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Research Article

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Very Low Density Lipoproteins in Intestinal Lymph: Role in Triglyceride and Cholesterol Transport during Fat Absorption

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ABSTRACT The role of nonchylomicron very low density lipoproteins (VLDL, S_f 20–400) in the transport of triglyceride and cholesterol was studied during lipid absorption. Various long chain fatty acids were infused intraduodenally in the form of mixed fatty acid—monoolein–taurocholate micelles; control animals received saline or taurocholate.

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These studies demonstrate that dietary long chain fatty acids differ significantly in their effects upon the transport of triglyceride and cholesterol by lipoproteins of rat intestinal lymph. These findings, together with the observed differences in rates of removal of chylomicrons and VLDL from plasma, suggest that variations in lipoprotein production at the intestinal level may be reflected in differences in the subsequent metabolism of absorbed dietary and endogenous lipids.

INTRODUCTION

In previous studies (1) we have shown that in the fasting state very low density lipoproteins (VLDL) play a major role in the transport of endogenous lipids in intestinal lymph. This class of triglyceride-rich lipoproteins can be operationally distinguished from the less dense chylomicrons by its flotation rate (VLDL, S_f 20–400; chylomicrons, $S_f > 400$). Although it is possible that VLDL and the more often studied chylomicrons represent two ends of a single spectrum of particle size and density, these two fractions differ also in other respects, including electrophoretic mobility in agarose gel and per cent lipid and protein composition (1, 2). Accordingly, intestinal lymph VLDL bear a greater similarity to plasma VLDL than to chylomicrons. These observations have suggested that the metabolic role of intestinal lymph VLDL may differ, at least quantitatively, from that of chylomicrons, and that they may contribute to plasma VLDL levels.

In the present studies we have extended our observations of intestinal lymph VLDL to an assessment of their role in the transport of triglyceride and cholesterol during the absorption of exogenous lipid. The results indicate that in this additional respect significant differences exist between lymph VLDL and chylomicrons.

TABLE I

Effect of Intraduodenal Infusions on Triglyceride in Whole Lymph and Lymph Lipoprotein Fractions

Infusion	Whole lymph	Triglyceride		
		Chylomicrons	VLDL	d > 1.006
	mg/hr	*mg/hr*	*mg/hr*	*mg/hr*
NaCl*	5.90 ±1.47	2.52 ±1.50	2.66 ±0.87	0.72 ±0.38
Taurocholate (TC)	6.58 ±1.92	3.02 ±1.36	2.57 ±0.64	1.00 ±0.61
Palmitate-monoolein (PM)	17.36 ±3.53	11.45 ±2.61	5.15 ±1.86	0.77 ±0.15
Oleate-monoolein (OM)	19.45 ±4.60	15.44 ±3.98	3.08 ±1.64	0.93 ±0.28
Linoleate-monoolein (LM)	17.56 ±3.55	13.60 ±3.77	2.87 ±0.31	1.12 ±0.42
Analysis of variance	$F = 23.34$ $P \ll 0.005$	$F = 25.9$ $P \ll 0.005$	$F = 4.51$ $P < 0.01$	$F = 0.96$ $P \gg 0.05$
Significant differences ($P < 0.05$)	NaCl, TC vs. PM, OM, LM	NaCl, TC vs. PM, OM, LM	NaCl, TC, LM vs. PM‡	None

Animals with intestinal lymph fistulas received test solutions intraduodenally over a 3 hr period as described in Methods. Except for the NaCl controls, all groups received a total of 200 μmoles of taurocholate. Administered mixed micelles contained a total of 192 μmoles fatty acid and 96 μmoles monoolein. Values shown are means ±1 SD of six experiments (except five in TC, LM-VLDL, and LM-d > 1.006, and four in OM). Whole lymph phospholipids (mg/hr): NaCl, 1.85 ±0.48; TC, 1.66 ±0.59; PM, 3.28 ±0.96; OM, 3.08 ±0.34; LM, 2.72 ±0.34.

* Data from Ockner, Hughes, and Isselbacher (1) included for comparison and statistical analysis.

‡ OM was not significantly different from any other group.

METHODS

Materials. Sodium taurocholate, oleic acid, linoleic acid, palmitic acid and glyceryl monooleate were obtained commercially[1] and were tested for purity by thin-layer chromatography. Results were as previously described (3). Methyl esters of linoleic acid and palmitic acid were also subjected to gas-liquid chromatography (4), and were found to be more than 98% pure.

