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CONTRIBUTION OF PLASMA PROTEIN AND LIPOPROTEINS TO INTESTINAL LYMPH: COMPARISON OF LONG-CHAIN WITH MEDIUM-CHAIN TRIGLYCERIDE DUODENAL INFUSION

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ABSTRACT

In rats with intestinal lymph-fistula and electrolyte and protein losses continuously replaced by I.V. infusion, the plasma to lymph filtration of total protein, albumin, and cholesterol was similar after duodenal infusion of either long-chain (LCT) or medium-chain (MCT) triglyceride. Filtration of cholesterol into intestinal lacteals was also measured after pulsed I.V. administration of ^{14}C-β-sitosterol as an indirect marker for passage of lipoproteins into the lymph system. During intraduodenal administration of LCT and constant I.V. infusion of ^{125}I-apo high density lipoprotein (HDL), intact plasma HDL appeared in lymph and contributed apo-LP to chylomicron formation. Nevertheless, most lymph apo-LP originated from local mucosal synthesis.

Studies in chyluric subjects (1) and in lymph fistula rats (see previous article) demonstrate that cholesterol of plasma origin appears in intestinal lymph by transudation. It has also been suggested that the intestinal lymph volume increases whenever fat is absorbed, but the difference between medium-chain triglycerides (MCT) and long-chain triglycerides (LCT) in an experimentally perfused duodenum seems to be negligible (2-5). Because lymph contains solutes and water originating from plasma filtration, this study aimed to measure the contribution of plasma lipoprotein components including cholesterol to the intestinal lacteals under conditions where fat absorption during digestion was transported through different routes: a) lymph system for LCT and b) portal system for MCT. In these experiments, special care was taken not to deplete the host fluid and solute volume. Thus, throughout the experiment electrolytes and bovine albumin were continuously infused so as not to disturb the plasma colloid osmotic pressure, which may have occurred in some earlier reports (6-8).

Lymph cholesterol derived from plasma is closely linked to plasma lipoproteins that percolate into the intestinal submucosal lacteals. This phenomenon was investigated by a constant intravenous infusion of plasma high density lipoproteins labeled in the protein component with ^{125}I. Furthermore, upon comparing lymph and plasma levels of apo-LP specific activity, a semi-quantitative measurement of mucosal apo-LP synthesis was possible since most of the apo-LPs found in intestinal lymph is generated in the intestinal mucosa (9,10).

MATERIALS AND METHODS

Male Wistar rats weighing 250-400g were lightly anesthetized intraperitoneally with pentobarbital (5mg/100g body weight) and the following procedures performed: catheters were placed into a) the main intestinal lymph duct (11); b) the duodenum for the infusion of fat; c) the jugular vein for the continuous administration of bovine albumin and electrolyte solution; and d) the carotid artery for blood collection. Chloramphenicol was administered intraperitoneally after the surgical procedure, and the rats were restrained in Bollman-type cages (12).

First experiment

The rats received the following solution I.V. at the rate of 1.15ml/h: Ringer (90ml); 50% glucose (10ml); 1.25g or bovine albumin (Sigma Chemical Co., St. Louis, MO), and KCl (4mEq). These solutions were sterilized through a Millipore filter (45nm). Duodenal administration of 10% glucose solution (2.2ml/h) was maintained for 18 hours. Thereafter, it was discontinued, and 2 hours later duodenal infusion of oil was initiated (.035ml/h for 10 hours).

The LCT administered was cottonseed oil and the MCT (kindly supplied by Unilever, Vlaardingen, Holland) contained fatty acids of the following composition: 5% C_8, 70% C_{10}, 20% C_{12}, 2.5% C_{16}, .5% $C_{18:2}$, 2.0% C_{18} as shown by GLC analysis. Sequential aliquots of blood were drawn (200μl) in 10μl of heparin. Experiments were ended by aortic exsanguination.

Cholesterol transudation was measured by an I.V. bolus of 4-^{14}C-β-sitosterol (Amersham, Buckinghamshire, England), 5μCi, dissolved in 20μl of ethanol, and 0.5ml of saline as described earlier (1).

The final blood sample drawn was used to determine the total serum protein by the Lowry method (13), and protein was fractionated by densitometry after electrophoresis in Cellogel (14). In this system a complete separation of the endogenous rat albumin from the infused bovine albumin was obtained.

