Studies on plasma lipoproteins during absorption of exogenous lecithin in man

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Abstract Human subjects were infused intraduodenally with either lecithin (150 mg/kg/hr) or safflower oil (100 mg/kg/hr) of similar fatty acid composition, and plasma lipoproteins were studied when constant plasma lipid levels were reached. Both types of fat induced increases of lipoproteins of $S_f > 400$ (chylomicrons) and S_f 20–400 (VLDL). Lecithin infusions produced increases predominantly in VLDL, whereas infusion of safflower oil induced mainly chylomicrons. Chylomicrons derived from lecithin were generally smaller and had a higher phospholipid:triglyceride ratio (mean 0.15) than those produced during safflower oil infusions (mean 0.08). The increases in VLDL from both lipids occurred mainly in larger particles of this density range. This "incremental VLDL" had a lower cholesterol: triglyceride ratio (0.098) than preinfusion VLDL (0.283) and probably represented "small chylomicrons" of gut origin. The differences in lipoproteins resulting from infusion of lecithin and safflower oil in human subjects were not observed in rats; in the latter, lecithin induced large chylomicrons to the same extent as did safflower oil. Lecithin absorption measured over 50- or 100-cm intestinal segments averaged 41%, but was probably greater over the whole of the small intestine. Lecithin infusion unexpectedly was found to decrease markedly the absorption of cholesterol in the upper part of the small intestine.—**Beil, F. U., and S. M. Grundy.** Studies on plasma lipoproteins during absorption of exogenous lecithin in man. *J. Lipid Res.* 1980. **21:** 525–536.

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After ingestion of a fat-containing meal, dietary lipids are partially hydrolyzed and absorbed into the intestinal mucosa. In the mucosa, the hydrolytic products are resynthesized into triglycerides, cholesteryl esters, and phospholipids. The resulting lipids are combined with apoproteins to form intestinal lipoproteins, primarily chylomicrons and very low density lipoproteins (VLDL) (1, 2). Triglycerides (TG) are the major source of lipids in the diet, and mechanisms of TG absorption have been studied in great detail. On the other hand, only a few studies have been carried out on the absorption of lecithin, a lesser lipid of the diet but one that also is supplied by the bile. In the lumen of the small intestine, lecithin is known to be hydrolyzed by pancreatic phospholipase A_2 to lysolecithin and fatty acids, and both are taken up by mucosal cells (3). Several different fates for lysolecithin have been proposed; these include: *a*) reesterification with a fatty acid to form lecithin which can be utilized as surface coat for chylomicrons or for intracellular membranes (4, 5); *b*) complete hydrolysis and use of the released fatty acid for TG synthesis (6–8); and *c*) direct absorption of lysolecithin into the portal circulation (9).

The role of intraluminal lecithin in formation of chylomicrons has been difficult to assess because phospholipids can be synthesized de novo by the mucosa itself (10, 11); thus the relative contributions of intraluminal and newly-synthesized lecithin to chylomicron phospholipids are still controversial (4, 5). Studies in animals (6–8) indicate that during fasting the intestine produces relatively small chylomicrons with a high phospholipid:TG ratio. The lipids for these particles could be derived in part from biliary lecithin. During fat feeding, dietary TG contributes most of the chylomicron TG, and the size of chylomicrons increases with a concomitant decrease in phospholipid:TG ratios (12–14).

In the present study in human subjects, we have attempted to determine whether large amounts of dietary lecithin unaccompanied by TG can induce formation of chylomicrons in man. Our data indicate that lecithin can serve as the sole precursor for the synthesis of chylomicrons; however, the major products of lecithin absorption would appear to be particles in the range of VLDL. In other words, lecithin appears to promote production of VLDL-sized "small chylomicrons" that have a higher ratio of phospholipid to TG than found in large chylomicrons produced during ingestion of dietary TG.

Abbreviations: PL, phospholipid; TG, triglyceride; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

TABLE 1. Fatty acid composition of lecithin and safflower oil

Fatty Acid	Lecithin			Safflower Oil Total
	α-position	β-position	Total	
	%	%	%	%
C16:0	24	2	13	
C18:0	8	1	4	3
C18:1	11	10	10	28
C18:2	52	80	67	68
C18:3	5	7	6	1

METHODS

Patients

Studies were carried out on 23 patients (22 men and 1 woman) on the Special Diagnostic and Treatment Unit (metabolic ward) of the Veterans Administration Medical Center, San Diego, CA. All patients had normal functions of the gastrointestinal tract and liver. However, several had other chronic conditions (e.g., hyperlipidemia, obesity, ischemic heart disease, slightly impaired glucose tolerance, a seizure disorder, and hypertension). None had acute illness, and the other illnesses did not interfere with their studies. Eight subjects were normotriglyceridemic (fasting plasma TG < 200 mg/dl); the remaining were hypertriglyceridemic. Only one patient had a fasting plasma cholesterol exceeding 280 mg/dl. All subjects gave informed written consent for their studies.

Diets

Between their studies, 16 patients maintained their weight on an ad libitum diet of solid food. Before and between studies, five subjects were on a liquid, hypocaloric diet consisting of 20% of calories as protein, 20% as fat from soy oil, and 60% as carbohydrates (Sustacal, Mead Johnson). These latter patients lost weight continuously during hypocaloric feeding of 900 Kcal/day.

Experimental procedure

Effects of phospholipids on plasma lipoproteins. For this study, patients were intubated with a single-lumen tube on the night before the study. The tube was allowed to pass into the duodenum, and it was positioned by X-ray so that the outlet was in the region of the ampulla of Vater. Next morning a fasting sample of venous blood was obtained, and infusion of lipid was begun. Lipid consisted of either lecithin or safflower oil. Infusion rates were 150 mg/kg ideal weight/hr for lecithin or 100 mg/kg ideal weight/hr for safflower oil; thus, both diets provided equivalent intakes of fatty acids. These lipids were infused at a constant rate for 10 hours. Blood sampling was begun at the fifth hour of infusion and hourly sampling continued for the next 5 hr (15).

Lecithin used in these studies was purified from soybeans; it was prepared by Nattermann, Cologne, Germany and was kindly supplied by Dr. Hans Genthe. The fatty acid composition as determined by Lekim and Betzing (16) is shown in **Table 1.** This lecithin had a waxy consistency at room temperature. It was prepared into a liquid "formula" consisting of lecithin, water, and triglycerol monoleate (Capital City Products, Columbus, OH); these were mixed in a ratio (wt/wt/wt) of 100:1000:7, respectively, and blended in a Waring blendor for 10 min at high speed. This procedure gave a homogenous suspension which was stable throughout the period of infusion. In addition, essential amino acids were included in the infusate. They were given in concentrations to maximalize pancreozymin secretion as reported by Go, Hofmann, and Summerskill (17).