Preparation of mixed micelles. Mixed micelles were prepared as previously described (3), except that twice the amounts of fatty acid and monoolein were used in these experiments. The final concentrations in the micellar solutions were: taurocholate, 20 mmoles/liter; fatty acid, 19.2 mmoles/liter; monoolein, 19.2 mmoles/liter. Although at these concentrations occasional clouding occurred in the micelles prepared with palmitic acid, suggesting partial transition to a crystalline state, this could be prevented by raising the pH of the solution to approximately 7.4. For this reason, all administered micelles were so treated.

Experimental procedure. Male albino CD strain rats,[2] 300–400 g, were used in all experiments. Cannulation of the main mesenteric lymph duct and duodenum was performed, and the animals were handled postoperatively as described previously (1). Animals were allowed to drink ad lib. until 1 hr before the start of the experiment. At this time fluid was withdrawn, but sufficient amounts were given intraduodenally if needed over the ensuing hour in order to maintain a lymph flow of about 3 ml/hr. At the start of the experiment, 5 ml of the mixed micellar or control (0.85% NaCl or 20 mM taurocholate) solutions were infused into the duodenum over a 30 min period, and washed in with 0.5 ml 0.85% NaCl. Over the next 30 min no fluids were administered. Beginning at 60 min a mixture of 5 ml of test solution and 5 ml of 0.85% NaCl (total volume, 10 ml) was infused continuously at the rate of 5 ml/hr over the next 2 hr. In the animals receiving mixed micelles, the total amount of lipid infused over the 3 hr period was approximately 80–90 mg. Under these conditions mean lymph flow in the several groups varied from 2.2 to 3.5 ml/hr, but no differences were significant, as determined by analysis of variance (see "statistical methods").

Lymph samples were collected and analyzed for lipid content, lipoprotein distribution of various lipids and, in some cases, triglyceride fatty acid composition by the methods previously described (1).

[1] Calbiochem, Los Angeles, Calif.

[2] Charles River Laboratories, Wilmington, Mass.

TABLE II

Effect of Intraduodenal Infusions on Cholesterol in Whole Lymph and Lymph Lipoprotein Fractions

Infusion	Whole lymph	Cholesterol		
		Chylomicrons	VLDL	d > 1.006
	mg/hr		*% of total*	
NaCl*	0.519 ±0.128	16.8 ±12.3	53.9 ±7.2	29.3 ±6.7
Taurocholate (TC)	0.592 ±0.077	25.2 ±4.9	41.7 ±8.2	33.1 ±10.4
Palmitate-monoolein (PM)	0.688 ±0.139	40.3 ±9.1	40.3 ±6.1	19.5 ±4.6
Oleate-monoolein (OM)	0.631 ±0.106	54.1 ±3.0	25.9 ±4.7	20.0 ±5.3
Linoleate-monoolein (LM)	0.634 ±0.118	53.7 ±2.3	25.5 ±6.1	20.9 ±6.5
Analysis of variance	$F = 1.64$ $P > 0.05$	$F = 25.3$ $P \ll 0.005$	$F = 16.8$ $P \ll 0.005$	$F = 4.12$ $P < 0.01$–0.05
Significant differences ($P < 0.05$)	None	NaCl, TC vs. PM vs. LM‡	NaCl vs. TC, PM vs. OM, LM	TC vs. PM

Animals with intestinal lymph fistulas received intraduodenal infusions of control or mixed micellar solutions over a 3 hr period (see Methods and Table I). Values shown are means ±1 SD of six experiments (except five in TC, LM-VLDL and LM d > 1.006, and four in OM).

* Data from Ockner, Hughes, and Isselbacher (1) included for comparison and statistical analysis.

‡ For OM vs. PM, Tukey's t just fails to reach conventional significance ($t = 2.77$; t_{tuk}, $0.05 = 2.82$); OM *does* differ significantly from NaCl and TC.

