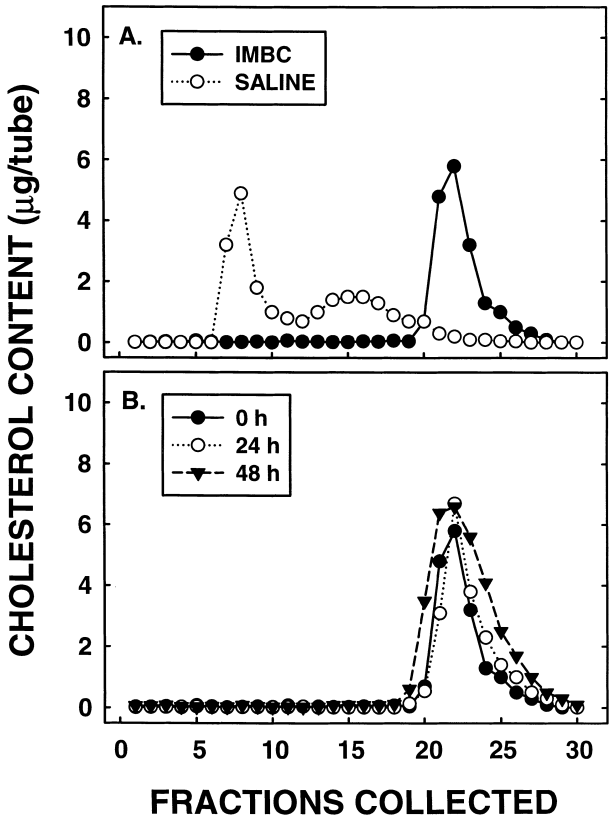

Fig. 3. Cholesterol carried as vesicles or micelles in lumenal samples that were maintained and/or eluted under different conditions. A: Lumenal samples were eluted from the Sepharose® 4B gel filtration columns using either the IMBC or 0.9% saline to elute the fractions. Cholesterol concentrations in the fractions from the gel exclusion chromatography were measured enzymatically and by GLC. B: Lumenal samples were maintained at 37°C for 0, 24, or 48 h and separated into micelles using gel filtration. The eluant was comprised of the IMBC. Cholesterol concentrations in the fractions from the gel exclusion chromatography were measured enzymatically or by GLC.

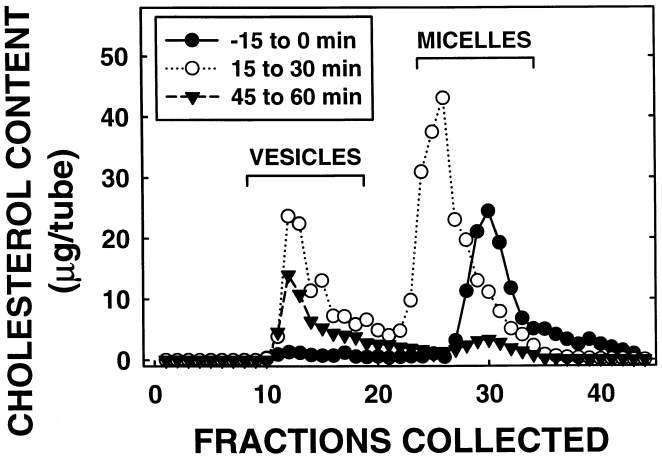

Fig. 4. Cholesterol carried as vesicles or micelles in lumenal samples before the meal and 15–30 and 45–60 min after the meal. Lumenal samples were separated into micelles and vesicles using Sepharose® 4B gel filtration at 37°C. The eluant was comprised of the IMBC described in the text. Cholesterol concentrations in the column fractions from lumenal samples from −15–0 (A), 15–30 (B), and 45–60 (C) min after meal consumption were measured enzymatically and by GLC. Data are presented as an example for one of the six subjects.

meal, there was an even distribution of cholesterol carried as micelles or vesicles. It appeared that the samples collected 45–60 min after the meal carried more cholesterol as vesicles as compared with that carried by micelles.