For the safflower-oil infusion, safflower oil, water, and triglycerol monoleate were homogenized in a ratio of 100:1000:7 (wt/wt/wt): this mixture yielded an emulsion that remained stable throughout the study. The fatty acid composition of the safflower oil is also shown in Table 1; it corresponds closely to that of the lecithin.

Venous blood samples obtained before and during infusion of lecithin and safflower oil were collected into EDTA-containing tubes, and plasma was separated by centrifugation at 4°C.

For one set of studies, lipoproteins were separated into three fractions: $S_f > 1400$, S_f 400–1400, and $S_f < 400$. The procedure was as follows: 6 ml plasma was transferred to 13-ml cellulose nitrate tubes. The plasma was layered with 6 ml NaCl solution (d = 1.0063 g/ml), and tubes were subjected to ultracentrifugation at 23°C in a Beckman SW-41 rotor for 1.6×10^6 g-avg min. With this procedure, all particles of $S_f > 1400$ should be in the top 1 ml; most of those of $S_f > 400$–1400 should be in the next 3 ml, and particles of $S_f < 400$ should be in the lower 8 ml. These calculations are based on the ultracentrifugation techniques of Dole and Hamlin (18) and Lossow et al. (19). The lower 8 ml ($S_f < 400$) was removed quantitatively through a pinhole in the bottom of the tube and analyzed for TG and cholesterol; TG in this fraction was designated lipoprotein-TG. In most cases, the top 1 ml was removed and analyzed for TG and cholesterol as well; this fraction should represent particles of $S_f > 1400$, and it was taken to reduce the possibility of contamination with VLDL.

In some studies, lipoproteins of $S_f > 400$ were separated into three subfractions (designated A, B and C) by density gradient centrifugation according to Los-

sow et al. (19). Electron microscopy with negative staining of particles, as recently described in our laboratory (20), showed that most particles in these subfractions had diameters of >2000 Å (fraction A), 1200–2000 Å (fraction B), and 750–1200 Å (fraction C). There was less than 10% overlap between each of these fractions.

A method was also employed in some of the studies to estimate mean diameters of chylomicrons during infusion of lecithin and safflower oil. These estimated mean diameters were based on the structure of chylomicrons, assuming that triglycerides make up the core material and phospholipids contribute only to the surface area (21). Fraser (14) has shown that phospholipid:triglyceride (PL:TG) ratios in subfractions of lymph chylomicrons from rabbits are well correlated with their volume:surface area ratios and their mean diameters; in general, the lower the PL:TG ratio the greater the diameters of chylomicrons. Our estimations were based on two equations. Equation 1 was derived by Fraser (14), and Equation 2 was derived by us from data in Table 1 of the publication by Fraser (14).

$$\frac{V}{SA} = 0.211 \times \frac{TG\ (mg/dl)}{PL\ (mg/dl)} + 0.27 \quad (r = 0.95) \qquad \text{(Equation 1)}$$

$$\frac{V}{SA} = 0.00213 \times DM - 0.11 \quad (r = 0.99) \qquad \text{(Equation 2)}$$

where V = mean volume [Å^3]; SA = mean surface area [Å^2]; DM = mean diameter [Å]; and the ratio V/SA is expressed as Å $\times$ 10^{-2}.

In other studies, three lipoprotein fractions (VLDL, LDL and HDL) were isolated and analyzed after removal of chylomicrons. VLDL were isolated by ultracentrifugation at 1.8 $\times$ 10^8 g-avg min (d = 1.006 g/ml), and LDL and HDL were isolated from the infranatant according to the methodology of the Lipid Research Clinics (22).

Cholesterol and TG on each fraction was estimated on a Technicon Autoanalyzer II (23, 24) (Technicon Instruments Corp., Tarrytown, NY). Phospholipids were extracted with ethanol–ether (3:1) or isopropanol, and phosphorus analysis was carried out according to Rouser, Fleischer, and Yamamoto (25).

Effects of lecithin infusion on absorption of lecithin and cholesterol. In these studies, absorption of lecithin and cholesterol over a segment of intestine was estimated by an intestinal perfusion technique described by Grundy and Mok (26). Patients were intubated with a 3-lumen tube on the night before the study, and the tube was allowed to pass into the small intestine overnight. The locations of the outlets were as follows: the most proximal outlet (site of infusion) was adjacent to the ampulla of Vater; the second outlet was 10 cm distally; and the most distal outlet was either 50 or 100 cm beyond the second.

In this study, the lecithin infusate was prepared as described above. Beta-sitosterol was used as a nonabsorbable flow marker for both lecithin and cholesterol; its use for this purpose has been described in detail previously (27). Beta-sitosterol (supplied by Eli Lilly Company, Indianapolis, IN, courtesy Mr. Erold Diller) was dissolved in triglycerol monoleate in a weight ratio of 1:20, and this solution was suspended in water by homogenization in a Waring blendor.

For absorption measurements, lecithin was infused along with amino acids through the proximal outlet at a rate of 150 mg/kg ideal weight/hr. The volume of infusion was about 125 mg/hr. Aspiration of intestinal contents was begun through the middle and distal outlets after 3 hr of infusion. About 10 ml of intestinal contents per hr were aspirated constantly and transferred immediately to glass vials containing 30 ml chloroform–methanol 2:1. Aspirations were divided into hourly samples from the 3rd to the 10th hour of infusion.

For analysis of lipid phosphorus in intestinal contents, the same procedure was employed as for intestinal phospholipids of biliary origin (27). Basically, samples were extracted with chloroform–methanol 2:1, and phosphorus content was determined on the chloroform phase according to Rouser et al. (25). We have repeatedly demonstrated that complete extraction of both lecithin and lysolecithin is obtained with this procedure.

Beta-sitosterol was used as an internal marker to estimate lecithin absorption. The net absorption of phospholipids over the absorbing segment was as follows: phospholipid absorption (mg/hr) = phospholipid inflow at the second outlet (mg/hr) − phospholipid outflow at the distal outlet (mg/hr). The influx and efflux of phospholipids at these two sites were determined by their ratios to beta-sitosterol which was infused at a constant rate. The basic equations for these measurements have been presented previously (26).

The necessity of amino acids in the phospholipid infusate was shown by the results in one study not presented in the Results section. In this study, amino acids were omitted from the infusate which resulted in a low lecithin absorption (less than 10%) and a low lysolecithin/lecithin ratio at the distal outlet (0.044). The lack of absorption and hydrolysis of lecithin in this patient can probably be explained by a failure of lecithin alone to stimulate pancreatic secretion.

The ratios of lecithin and lysolecithin in intestinal contents were estimated according to the following procedure. Chloroform extracts were subjected to one-dimensional thin-layer chromatography on Silica Gel H plates (Merck, Darmstadt, GFR) in chloroform–methanol–acetic acid–water 25:15:4:2 (v/v). Spots were detected by iodine vapor, identified by reference standards, and scraped off the thin-layer plates for phosphorus analysis (25).