Serum cholesterol was determined by an enzymatic method (15) in which 20μl

Table 1
Intestinal Protein Flux and Lymph Volume in Rats After Duodenal Infusion of Either LCT or MCT Over 10 Hours

		LCT (n=7)	MCT (n=9)	p[a]
Plasma	(mean mg/l ± SD)			
	Total protein	5.55 ± 5.0	53.5 ± 6.3	NS
	Endogenous protein	13.5 ± 3.4	11.4 ± 2.9	NS
	Bovine albumin[b]	9.9 ± 4.1	7.9 ± 2.6	NS
Lymph	(mean mg/l ± SD)			
	Total protein	23.5 ± 5.5	23.1 ± 5.0	NS
	Endogenous protein	8.3 ± 1.6	6.8 ± 1.2	NS
	Bovine albumin	3.2 ± 1.9	3.5 ± 1.7	NS
Ratio	lymph/plasma			
	Total protein	0.42 ± 0.10	0.43 ± 0.70	NS
	Endogenous albumin	0.69 ± 0.16	0.66 ± 0.21	NS
	Bovine albumin	0.39 ± 0.12	0.48 ± 0.18	NS
Lymph	loss in 10 hours (mean mg ± SD)			
	Total protein	211.0 ± 63.8	174.6 ± 39.7	NS
	Endogenous albumin	77.4 ± 27.4	57.2 ± 17.8	p<0.05
	Bovine albumin	36.2 ± 24.8	27.9 ± 13.8	NS
	Volume (ml)	10.3 ± 4.7	8.4 ± 2.8	NS
Animal weight (mean ± SD)		378 ± 50	368 ± 23	NS

a - probability according to Student's "t" test (p<.05); b - bovine albumin was infused continuously I.V. to replenish exogenous lymph protein loss.

Table 2
Transudation of Cholesterol from Plasma into Intestinal Lymph in Rats After Duodenal Infusion of Either LCT or MCT

	LCT (n=8)	MCT (n=7)	p[a]
Cholesterol content (mean mcg/ml ± SD)			
Plasma	673 ± 171	663 ± 121	NS
Lymph	214 ± 76	231 ± 82	NS
Lymph cholesterol in 10 hours (mean mcg ± SD)	1865 ± 837	1964 ± 1008	NS
Cholesterol transudation[b]			
mcg/h (mean ± SD)	53.0 ± 26.9	39.9 ± 19.0	NS
mcg/ml (mean ± SD)	53.4 ± 25.3	41.9 ± 14.3	NS

a - probability according to Student's "t" test ($p < .05$); b - cholesterol transudation = lymph ^{14}C-ßS as d.p.m./hour ÷ plasma ^{14}C - ßS as d.p.m./mg of cholesterol

of serum in 15ml of Aquasol (New England Nuclear, Boston, MA) was counted for radioactivity in a beta liquid scintillation counter (Model LS-100, Beckman).

Lymph samples were continuously collected over ice in EDTA (.01%) and sodium azide (.01%). These solutions were utilized for the measurements described above. However, cholesterol mass and radioactivity determinations were done in lymph previously lyophilized and extracted with ethyl ether (2 x 5ml).

Second experiment

Three rats were infused with I.V. ^{125}I-apo HDL while LCT was perfused into the duodenum. Rats were prepared as in the first experiment. HDL was obtained from donor rats by preparative ultracentrifugation (16) and labeled with ^{125}I (17). About 18-24 hours after the surgical procedure, a duodenal infusion of LCT was started, and 2 hours later, a pulsed I.V. infusion of ^{125}I-apo HDL was administered, followed by a continuous injection, during 6 hours, of 1/3 of the radioactivity initially administered. Throughout the experiment the radioactivity of lipoproteins in plasma and lymph aliquots was determined after precipitation with trichloroacetic acid in order to exclude free ^{125}I (18). These experiments also were terminated by exsanguination.

The larger plasma aliquot was used for the HDL analysis together with pooled lymph samples collected during the last 3 hours of the study period. Samples were submitted to exhaustive dialysis against saline EDTA solution to discard free ^{125}I prior to ultracentrifugation for lipoprotein isolation (16). Radioactivity was measured in each apo-LP identified, after delipidation, by polyacrylamide gel electrophoresis (PAGE). Scanning densitometry of the gel (19) and protein measurement by the Lowry procedure (13) allowed for the quantitative analysis of each

Table 3
Percent distribution ± C.V. of radioactivity (^{125}I - apo -HDL) infused I.V. in plasma and lymph of 3 rats shown in *Fig. 1.* Values in plasma were obtained at the end of experiment. Lymph data of each rat represent the mean values of the last three hours of collection.