To verify the results from the gel exclusion chromatography, the molar percentage of cholesterol, bile acids, and TDP+phospholipid (PL) from Fig. 1 was calculated (**Table 2**), and the resulting data were plotted on a phase diagram previously described for lumenal samples (15). As seen in **Fig. 5**, a sample with the same molar percentages of the lipids in the pre-meal will result in a solution that contains both micelles and vesicles (the gray area of the diagram). The sample taken 15–30 min after the meal had slightly more bile acid and TDP+PL and slightly less cholesterol. The position of this data point on the phase diagram is very close to the pre-meal sample due to the relative similar increases in both bile acid and TDP+PL. By 45–60 min after the meal, there was proportionally more TDP+PL as compared with bile acids. Consequently, these data were positioned at a different location in the phase diagram of Fig. 5, though still in the region of the diagram that represents the presence of both micelles and vesicles. Thus, results obtained from the phase diagram

TABLE 1. Percent cholesterol carried by micelles and vesicles in lumenal samples taken before and after a liquid meal

Post-meal	Vesicles	Micelles
min	%	
−15–0	53 ± 27	47 ± 27
15–30	46 ± 17	54 ± 17
45–60	86 ± 3	15 ± 3

Values represent averages ± SEM for six subjects.

TABLE 2. Molar percentages of cholesterol, TDP[a] plus phospholipids, and bile acids after a liquid meal

Post-meal	CH	TDP+PL	BA
min		*molar %*	
−15–0	5.4	39.6	55.0
0–15	1.6	46.1	52.3
15–30	2.1	39.9	58.0
30–45	1.7	55.3	43.0
45–60	1.4	70.1	28.5
60–75	1.8	69.2	29.0
75–90	1.1	67.1	31.8
90–120	0.7	65.5	33.8

[a] TDP include free fatty acids, monoglycerides, and diglycerides.

demonstrated that the samples were in a two phase zone of the diagram. These results were consistent with those obtained from direct measurements of micelles and vesicles which showed that both micelles and vesicles were present in the subphase of the lumenal samples.

DISCUSSION

Cholesterol absorption is a complex, multistep process that begins in the intestinal lumen and ends in the circulation. There are many different regulable steps in the pathway, each of which could play a major role in defining cholesterol absorption. Some of the least studied, but more important, steps in this process are the intralumenal events leading to absorption. The environment of the lumen plays a major role in cholesterol absorption efficiencies in that it can alter the distribution of cholesterol between the aqueous and lipid phases within the gut, and can affect the type of particle within the aqueous phase that carries the cholesterol, such as the micelle or vesicle (12–14). One of the reasons for the lack of data on cholesterol distribution within the human intestine is the lack of tested methodology and the difficulty in performing well-controlled studies in humans.

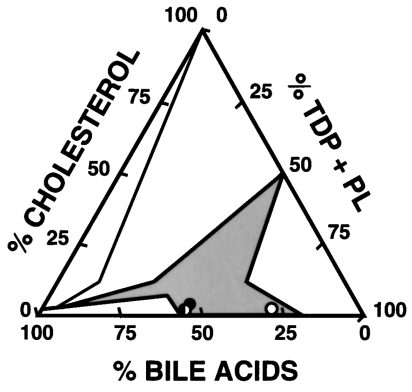

Fig. 5. Equilibrium phase diagram of the lumenal lipids plotted on triangular coordinates as described previously (15). The diagram represents a cut at 99% water of the original phase diagram. The closed data point represents the pre-meal sample, the half closed data point represents the 15–30 min post-meal sample, and the open data point represents the 45–60 min post-meal sample.

The studies presented here demonstrate the technique for collecting representative samples of lumenal contents during absorption, and define the solubility of cholesterol within the aqueous phase including the identification of micellar and vesicular composition. The main purpose of these studies was to verify that this methodology, which had been used previously for model bile, could be applied to human samples. To validate the methods for use in studies of human lumenal contents, several different parameters needed to be defined, including the position of the tube within the intestine, the importance of the elution buffer for the separation of micelles and vesicles by column chromatography, the length of time that the micelles and vesicles remain intact after removal from the gut, and the most representative times after a meal that can be used to examine the physical and chemical characteristics of the lumenal contents.