Preparation and administration of cholesterol-^{14}C-labeled lymph lipoprotein fractions. Tracer quantities of cholesterol-4-C[3] (approximately 3 μc) were administered intraduodenally to lymph fistula rats. Lymph was collected over a 1–3 hr period and the chylomicron and VLDL fractions were separated and "washed" once by layering them under 0.85% NaCl and repeating the ultracentrifugation. The per cent esterification of the *radioactive* cholesterol in the administered lipoprotein fractions was similar (means of two determinations: chylomicrons 66.7%, VLDL 64.2%). Tracer quantities of these fractions, containing less than 0.2 mg of cholesterol, were injected intravenously into nonfasted, nonoperated recipient rats under light ether anesthesia. Samples of blood (approximately 200 μl) were collected in heparinized capillary tubes from the tail vein every 5–6 min for a 30 min period. The tubes were then sealed, the red blood cells sedimented, and 60–100 μl of plasma assayed for radioactivity. The logarithms of the values obtained (dpm/ml plasma) bore a linear relationship to time. The linear regression was calculated for each experiment (5), and from this the half-time ($t_{\frac{1}{2}}$) of disappearance of radioactivity was determined. Radioassay techniques were those reported previously (3).

[3] New England Nuclear Corp., Boston, Mass.

Statistical methods. Student's t test was used to assess the significance of differences between groups, when only two groups were being compared in a given experiment. In those experiments in which more than two groups are compared, this test is not sufficiently stringent in its requirements for significance. Accordingly, in such experiments the analysis of variance method was used, in order to assess the null hypothesis that all groups were derived from only one population (i.e. that no significant differences were present among them). In those cases in which the probability (P) of this was found to be less than 0.05 (suggesting that one or more significant differences did exist), all groups were compared with each other by means of Tukey's method of multiple comparisons (6). Groups which differed significantly from each other ($P < 0.05$) by this method are indicated in the tables. In appropriate experiments, regression lines and correlation coefficients were determined by standard methods (5).

RESULTS

Effect of intraduodenal infusions on lipids of intestinal lymph. Lymph collected during the 3 hr test infusion period was analyzed for total content of triglyceride,

cholesterol and phospholipid, and the results were expressed in milligrams per hour (Tables I and II). It is seen that whole lymph lipid levels in NaCl and taurocholate-infused groups did not differ significantly from each other. Similarly, no significant differences were observed among the three lipid-infused groups. As expected, lipid-infused groups showed significant increases in lymph triglyceride and phospholipid levels, averaging approximately three and two times control levels, respectively (Table I). There were no statistically significant differences in lymph cholesterol levels among any of the groups (Table II).

Distribution of triglyceride among lymph lipoproteins. The absolute amount of triglyceride carried in chylomicrons, VLDL, and lipoproteins of density greater than 1.006 ($d > 1.006$) was determined for each of the groups during the test period, as shown in Table I. As was noted previously in the NaCl-infused control group, VLDL and chylomicrons carry approximately equal amounts of triglyceride. Although taurocholate infusion appeared to result in a slight increase in chylomicron triglyceride, the differences between the taurocholate and NaCl-infused group were not statistically significant.

Among the lipid-infused groups, chylomicron triglyceride did not differ significantly, and as expected was four to five times greater than in the NaCl and taurocholate-infused groups. Of particular interest was the finding that in the palmitate group VLDL triglyceride levels were significantly higher than in either the control or linoleate groups. The oleate group appeared to occupy an intermediate position in this respect, differing significantly from neither the palmitate nor the other groups. The triglycerides in lipoproteins of $d > 1.006$ were not significantly affected by lipid absorption.

These results indicate that administered palmitic and linoleic acids lead to significant differences in the lipoprotein distribution of the triglycerides subsequently appearng in intestinal lymph. In order to visualize this difference more clearly, the data were analyzed in another manner, as shown in Fig. 1. Here, the mean values for total lymph, chylomicron, and VLDL triglycerides in the taurocholate control group (representing *endogenous* triglyceride) have been subtracted from the respective values for each individual animal in the palmitate and linoleate groups, and the remainders, designated *exogenous* triglycerides, have been plotted as shown. Although the calculated exogenous triglyceride values may actually include small amounts of endogenous lipid, the increases over control values nevertheless reflect the administration of the exogenous fatty acids. It is seen that for linoleate, increases in exogenous triglyceride levels of whole lymph are entirely accounted for by increases in exogenous *chylomicron* triglyceride, with *no* increase in *VLDL* triglyceride. In the case of palmitate, however, increases in exogenous lymph triglyceride levels are reflected in *both* chylomicrons and VLDL.