To determine whether lecithin is mostly absorbed over the entire length of the gut, balance studies were carried out in two additional patients. These patients were given large quantities of lecithin by mouth. The first patient was investigated during two periods. In the first period of 1 month, he received a liquid diet containing 40% of calories as fat in the form of lard. In the second month, fat content of the diet was reduced to 20% of total calories, and the removed fat was replaced by 30 g of lecithin given in four divided doses with the liquid diets. The design in the second patient was the same except that in a third period of 2 weeks, the fat intake was further reduced and oral lecithin was increased to 75 g/day. In both patients, stools were collected throughout all periods and were pooled into six pools per period for analysis of lipid; chromic oxide was used as a fecal-flow marker (28). The stools were saponified, acidified, and fatty acids (which might have been derived from either triglycerides or lecithin), were extracted with an organic solvent and determined gravimetrically.

The absorption of cholesterol over the perfused segment was estimated by the technique of Grundy and Mok (26). This method also employs beta-sitosterol as an internal marker. Samples obtained from proximal and distal ends of the absorption segment were analyzed for cholesterol and beta-sitosterol. These sterols were isolated by petroleum ether extraction from liquid formula and intestinal contents after saponification in 1 N NaOH at 65°C for 1 hr. The extracts were evaporated under nitrogen, and sterols were measured by gas–liquid chromatography as trimethylsilyl ethers, as described by Miettinen, Ahrens, and Grundy (29). Calculations of cholesterol absorption used the same equations as described above for lecithin and presented previously by Grundy and Mok (26).

Rat studies

Absorption of lecithin and safflower oil was compared also in lymph fistula rats. The intestinal lymph duct was cannulated as described by Warshaw (30). Fasting male Sprague-Dawley rats weighing 300–350 g were anesthetized with ether. Silastic cannulae were inserted into the main mesenteric lymph duct and into the duodenum. The rats were kept in restraining cages after the operation and perfused with 0.9% NaCl through the duodenal cannula at a rate of 5 ml per hour. Fat infusions into the duodenum were begun 6–8 hr after the operation and hourly intestinal lymph samples were collected on ice. Clotted material was removed by filtration through glass wool. Continuous infusion with safflower oil and lecithin was carried out in a way similar to that described for human subjects; infusion rates were 150 mg/kg/hr for lecithin and 100 mg/kg/hr for safflower oil. The same rats were used for both lecithin and safflower oil infusions; the interval between infusion was 6–8 hr to allow removal of residual fats in lumen and intestinal mucosa (31).

For analysis of lymph lipoproteins, lymph was brought to a density of 1.019 g/ml with NaCl solutions of higher density (combining 0.01% EDTA). Six ml of this lymph-saline mixture was overlayered with 6 ml of NaCl solution (d = 1.0063) in a 13-ml cellulose nitrate tube, and the tubes were subjected to ultracentrifugation at 23°C in a SW-41 rotor at 1.6×10^6 g-avg min. The upper 4 ml ("chylomicrons") was removed, and the lower 8 ml was raised to a background density of 1.019 g/ml and centrifuged in a 40.1 rotor at a force of 1.8×10^8 g-avg min. The top 1 ml was removed as VLDL. Chylomicrons and VLDL were analyzed for triglycerides and cholesterol as described above.

Fatty acid composition

Individual fatty acids of triglycerides in lipoproteins were determined by gas–liquid chromatography (15% OV-275 on 100/120 Chromosorb P AW-DMCS-Supelco), after transmethylation with boron trifluoride in methanol (Applied Science Laboratories Inc., State College, PA).

RESULTS

Lecithin absorption

Table 2 shows the data for lecithin absorption in seven studies; in the first five patients the absorption segment was 50 cm, and in the latter two it was 100 cm. Rates of infusion were 150 mg/kg/hr, and on the basis of previous studies (28), the input of biliary lecithin would be in the range of 3 to 5 mg/kg/hr. For all patients, the mean influx at the middle tube was 9.5 g/hr. Percentage absorption over 50 cm ranged from 19 to 66% (mean 36%) and the mass absorbed averaged 3.2 g/hr. In the two patients in whom the absorption segment was 100 cm, percentage absorption averaged 55% and mass uptake was 5.8 g/hr. At the distal port, the molar lysolecithin/lecithin ratio ranged from 0.54 to 2.23 (mean 0.94).

Results for the two patients studied for lecithin balance were the following. In the first patient, lipid excretion (as fatty acids) in the control period averaged 4.5 ± 0.3 (SD) g/day, and 4.6 ± 0.6 g/day during intake of 30 g/day of lecithin. In the second patient, lipid excretion in the lecithin-free period averaged 5.2 ± 0.5 g/day; with 30 g/day of lecithin, it was 4.7 ± 1.4 g/day; and on 75 g/day, it was 6.2 ± 1.5 g/day. Thus when relatively large quantities of lecithin were given by mouth, no malabsorption of lipids could be detected.

These data show that significant amounts of infused lecithin (or dietary lecithin) were absorbed. Absorption over the 100-cm segment appeared to be greater than over the 50-cm segment, and the balance studies in two patients gave no evidence of malabsorption. In view of the high ratios of lysolecithin to lecithin at the distal port of most patients, it seems likely that significant absorption would continue beyond the measured segment. The data nonetheless suggest that absorption of lecithin must have occurred over a long length of intestine and was not almost complete in the upper intestine as may occur for triglyceride absorption.

Cholesterol absorption

Cholesterol absorption was estimated simultaneously with lecithin absorption in seven subjects (**Table 3**). In five studies using the 50-cm perfusion segment, absorption of cholesterol was essentially blocked; in fact, cholesterol outputs at the distal port exceeded those at the inlet. This suggests that large quantities of luminal phospholipids not only blocked absorption but actually "extracted" cholesterol from the intestinal mucosa. These "negative" absorption values (mean = −45 ± 2 (SEM)%) contrasted greatly to those obtained in four control subjects, previously studied in our laboratory (32), who had a mean absorption of +40 ± 4 (SEM)% on TG-containing infusions.

TABLE 2. Lecithin absorption during lecithin infusion

Patient	Segment Length	Proximal Input	Distal Output	Absorption		% Lysolecithin Distal Port
	cm	*mg/hr*	*mg/hr*	*mg/hr*	*%*	*molar %*
1	50	9354	6844	2509	27	44
2	50	9701	7858	1842	19	35
3	50	9381	6070	3310	35	38
4	50	9125	6301	2823	31	37
13	50	8125	2743	5381	66	49
14	100	11000	6336	4664	42	46
15	100	9975	3048	6927	69	69

In two patients in whom a 100-cm segment was employed, a positive cholesterol absorption was noted. This positive absorption may have been due in part to the fortuitous high inputs of cholesterol in these patients, and it might be noted that their percentage absorptions of cholesterol (17 and 34%) were generally lower than those of 20 control patients in whom 100-cm segments were used (mean absorption = 43 ± 5) (26). On the other hand, cholesterol absorption may have begun to increase lower in the intestine as lecithin absorption proceeded and intraluminal concentrations of phospholipids diminished.