One of the most critical aspects of these studies is the position of the tube within the intestine because the contents retrieved through the tube must provide an accurate account of the lumenal physico-chemical conditions during absorption. It is also essential to pick a position that could be reproducible between subjects to reduce subject-to-subject variation. Thus, the sample should be collected from a relatively uniform length of small intestine (distance from the pylorus to the collection ports) that is representative of a site of absorption and is easily located in each subject. The position chosen for tube tip location in the present studies was the ligament of Trietz for several reasons. First, the ligament of Trietz is a radiographically readily identifiable site. Second, this location is distal to the ampulla of Vater. Third, this site would be representative of absorption occurring in a significant portion of the distal duodenum because the collection ports are present over ≈6 cm of tubing. Fourth, tube placement to this distance does not expose the subject to excessive radiation exposure. Fifth, the passage of the tube to this distance is well tolerated by adults as well as children (personal observation, J. Heubi), and many volunteers have agreed to repetitive placement with or without sedation and only topical anesthesia. In addition to the positioning of the tube, the composition of the tube is very important. The soft, pliable consistency of the Frederick Miller tube minimizes discomfort during placement, and the addition of 10 more ports for collection has allowed reproducible collection of volumes that have mostly been collected by siphonage.

In addition, we have shown that, as with model bile, the IMBC must be used as the elution buffer during column chromatography in order to get an accurate representation of the amount of cholesterol carried in micelles or vesicles in the lumen. When 0.9% saline was used as the elution buffer instead of the IMBC, micelles were converted to vesicles, thus underestimating the amount of cholesterol carried as micelles and overestimating the amount of cholesterol carried as vesicles. We have also demonstrated that the micelles and vesicles must be separated from one another within 48 h after being removed from the intestine. If the samples were maintained for longer periods of time, such as up to 144 h, the particles began to disperse. Finally, with the tube tip placement at the ligament of Trietz, the most appropriate times to examine the micelles and vesicles during these types of studies were found to be the baseline or the pre-meal, 15–30 min after the meal (which corresponds to the time of the greatest concentration of lumenal lipids after the meal and the contraction of the gallbladder), and 45–60 min, which corresponds to lumenal contents after the initial bolus of digested meal and bile has passed through the intestine.

Interestingly, micelles were present in some of the subjects even in the fasted state when bile acid concentrations were low and one might expect vesicles. The presence of micelles could be possibly due to the presence of TDP and phospholipids in the lumenal samples with low concentrations of bile acids; the presence of lipids in the fasted state was the result from biliary secretion that bypasses the gallbladder. The composition of these samples (Table 2) does most resemble gallbladder bile (25). By 45–60 min after the meal, there were more vesicles within the lumen due to the maintained high concentrations of TDP relative to lower bile acid concentrations. Thus, even in the absence of micelle formation, cholesterol could still be solubilized within the aqueous phase as vesicles. Results obtained from the column are physiological and can be located in the same position on the phase diagram as that obtained from the sizing columns.

Knowing the physical and chemical characteristics of digested products in the lumen under different conditions could help better delineate intralumenal processes leading to cholesterol absorption. Understanding the role of bile acid composition on solubilization of dietary cholesterol, as well as the influence of dietary fat compositional changes on cholesterol absorption, can only be determined by careful analysis of intralumenal events. Better understanding of mechanisms underlying intralumenal solubilization of dietary and biliary cholesterol could lead to the development of novel agents that would potentially interfere with cholesterol and/or fat absorption and prove to be effective new agents to treat hypercholesterolemia such as ezetimibe (26). Other applications of this information regarding intralumenal events could be helpful in examining pathologic conditions. For example, the actual effects of various therapeutic bile acids on lumenal cholesterol solubility and the partitioning of cholesterol between the micelles and vesicles is unknown in human lumenal contents. Current efforts in our laboratories are now directed at examining the role of individual bile acids as well as the effect of dietary fat composition on cholesterol absorption and metabolism.

The authors wish to thank Julie McConihay and Amy Honkomp for their expert technical assistance, and Cindy Deeks, R. D., Victoria Brickweg, R. D., and Suzanne Gilley for nutritional consultation and diet preparation. This work was supported by National Institutes of Health awards P01 DK-46405 and National Center for Research Resources Grant

#M01 08084. L.A.W. is a recipient of an Established Investigator Award from the American Heart Association.

Manuscript received 2 October 2001 and in revised form 10 January 2002.

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