Triglyceride fatty acids of lymph chylomicrons and VLDL during lipid absorption. It has been shown previously that in fasting intestinal lymph, the fatty acids of chylomicrons and VLDL triglycerides are identical (1). In the present studies, however, the differences in distribution of triglycerides among lymph lipoproteins after palmitate and linoleate administration suggested that during lipid absorption, chylomicrons and VLDL might

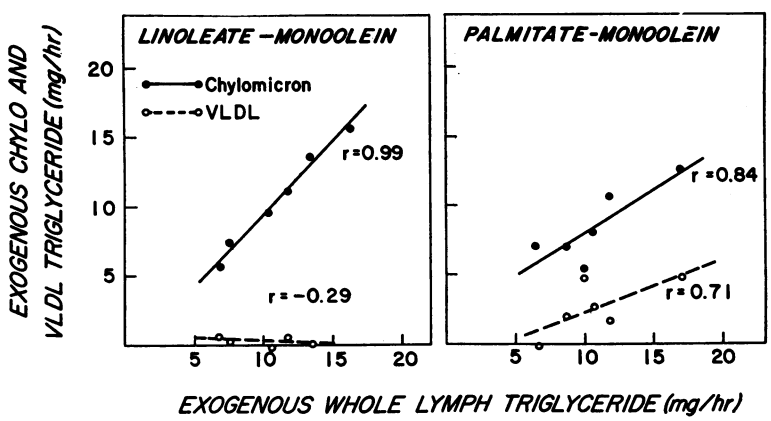

FIGURE 1 Lipoprotein distribution of exogenous triglycerides in intestinal lymph. Total triglyceride in lymph chylomicrons and VLDL was determined for mixed micelle-infused animals (see Methods, and Table II). "Exogenous" triglyceride in these fractions was calculated for each animal by subtracting the mean control (taurocholate infused) values from the respective individual values. The resulting values, representing the increase in triglyceride in each fraction due to the absorption of the fatty acid and monoglyceride, are plotted, along with the calculated regression line.

TABLE III

Triglyceride Fatty Acid Composition of Lymph Lipoproteins during Intraduodenal Infusions

Infusions	Triglyceride fatty acids								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
					% of total				
NaCl									
Chylomicrons	tr.*	tr.	27.5	tr.	13.1	12.6	30.2	4.1	12.6
VLDL	tr.	tr.	26.9	tr.	14.2	14.2	28.1	3.1	13.0
Linoleate-monoolein									
Chylomicrons	tr.	tr.	8.2	tr.	3.6	23.6	54.7	tr.	3.7
VLDL	tr.	tr.	20.3	tr.	10.8	19.0	39.4	tr.	8.3
Palmitate-monoolein									
Chylomicrons	tr.	tr.	55.9	tr.	5.6	30.7	7.0	tr.	tr.
VLDL	tr.	tr.	45.7	tr.	10.2	19.3	17.3	tr.	6.1

Animals with intestinal lymph fistulas received intraduodenal infusions of NaCl or mixed micellar solutions over a 3 hr period (see Methods and Table I), and in each experiment chylomicrons and VLDL were isolated from the same lymph sample. Triglyceride fatty acid methyl esters were analyzed by gas-liquid chromatography. Percent composition represents ratio of area under curve for each fatty acid to sum of areas for all fatty acids as determined by planimetry.

* tr. = trace, i.e., <1%.

differ in their triglyceride fatty acid composition. Therefore lymph chylomicrons and VLDL were isolated after intraduodenal infusion of linoleate-monoolein and palmitate-monoolein micelles, and triglyceride fatty acid composition was determined by gas-liquid chromatography. The results are shown in Table III. It is seen that after linoleate-monoolein infusion the chylomicron fatty acid pattern has been markedly altered, as compared with the NaCl-infused control, reflecting the presence of the administered lipid. In the same lymph sample, however, the VLDL pattern differs significantly from that of the chylomicrons, and more closely reflects the endogenous (NaCl-infused) pattern. With palmitate-monoolein infusion, differences between chylomicron and VLDL patterns are also observed.

Effect of intraduodenal infusions on the distribution of cholesterol in lymph lipoproteins. It would be expected that the differences in lymph lipoprotein triglyceride distribution between the palmitate and linoleate groups would be accompanied by differences in the distribution of other lipids as well. Accordingly, the distribution of endogenous cholesterol among lymph lipoproteins was determined for the various experimental groups. The use of percentages simplifies the comparisons, eliminates the effect of biologic variations in whole lymph cholesterol, and is justified because these studies have shown that there are no significant differences in lymph cholesterol content among the various groups. It is seen (Table II) that lipid administration resulted in increased chylomicron cholesterol compared with NaCl and taurocholate infusion. This increase was more pronounced with oleate and linoleate than with palmitate. Conversely, VLDL cholesterol was *decreased* with lipid infusion. It is noteworthy, however, that whereas oleate and linoleate infusions resulted in VLDL cholesterol levels significantly below the taurocholate group, the palmitate group was not different from this control group. Cholesterol in lipoproteins of $d > 1.006$ was also reduced by lipid absorption, although statistical significance is achieved only in the comparison between the taurocholate and palmitate groups. These findings indicate that, in addition to differences in triglyceride distribution, there were also differences in the distribution of cholesterol in lymph lipoproteins among the various lipid-infused groups.