Effects on plasma lipoproteins

Table 4 shows effects of lecithin infusion on plasma total cholesterol, lipoprotein-TG, and chylomicron-TG. Mean values in steady-state with infusion are compared to fasting values. Paired analysis showed a small but significant increase in cholesterol levels on lecithin infusion. Lipoprotein-TG was significantly increased during lecithin infusion (283 versus 369 mg/dl; $P < 0.01$). Likewise, the increase over fasting values in chylomicron-TG was significant although small (5 versus 23 mg/dl; $P < 0.01$). For comparison, effects of infusion of an equivalent amount of fatty acids from safflower oil were studied (**Table 5**). Paired analysis again showed a slight increase in cholesterol

TABLE 3. Cholesterol absorption during lecithin infusion

Patients	Length of Absorption Segment	Cholesterol Input	Cholesterol Output	Cholesterol Absorption	
	cm	*mg/hr*	*mg/hr*	*mg/hr*	*%*
Lecithin infusions[a]					
Mean ± SEM (n = 5)	50	66 ± 6	95 ± 7	−29 ± 3	−45 ± 2
Control subjects[b]					
Mean ± SEM (n = 4)	50	67 ± 11	40 ± 6	27 ± 6	40 ± 4
Patient 14	100	182	150	32	17
Patient 15	100	195	127	68	34
Control subjects[c]					
Mean ± SEM (n = 20)	100	99 ± 13	56 ± 10	43 ± 5	48 ± 3

[a] Includes patients 1, 2, 3, 4, and 13.
[b] Includes subjects previously studied in this laboratory (32).
[c] Subjects also studied with the same technique (26).

TABLE 4. Effects of lecithin infusion on lipoprotein- and chylomicron-triglycerides

Patient	Total Cholesterol			Lipoprotein-TG			Chylomicron-TG[a]		
	Fasting	Infusion	Δ	Fasting	Infusion	Δ	Fasting	Infusion	Δ
		mg/dl			*mg/dl*			*mg/dl*	
1	148	155	+7	72	107	+35	0	5	+5
2	217	253	+36	173	171	−2	4	8	+4
3	207	233	+26	151	184	+33	1	8	+7
4	153	175	+22	115	165	+50	0	7	+7
5	217	227	+10	187	278	+91	1	19	+18
6(a)	235	234	−1	145	207	+62	0	36	+36
6(b)	249	254	+5	166	185	+19	0	8	+8
7	220	209	−11	126	189	+36	0	4	+4
8	135	139	+4	236	247	+11	1	1	0
9	230	247	+17	277	451	+174	19	33	+14
10	328	347	+19	343	423	+80	17	39	+22
11	207	236	+29	343	533	+190	7	95	+88
12	269	285	+16	467	505	+38	10	11	+1
13(a)	267	279	+12	527	689	+162	27	58	+31
13(b)	292	311	+19	699	965	+266	0	30	+30
13(c)	318	329	+11	502	615	+113	0	7	+7
Mean ± SEM	231 ± 14	244 ± 15	14 ± 3	283 ± 45	369 ± 60	87 ± 19	5 ± 2	23 ± 6	18 ± 5
Significance[b]			$P < 0.01$			$P < 0.01$			$P < 0.01$

[a] Chylomicron-TG by difference total TG and lipoprotein-TG.
[b] The significance of the differences between fasting and infusion was determined by paired analysis.

levels during fat infusion; lipoprotein-TG was increased significantly and to a similar degree as with lecithin infusion (348 versus 425 mg/dl, $P < 0.05$); but in contrast to lecithin infusion, the increment in chylomicron-TG was marked (21 versus 215 mg/dl; $P < 0.01$).

Although lecithin infusion was found to produce a significant increase in chylomicron-TG (the values shown in Table 5), values were calculated indirectly (by difference). To verify the formation of chylomicrons from lecithin, chylomicron-TG was measured directly in the top 1 ml of the supernatant and compared to the results calculated by difference. Sixteen paired studies were done. Fasting chylomicron-TG by difference averaged 6 ± 3 (SEM) mg/dl and by direct measurement was 5 ± 2 mg/dl. During lecithin infusion, chylomicron-TG increased to 27 ± 9 mg/dl by difference, which was significantly greater than the fasting value ($P < 0.01$). By direct measurement, the value increased to 16 ± 5 mg/dl, which was also significantly greater than fasting ($P < 0.01$). The reason for the lesser increment by direct measurement was that it determined only TG that floated in the top 1 ml of the centrifuge tube. A small quantity of chylomicron-TG undoubtedly was present in the 3 ml about the upper layer of plasma but below the top 1 ml. This study indicates that while the increment in chylomicron-sized particles on lecithin infusion was small, it nevertheless was real.

TABLE 5. Effects of safflower oil infusion on lipoprotein- and chylomicron-triglycerides

Patient	Total Cholesterol			Lipoprotein-TG			Chylomicron-TG[a]		
	Fasting	Infusion	Δ	Fasting	Infusion	Δ	Fasting	Infusion	Δ
		mg/dl			*mg/dl*			*mg/dl*	
1	114	126	+12	195	156	−39	0	16	16
4	163	180	+17	121	138	17	0	10	10
5	198	195	−3	192	312	120	0	35	35
6	231	231	0	131	250	119	0	67	67
7	196	212	+16	319	345	26	27	415	388
8	157	160	+3	263	356	93	2	32	30
11	225	239	+14	633	842	209	19	333	314
12	247	249	+2	332	268	−64	105	797	692
13(a)	297	293	−4	559	604	45	38	103	65
13(b)	336	345	+9	743	983	240	22	347	325
Mean ± SD	216 ± 21	223 ± 20	7 ± 3	348 ± 70	425 ± 91	84 ± 29	21 ± 10	215 ± 81	194 ± 72
Significance[b]			$P < 0.05$			$P < 0.05$			$P < 0.05$

[a] Chylomicron-TG by difference between total TG and lipoprotein-TG.
[b] The significance of the differences between fasting and infusion studies was determined by paired analysis.

TABLE 6. Triglyceride content of chylomicron subfractions and VLDL during lecithin and safflower oil infusions

Patient	Chylomicron Subfraction A	B	C	Δ VLDL-TG
	mg/dl			*mg/dl*
Lecithin				
6	6	25	46	31
9	9	30	55	185
Safflower oil				
6	22	20	21	63
11	82	88	83	270
13(a)	25	49	82	98
13(b)	149	150	142	338
17(a)	15[a]		6	11
17(b)	22[a]		4	8

[a] In the two studies in patient 17, fractions A and B were combined.