Plasma survival of cholesterol-^{14}C–labeled lymph lipoproteins. In order to determine if the lipoprotein form in which cholesterol entered the plasma might influence its subsequent metabolism, intestinal lymph lipoproteins labeled with cholesterol-^{14}C were injected intravenously into intact recipient rats, and the half-life ($t_{\frac{1}{2}}$) of the injected radioactivity was determined. In Fig. 2 it is seen that the half-time of plasma survival of cholesterol-^{14}C–labeled VLDL was twice that of chylomicrons, indicating a more rapid removal of the larger particles.

DISCUSSION

In the present studies, lipid transport in lymph lipoprotein fractions has been examined and compared during the absorption of exogenous lipids administered intraduodenally in the form of mixed micelles. Under these conditions it was found that the quantitative importance of VLDL and chylomicrons in lipid transport depended

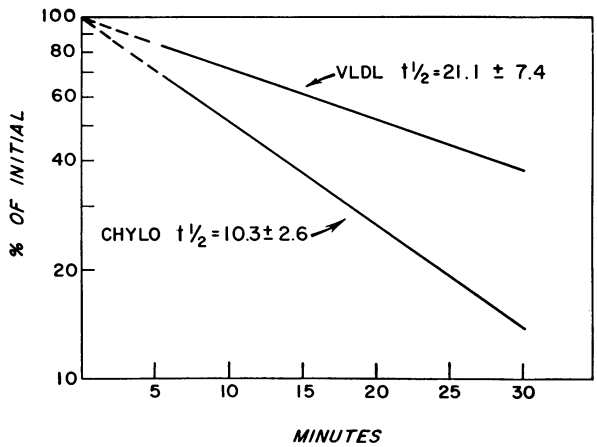

FIGURE 2 Plasma radioactivity after i.v. cholesterol-^{14}C–labeled lymph lipoproteins. After intraduodenal infusion of tracer quantities of cholesterol-^{14}C, lipoprotein fractions were isolated from intestinal lymph and infused into nonoperated rats. Tail vein plasma was assayed at intervals for radioactivity. Shown are the calculated mean half-times of plasma disappearance for six (VLDL) and five (chylomicrons) experiments, ±1 SD. The differences were significant ($0.01 < P < 0.025$).

upon the administered fatty acid. Linoleic acid administration resulted in increased lymph triglyceride levels confined entirely to the chylomicron fraction, whereas with palmitic acid triglyceride increased in both chylomicrons and VLDL. Oleic acid appeared to occupy a somewhat intermediate position, in this regard, but more closely resembled linoleic acid.

These differences in distribution of lymph triglyceride among the lipoprotein fractions were accompanied by equally significant differences in the distribution of endogenous lymph cholesterol. Thus, during the absorption of palmitic acid the quantities of cholesterol transported by chylomicrons and VLDL were equal. In contrast, during the absorption of oleic and linoleic acids chylomicron cholesterol was more than twice that in the VLDL fraction. In preliminary experiments, the lipoprotein distribution of *exogenous* cholesterol was also found to be influenced by absorbed fatty acids.[4]

Other workers previously observed that in the absence of dietary fat, most lymph cholesterol was carried in the chylomicron-free subnatant, and that the chylomicron cholesterol increased during fatty acid absorption (7). Most studies, however, have shown that lymph cholesterol is transported mainly in the chylomicron fraction (8). On the basis of the present experiments it seems likely that these latter observations reflect the substantial quantities of triglycerides rich in oleate and linoleate (corn oil or olive oil) which have usually been administered. Indeed, Zilversmit, Courtice, and Fraser (9) observed that a rabbit eating a diet containing corn oil carried 51% of its thoracic duct cholesterol in chylomicrons, whereas on a diet rich in palmitate only 27% was so transported. Although the conditions employed in this and the present studies differed markedly, the results are in essential agreement. Both studies indicate that dietary fats, acting at the *intestinal* level, are capable of significantly affecting the lipoprotein form in which absorbed cholesterol is transported, and in which it enters the circulating plasma.