TABLE 7. Phospholipid:TG ratios in chylomicrons during lecithin and safflower oil infusions

Study	PL	TG	PL:TG	Estimated Mean Diameter
		mg/dl		*Å*
Lecithin				
18a	11	93	0.12	1000
14	13	86	0.15	840
13e	2	15	0.13	940
13f	6.5	33	0.19	700
Mean ± SEM	8 ± 2	57 ± 19	0.15 ± 0.015	870 ± 65
Safflower oil				
13b	16	341	0.05	2160
16	9	76	0.12	1000
20	12	129	0.09	1280
21	16	224	0.07	1600
Mean ± SEM	13 ± 2	192 ± 58	0.08 ± 0.015	1510 ± 250

These results are in accord with those shown in **Table 6.** When lecithin was infused in two patients, the increase in TG in the lightest fraction (A) was slight and was greatest in the heaviest fraction (C). Different results were obtained with safflower oil infusion in four similar studies; in these, increments were equally distributed between the three chylomicron subfractions. As shown in two studies using safflower oil (patient 17), the increments in fractions A and B during safflower oil infusion were not due simply to a greater increase in total chylomicron-TG than with lecithin; in this patient chylomicron-TG increased only slightly, but in contrast to patients given lecithin, the increments occurred predominantly in fractions A + B. For both lecithin and safflower oil, the rise in VLDL-TG was usually in proportion to the total increase in chylomicron-TG.

The phospholipid:triglyceride ratios in chylomicrons (lipoproteins S > 400) are presented in **Table 7.** This ratio was 0.15 ± 0.015 for lecithin infusion and considerably lower for safflower oil infusion, 0.08 ± 0.015. Also given in Table 7 are "estimated mean diameters" of the respective chylomicrons; these diameters were derived from Equations 1 and 2, Methods section. The calculated diameter of chylomicrons obtained during lecithin infusions is lower (mean of 870 Å) than during safflower oil infusions (mean of 1510 Å).

Effects of infusion of lecithin and safflower oil on the composition of VLDL are shown in **Table 8.** The

TABLE 8. Lipid composition of VLDL during infusion of lecithin and safflower oil

Patient	Fasting VLDL CH[a]	TG	CH:TG	Infusion VLDL CH	TG	CH:TG	Difference CH	TG	CH:TG
	mg/dl			*mg/dl*			*mg/dl*		
Lecithin									
5	24	84	0.285	26	102	0.255	2	18	0.111
7	16	65	0.246	17	89	0.190	1	24	0.040
8	35	167	0.212	37	178	0.205	2	11	0.109
9	33	157	0.210	36	258	0.140	3	157	0.030
10	73	252	0.289	76	322	0.236	3	70	0.042
12	66	224	0.294	83	313	0.265	17	89	0.190
15	50	147	0.353	57	187	0.305	7	45	0.155
18	31	82	0.378	34	114	0.298	3	32	0.108
Mean ± SEM	41 ± 7	147 ± 24	0.283 ± 0.021	46 ± 8	195 ± 33	0.237 ± 0.019	5 ± 2	56 ± 17	0.098 ± 0.020
Safflower oil									
5	27	154	0.175	41	244	0.168	14	90	0.155
8	41	208	0.197	43	285	0.147	2	77	0.044
12	60	223	0.269	67	301	0.220	7	78	0.089
13	144	473	0.304	162	803	0.200	18	330	0.050
18(a)	30	77	0.385	37	137	0.270	7	60	0.112
18(b)	45	115	0.390	52	176	0.295	7	61	0.114
Mean ± SEM	58 ± 18	208 ± 57	0.287 ± 0.037	67 ± 19	324 ± 99	0.217 ± 0.023	9 ± 2	116 ± 84	0.094 ± 0.017

[a] CH cholesterol.

TABLE 9. Fatty acid composition of (VLDL + Chylo) triglycerides before and during lecithin infusions

Fatty acid	C16:0	C18:0	C18:1	C18:2
		molar %		
A. Before infusion	33.4	3.8	44.5	18.0
B. During infusion	34.1	2.8	38.0	24.9
C. Lecithin composition	20.6	5.4	13.4	60.5
D. Predicted B[a]	31.5	4.0	39.8	24.4

[a] D. (predicted B) = (0.85 × A) + (0.15 × C).

cholesterol and TG content, along with the cholesterol:TG ratio, are given for VLDL during fasting and lipid infusion. In eight subjects given lecithin, fasting VLDL had a ratio of 0.283 ± 0.061 (SD), and with infusion of lecithin it decreased to 0.237 ± 0.056. This change was associated with a mean increment in VLDL-TG of 56 mg/dl, and the difference in ratios of cholesterol to TG was 0.098 ± 0.057. This implies that the increment in VLDL was due to particles with a much lower cholesterol:TG ratio than those usually found in circulating VLDL. A similar result was obtained in six studies on seven patients given safflower oil. In these studies, the mean increment in VLDL-TG of 116 mg/dl was associated with a change in cholesterol:TG ratio of 0.094 ± 0.042. Again, the incremental particles appeared to have a much lower ratio than did the fasting VLDL.

Further evidence that lecithin-derived fatty acids served as a precursor for the increase in TG during lecithin infusion was obtained from the fatty acid composition of the incremental TG obtained during lecithin infusions in patient 14 (**Table 9**). If increases in TG were due to products of exogenous lecithin, the fatty acid composition of plasma TG should shift towards linoleic acid (18:2) which is the predominant fatty acid of infused lecithin. In this study, VLDL and chylomicrons were pooled since the increment in chylomicrons during lecithin infusion was small. The increment in TG of VLDL + chylomicrons was 85 mg/dl which was 15% of the total TG in these fractions (Table 9). From this value, it was predicted that the 18:2 content of TG fatty acids should rise from 18 to 25% provided that all of the increment was derived from exogenous lecithin. The observed increase in 18:2 was almost exactly that predicted. This finding would seem to rule out the possibility that the increment in TG was due to a delayed removal of VLDL-TG of hepatic origin.

Particle diameters of VLDL were determined by electron microscopy in three patients given lecithin and two given safflower oil (**Table 10**). VLDL was isolated for analysis after an initial centrifugation to remove chylomicrons. Mean diameters were increased in every patient during infusion of both lipids. In general, the increase in mean diameter was a reflection of a greater fraction of larger particles (e.g., diameters greater than 400 Å). This finding is consistent with the addition of "small chylomicrons" to the VLDL pool, but of course the origins of these larger particles in VLDL during infusion cannot be discerned by electron microscopy alone.

In a subgroup of patients, concentrations of plasma phospholipids, ratios of PL:TG in VLDL, and cholesterol and TG in HDL also were measured. Phospholipids in total plasma were not increased significantly by infusion of either lecithin in five patients (plasma PL = 236 ± 20 (SEM) mg/dl (control) versus 249 ± 26 mg/dl (infusion)), or safflower oil in four patients (plasma PL = 252 ± 36 mg/dl (control) versus 263 ± 47 mg/dl (infusion)). PL/TG ratios in VLDL also were estimated during infusion of lecithin (four patients) and safflower oil (three patients). Like cholesterol/TG in VLDL, PL/TG ratios *decreased* during both types of infusion, as compared to fasting values. During lecithin infusion, ratios fell from 0.38 ± 0.4 to

TABLE 10. Particle distribution of VLDL diameters before and during duodenal lipid infusions

Patient		VLDL-TG	Particle Diameter Range (4¾A) <200	200–250	250–300	300–350	350–400	>400	Diameter Mean ± SD
		mg/dl			*% of total*				*Å*
Lecithin									
9	Fasting	156	4.76	4.02	15.45	37.23	21.26	17.02	339 ± 84
	Infusion	258	0.3	9.54	20.33	26.19	15.29	28.13	362 ± 109
10	Fasting	252	0	12.42	29.36	30.64	14.63	12.9	323 ± 70
	Infusion	322	0	5.08	26.26	32.13	19.59	16.81	339 ± 71
12	Fasting	224	1.05	11.67	21.44	26.94	30.14	8.67	324 ± 68
	Infusion	313	1.14	12.55	22.6	23.74	26.02	13.89	330 ± 80
Safflower oil									
13(a)	Fasting	508	11.6	27.75	23.73	18.16	8.82	9.82	286 ± 78
	Infusion	606	6.24	26.63	18.79	18.16	17.53	12.48	305 ± 82
13(b)	Fasting	473	1.85	25.8	23.22	26.5	14.53	7.91	302 ± 67
	Infusion	803	3.38	17.5	18.97	23.8	17.74	18.4	327 ± 84

TABLE 11. Lipid (fatty acid) recovery in intestinal lymph during intraduodenal infusions of lecithin and safflower oil in rats

Study	Lipid	Infusion Rate (Fatty Acids)	Recovery Rate (Fatty Acids)	Recovery (Fatty Acids)	Fraction in Chylomicrons (Fatty Acids)	18:2 Fatty Acids
		mg/hr	*mg/hr*	%	%	%
1	Safflower oil	35	32	91	72	71[a]
	Lecithin	35	33	94	81	71[b]
2	Safflower oil	35	23	65	70	
	Lecithin	35	17	49	60	
3	Safflower oil	35	31	88	80	

[a] % 18:2 fatty acids in safflower oil was 68% of total (see Table 1).
[b] % 18:2 fatty acids in lecithin was 67% of total (Table 1).

0.32 ± 0.02, and from 0.37 ± 0.06 to 0.33 ± 0.04 on safflower oil. Finally in 13 patients, concentrations of cholesterol and TG in HDL did not change during lecithin infusion; also, in eight patients given safflower oil, cholesterol in HDL was unchanged, but TG increased by an average of 6.1 mg/dl ($P < 0.01$, paired analysis).

Studies in rats

Table 11 presents the recoveries of lipids in intestinal lymph during intraduodenal infusion of lecithin and safflower oil in rats. The infusions of the two lipids were done in sequence in the same animal. After 4 hours of infusion, outputs of lipids in lymph had reached a steady-state. In both rats, Nos. 1 and 2, magnitudes of recovery of TG were similar during lecithin and safflower oil infusion. In No. 1, recovery was almost identical to input; but in No. 2 it was somewhat less for both lipids. The fraction of TG as chylomicrons varied between 60 and 81% and was the same for lecithin as for safflower oil. The fatty acid composition of lymph TG was essentially identical to that of the infused lipids in both cases. Thus, in contrast to man, the rat appeared to handle infused TG and lecithin in an identical manner; however, the difference was quantitative and not qualitative and might have depended to some extent on experimental design.

DISCUSSION

The purpose of this study was to determine the fate of intestinal lecithin in man. Several potential pathways for lecithin metabolism have been proposed by previous workers. It is well known that intestinal lecithin is degraded by phospholipase A_2 to lysolecithin and a fatty acid. Deacylation of lysolecithin, on the other hand, is thought to proceed very slowly if at all intraluminally (3); instead, lysolecithin and fatty acids, and possibly intact lecithin to a small extent (33), are absorbed by the mucosal cell. In man, the subsequent fates of lysolecithin and its accompanying fatty acid are not well understood. The fatty acid could be a source of chylomicron-TG or could become re-esterified again with lysolecithin to produce lecithin. Mucosal lysolecithin might follow at least three different pathways. *a*) It could be deacylated to glycerophospholcholine which could then be degraded into its component parts. *b*) It might be secreted directly into the portal circulation where it would be removed by the liver. Indeed, an enterohepatic circulation for lecithin has been postulated (9). This is to say, lecithin is secreted first into bile and is broken down to lysolecithin and absorbed by the intestine; lysolecithin in turn could be returned to the liver where it might be resynthesized into biliary lecithin. *c*) A third pathway for lysolecithin could be esterification with a fatty acid in the intestinal mucosa to reform lecithin; the later could then be incorporated into the surface coat of chylomicrons or intestinal VLDL. In accord with this third pathway, it has been proposed that availability of luminal lecithin is a requisite for formation of lymph chylomicrons. When lecithin is removed completely from the intestinal lumen in experimental animals, the clearance of newly absorbed fat from the intestinal mucosa is seriously curtailed (34). The intestinal mucosa apparently has the capacity to synthesize its own lecithin for formation of some chylomicrons; thus when fat intake is relatively small, absorption of triglycerides occurs rapidly from the upper intestine. However, if a fat load is excessively large, a much larger segment of small intestine may be required for complete absorption; this could be due in part to an overloading of the lecithin-synthesizing capacity of the intestine (35). However, since biliary lecithin is usually available, the rate and quantity of presentation of TG probably is a more important factor for determining the length of intestine over which TG is absorbed.

The present study was carried out primarily to determine whether one pathway for intestinal lecithin enables it to serve as a precursor for TG for chylomicrons. If the fate of all intestinal lysolecithin is direct transport to the liver via the portal vein, chylomicron formation would not be possible during the intestinal infusion of pure lecithin. Previously, however, Lekim (33) has shown in rats that oral administration of a single dose of lecithin, radiolabeled in both its fatty acid moieties, results in incorporation of about 75% of radioactivity into chylomicron-TG (or lipoprotein-TG), with the remaining 25% in lecithin; in his study, more than 90% of labeled fatty acids of lecithin appeared in association with lipoproteins, suggesting that direct transport of lysolecithin to the liver is not a significant pathway in rats. Our own study in rats confirms that lecithin is a good source of TG for chylomicrons. Following lymph cannulation, transport of TG into lymph was similar during infusion of either lecithin or safflower oil. For both, about 75% of administered lecithin was in chylomicrons with the remainder in VLDL. Thus, rats can degrade lecithin completely to provide fatty acids for these two types of fat particles.