The reasons for these variations in the quantitative importance of VLDL and chylomicrons in triglyceride and cholesterol transport have not been elucidated. The larger class of particles (chylomicrons), because of its lower surface-to-volume ratio and therefore lower protein-to-triglyceride ratio (1) requires less total apoprotein to transport a given amount of triglyceride. Theoretically, therefore, this is a more efficient process and because of the metabolic advantage to the mucosal cell would be expected to be the preferential transport particle. As has been noted, however, although chylomicrons were utilized exclusively during oleate and linoleate absorption, the smaller particles (VLDL) assumed greater significance during palmitate absorption.

This increased use of an apparently inefficient transport mechanism during palmitic acid absorption may reflect differences in physicochemical properties among the fatty acids. Thus, saturated and unsaturated fatty acids differ in molecular configuration (10) and in melting point (palmitic acid, 63°C; linoleic acid, −11°C). Alternatively, or perhaps as a result of these factors, it is possible that linoleic and oleic acids differ from palmitic acid with regard to the length of small intestine over which they are absorbed. If oleic and linoleic acids were absorbed over a short segment of proximal intestine, relatively few mucosal cells would be involved in the synthesis of the lipoprotein particles required for triglyceride transport. On the other hand, if palmitic acid were absorbed over a longer segment of intestine, the larger number of mucosal cells involved might result in the transport of the absorbed lipid in larger numbers of particles, which would therefore be of smaller size. In support of this concept, recent experiments suggest that under conditions similar to those employed in the present study, palmitic acid is absorbed over a greater length of intestine than linoleic acid.[5]

A third possibility is that the lipoprotein particles secreted by the mucosal cell during the absorption of linoleate have a greater tendency to coalesce in the tissue spaces to form larger, less dense particles. Recent electron microscopic studies of intestinal lymph lipoproteins[6] have confirmed differences in particle size during linole-

[4] Unpublished observations.

[5] Ockner, R. Unpublished observations.

[6] Ockner, R. and A. Jones. Submitted for publication.

ate absorption and in addition have demonstrated increased tendency of linoleate particles to coalesce. It is not certain, however, that such coalescing occurs in vivo, or has a direct effect on observed particle density.

The several differences noted between intestinal lymph VLDL and chylomicrons (1) suggested that the two fractions might differ at least quantitatively in their metabolism. In the present studies we have shown that cholesterol entering the plasma from the lymph in the form of VLDL is cleared significantly less rapidly than the cholesterol in chylomicrons. This finding is consistent with the earlier observation of Quarfordt and Goodman (11) that larger chylomicrons are cleared from the circulation more rapidly than are smaller chylomicrons, even though the particles used in their studies did not include lipoproteins in the VLDL range. It is of particular interest in this regard that unsaturated fatty acids, which lower plasma cholesterol in man and experimental animals, were found in the present studies to favor the transport of intestinal lymph cholesterol in a lipoprotein form, the chylomicron, which has a relatively short plasma survival. It has been suggested (9) that such differences in the lipoprotein form in which lymph cholesterol enters the plasma may have a bearing on the development of atherosclerosis.

There is evidence that dietary triglyceride entering the circulation in the form of chylomicrons may be taken up directly by peripheral adipose tissue, thus bypassing the liver (12, 13). The exact quantitative significance of this phenomenon is not yet certain, but it may account for the removal of most of the chylomicron triglyceride from plasma. It seems quite likely that intestinal lymph VLDL, persisting as they do for longer periods of time in the circulation, would be even more likely than chylomicrons to have their triglyceride removed by peripheral tissues. Thus, not only the cholesterol, but also the triglyceride in lymph VLDL could be subject to metabolic fates differing quantitatively from those involving the same lipids in the chylomicrons.

The present studies have demonstrated that during the absorption of dietary lipid, the small intestine produces triglyceride-rich lipoproteins which include not only particles having characteristics of chylomicrons as ordinarily defined and studied, but also others which more closely resemble plasma VLDL. The VLDL (S_f 20–400) are particularly important in lymph triglyceride and cholesterol transport during the absorption of palmitic acid. These observations suggest that variations in lipoprotein production, occurring at the intestinal level, may have a significant effect upon the subsequent metabolism of absorbed dietary and endogenous lipids.

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