The current work also demonstrates that intestinal lecithin can serve as a source of chylomicron-TG in human subjects. Although multiple studies were required to prove formation of chylomicrons, the accumulated data indicate that chylomicrons do appear in plasma during lecithin infusion. However, lecithin was not as good a precursor for chylomicron formation as safflower oil, and in contrast to the rat, when lecithin and safflower oil supplied equal amounts of fatty acids, increases in chylomicron-TG were consistently greater with safflower oil. For our human studies, therefore, we might inquire into the reasons for the failure of infused lecithin to produce large chylomicrons, and to cause formation of small chylomicrons instead.

First, absorption of lecithin and its products may not have been complete. If the total quantity of absorbed lipid was small, the resulting chylomicrons also might have been reduced in size. Although previous investigators (3), using lesser amounts of lecithin, showed that complete absorption occurs in the upper intestine, the larger quantities used in our study may have overloaded absorptive mechanisms. There are, however, several reasons for believing that a significant portion, if not most of the infused lecithin, was absorbed. First, when absorption over only a 50-cm segment of upper intestine was examined, a mean uptake of 35% was found (studies 1, 2, 3, 4 and 13); in two additional studies, mean absorption over a 100-cm segment was 55% (studies 14 and 15). In most patients, a major fraction of unabsorbed phospholipid at the distal port was in the form of lysolecithin, which should have been absorbed at a lower level. If we can assume that all lysolecithin at the distal port was absorbed, the measured absorption plus lysolecithin averaged 70% of the input. This should represent the minimal absorption. Second, in similar intubation studies carried out in man, Arnesjo et al. (3) examined intestinal phospholipase at various levels of the small intestine after a test meal and found that enzymatic activity was present further down in the intestine. Indeed, activities of phospholipase A_2 were relatively low in the duodenum but increased progressively at lower levels; this was attributed to either inhibition of enzymatic activity in the upper intestine by high concentrations of bile acids or by the secretion of phospholipase A_2 by the intestine itself. Therefore, considerable absorption of lecithin might be expected beyond the proximal 50–100 cm. Third, in two patients fed lecithin at 30 g or 75 g per day, no evidence of lipid malabsorption was detected by fecal lipid balance. And finally, our studies in rats showed that when pure lecithin was infused in comparable amounts (per kg) as in man, complete absorption occurred. Hence, the marked differences in plasma-TG response between lecithin and safflower oil infusions probably cannot be explained by differences in quantities of lipids absorbed.

A second possible reason for the failure of lecithin to produce large chylomicrons is that lysolecithin might have been absorbed directly from mucosal cells into the portal circulation (9). The work of Lekim (33) suggests that this pathway does not occur to an appreciable extent in rats, and our studies in this animal are in accord; however, the possibility of portal absorption in human subjects was not ruled out by the current work, and further studies are needed to demonstrate the absence of this unlikely pathway in man.

A third reason for the production of smaller particles by lecithin is that absorption of lecithin might be extended over the whole of the small intestine instead of just in the upper intestine, as usually occurs with TG (36). This would lead to smaller quantities of lipid entering each cell; and since each cell would be relatively "deficient" in fatty acids, it might make smaller particles (13, 37). Our absorption data are consistent with this mechanism. While the lipid balance studies suggest that lecithin malabsorption was small or nonexistent, the intubation studies showed clearly that absorption was incomplete in the upper intestine.

A final reason for smaller particles with lecithin administration could be that an increase of lecithin

relative to fatty acids for TG synthesis might enrich the resulting particles with phospholipids. In other words, the coat-to-core ratio could be greater when there is an excess of lecithin or lysolecithin present in the cell. Again, our data are compatible with this mechanism. Consequently, the lack of large chylomicrons during lecithin infusion might be the result of multiple mechanisms.

Since the major effect of lecithin infusion on lipoproteins was to cause an increase in VLDL-sized particles and not chylomicrons, we might inquire about the origins of the former particles. The increase in VLDL could have been due to either an enhanced influx of VLDL or to a reduction in its removal. An increased input could result from gut secretion of "small chylomicrons" which float in the VLDL density range. However, we cannot completely exclude an increased hepatic production of VLDL; for theoretically, a greater production of VLDL by the liver could have been stimulated by excess fatty acids released from chylomicrons in peripheral tissues or from chylomicron remnants taken up by the liver. Nonetheless, data on VLDL composition during lecithin infusion would seem to favor the gut origin. If the increment in VLDL had been due to increased hepatic secretion, the cholesterol:TG ratios before and during infusion should have been nearly the same; yet, the calculated ratios for "incremental lipoproteins" in the VLDL fraction were very similar to those found in the chylomicron fraction in the same studies. Also, there was an average increase in mean particle size in this fraction with lecithin infusion, which was the result of a higher percentage of large particles greater than 350 Å. When all data are considered, it appears that during lecithin infusion particles are formed by the gut with diameters between 400 and 1500 Å, and this spectrum was divided by our centrifugation into the heavier chylomicrons (fraction C) and the lightest VLDL fraction. Thus, the compositional data suggest that the increment of TG in VLDL was due to the increased secretion of "small chylomicrons" by the intestine, and not to increased production of VLDL by the liver. By the same reasoning, the compositional data speak against the increment in VLDL being due to a delayed removal of hepatic VLDL by the lipoprotein lipase system of peripheral tissues. It might be noted that safflower oil infusions caused similar changes in the VLDL fraction. Thus, while most of the particles produced from safflower oil infusion were larger chylomicrons, safflower oil clearly stimulated the formation of smaller particles as well.

Our basic interpretation of the data is that pure lecithin without accompanying TG can cause production of TG-rich lipoproteins by the intestine, but these particles are smaller on the average than those produced from dietary TG. Most of the TG appearing in plasma during lecithin infusion would seem to be associated with "small chylomicrons" that float mostly with VLDL. In contrast to larger chylomicrons induced by dietary-TG, these particles have a high ratio to coat-to-core lipids. Thus, we have shown that it is possible to alter the size, and the ratio of coat-to-core lipids, of intestinal lipoproteins in man. This ability to manipulate the properties of chylomicrons could prove useful in studies of their metabolism. Of particular interest is the question of whether changes in the properties of chylomicrons might also affect the metabolism of other lipoproteins, such as HDL, since several workers have implied that chylomicron surface components (i.e., phospholipids, cholesterol, and apoproteins) can contribute to the formation of HDL (38).

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REFERENCES

1. Ockner, R. K., F. B. Hughes, and K. J. Isselbacher. 1969. Very low density lipoproteins in intestinal lymph: role in triglyceride and cholesterol transport during fat absorption. *J. Clin. Invest.* **48:** 2367–2373.
2. Tytgat, G. N., C. E. Rubin, and D. R. Saunders. 1971. Synthesis and transport of lipoprotein particles by intestinal absorptive cells in man. *J. Clin. Invest.* **50:** 2065–2078.
3. Arnesjö, B., A. Nilsson, J. Barrowman, and B. Borgström. 1969. Intestinal digestion and absorption of cholesterol and lecithin in the human. *Scand. J. Gastroenterol.* **4:** 653–665.
4. Scow, R. O., Y. Stein, and O. Stein. 1967. Incorporation of dietary lecithin and lysolecithin into lymph chylomicrons in the rat. *J. Biol. Chem.* **242:** 4919–4924.
5. Mansbach, C. M., II. 1977. The origin of chylomicron phosphatidylcholine in the rat. *J. Clin. Invest.* **60:** 411–420.

6. Shrivastava, B. K., T. G. Redgrave, and W. J. Simmonds. 1967. The source of endogenous lipid in the thoracic duct lymph of fasting rats. *Q. J. Exp. Physiol.* **52:** 305–312.
7. Baxter, J. H. 1966. Origin and characteristics of endogenous lipid in thoracic duct lymph in rat. *J. Lipid Res. Res.* **7:** 158–166.
8. Ockner, R. K., F. B. Hughes, and K. J. Isselbacher. 1969. Very low density lipoproteins in intestinal lymph: Origin, composition, and role in lipid transport in the fasting state. *J. Clin. Invest.* **48:** 2079–2088.
9. Boucrot, P. 1972. Is there an entero-hepatic circulation of the bile phospholipids? *Lipids.* **7:** 282–288.
10. Gurr, M. I., W. F. R. Pover, J. N. Hawthorne, and A. C. Frazer. 1963. The phospholipid composition and turnover in rat intestinal mucosa during fat absorption. *In* Biochemical Problems of Lipids. A. C. Frazer, editor. Elsevier Nederland, Amsterdam, 236–243.
11. Noma, A. 1964. Studies on the phospholipid metabolism of the intestinal mucosa during fat absorption. *J. Biochem. (Tokyo).* **56:** 522–532.
12. Yokoyama, A., and D. B. Zilversmit. 1965. Particle size and composition of dog lymph chylomicrons. *J. Lipid Res.* **6:** 241–246.
13. Fraser, R., W. J. Cliff, and F. C. Courtice. 1968. The effect of dietary fat load on the size and composition of chylomicrons in thoracic duct lymph. *Q. J. Exp. Physiol.* **53:** 390–398.
14. Fraser, R. 1970. Size and lipid composition of chylomicrons of different Svedberg units of flotation. *J. Lipid Res.* **11:** 60–65.
15. Grundy, S. M., and H. Y. I. Mok. 1976. Chylomicron clearance in normal and hyperlipidemic man. *Metabolism.* **25:** 1225–1239.
16. Lekim, D., and H. Betzing. 1974. Der Einbau von EPL-Substanz in Organe von gesunden und durch Galaktosamin geschadigten Ratten. *Arzneim. Forsch.* **24:** 1217– 1221.
17. Go, V. L. W., A. F. Hofman, and W. H. J. Summerskill. 1970. Pancreozymin bioassay in man based on pancreatic enzyme secretion: potency of specific amino acids and other digestive products. *J. Clin. Invest.* **49:** 1558–1564.
18. Dole, V. P., and J. T. Hamlin III. 1962. Particulate fat in lymph and blood. *Physiol. Rev.* **42:** 674–701.
19. Lossow, W. J., F. T. Lindgren, J. C. Murchio, G. R. Stevens, and L. C. Jensen. 1969. Particle size and protein content of six fractions of the $S_f > 20$ plasma lipoproteins isolated by density gradient centrifugation. *J. Lipid Res.* **10:** 68–76.
20. Groszek, E., and S. M. Grundy. 1978. Electron-microscopic evidence for particles smaller than 250 A in very low density lipoproteins of human plasma. *Atherosclerosis.* **31:** 241–250.
21. Zilversmit, D. B. 1965. The composition and structure of lymph chylomicrons in dog, rat, and man. *J. Clin. Invest.* **44:** 1610–1622.
22. Manual of Laboratory Operations: Lipid Research Clinics Program. Volume 1, Lipid and Lipoprotein Analysis. 1974. DHEW Publication No. (NIH) 75-628. National Heart and Lung Institute, Bethesda, MD.
23. Block, W. D., K. J. Jarret, and J. B. Levine. 1965. Use of a single color reagent to improve the automated determination of serum total cholesterol. *In* Automation in Analytical Chemistry. Mediad Inc., New York. 345–347.
24. Kessler, G., and H. Lederer. 1965. Fluorometric measurement of triglycerides. *In* Automation in Analytical Chemistry. Mediad Inc., New York. 341–344.
25. Rouser, G., S. Fleischer, and A. Yamamoto. 1970. Two dimensional thin-layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids.* **5:** 494–496.
26. Grundy, S. M., and H. Y. I. Mok. 1977. Determination of cholesterol absorption in man by intestinal perfusion. *J. Lipid Res.* **18:** 263–271.
27. Grundy, S. M., and A. L. Metzger. 1972. A physiological method for estimation of hepatic secretion of biliary lipids in man. *Gastroenterology.* **62:** 1200–1217.
28. Davignon, J. W., J. Simmonds, and E. H. Ahrens, Jr. 1968. Usefulness of chromic oxide as an internal standard for balance studies in formula-fed patients and for assessment of caloric function. *J. Clin. Invest.* **47:** 127–138.
29. Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6:** 411–424.
30. Warshaw, A. L. 1972. A simplified method of cannulating the intestinal lymphatic of the rat. *Gut.* **13:** 66–67.
31. Wu, A. L., S. B. Clark, and P. R. Holt. 1973. Transmucosal triglyceride transport rates in proximal and distal rat intestine in vivo. *J. Lipid Res.* **16:** 251–257.
32. Einarsson, K., and S. M. Grundy. Effects of feeding cholic acid and chenodeoxycholic acid on cholesterol absorption and hepatic secretion of biliary lipids in man. *J. Lipid Res.* **21:** 23–24.
33. Lekim, D. 1976. On the pharmacokinetics of orally applied essential phospholipids (EPL). *In* Phosphatidylcholine: Biochemical and Clinical Aspects of Essential Phospholipids. H. Peeters, editor. Springer-Verlag, Berlin, Heidelberg, New York. 48–65.
34. O'Doherty, P. J. A., G. Kakis, and A. Kuksis. 1973. Role of luminal lecithin in intestinal fat absorption. *Lipids.* **8:** 249–255.
35. Sabesin, S. M., P. R. Holt, and S. B. Clark. 1975. Intestinal lipid absorption: evidence for an intrinsic defect of chylomicron secretion by normal rat distal intestine. *Lipids.* **10:** 840–846.
36. Borgström, B., A. Dahlqvist, G. Lundh, and J. Sjövall. 1957. Studies of intestinal digestion and absorption in the human. *J. Clin. Invest.* **36:** 1521–1536.
37. Zilversmit, D. B. 1978. Assembly of chylomicrons in the intestinal cell. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, Jr., and J. A. Ontko, editors. American Physiological Society, Bethesda, MD. 69–81.
38. Tall, A. R., and D. M. Small. 1978. Plasma high-density lipoproteins. *New Engl. J. Med.* **299:** 1232–1236.