Plasma	
(d < 1.063)	1.4 ± .4
(1.063 < d < 1.210)	98.4 ± .5
Lymph	
(d < 1.063)	30.0 ± 4.4
(1.006 < d < 1.210)	70.0 ± 4.4
Transudation[a]	16.6

[a]Transudation of HDL from plasma to lymph = ^{125}I -apo HDL in d.p.m./ml lymph/plasma x 100.

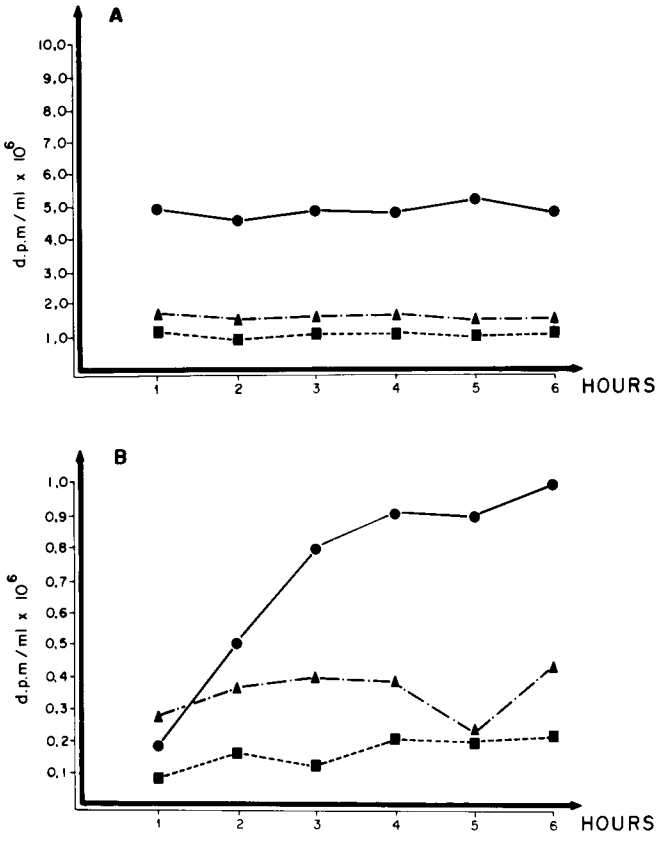

Fig. 1. Plasma (A) and lymph (B) radioactivity of I.V. infused ^{125}I-apo-HDL in three rats. The geometric signs conform to the findings (plasma and lymph) for each respective rat.

apo-LP and expression of the results as c.p.m./mg of protein.

RESULTS

Total lymph flow and plasma protein flux into the intestinal lymph system of rats after duodenal infusion of LCT as compared to MCT are shown in *Table 1.* The total rat endogenous albumin mass appearing in lymph throughout the 10-hour study period was slightly less with MCT as compared with LCT infusion. However, the lymph volume and the lymph output where bovine albumin was administered were similar in both groups. The lymph/plasma ratio of total protein, rat endogenous albumin and bovine albumin also did not differ in the two experimental groups.

The radioactive appearance in lymph of labeled β-sitosterol after intravenous infusion as a direct measure of plasma lipoprotein transudation was similar after either MCT or LCT administration *(Table 2).* Evidence that whole lipoproteins "leak" from plasma into intestinal lacteals was seen after blood infusion of autologous labeled apo-HDL (^{125}I apo-HDL) to 3 rats undergoing chronic duodenal infusion with LCT. Data were analyzed after the steady-state of radioactivity was reached in plasma and lymph as shown in *Fig. 1. Table 3* shows that the average radioactivity per ml of lymph was 16.6% of the plasma value, and was roughly twice as much as that obtained for cholesterol on a similar experimental group (7.66% on LCT).

Table 3 shows that 98.4% of the infused ^{125}I was in the HDL fraction at the end of the experiment, whereas 30% of the radioactivity appeared in lymph in the chylomicron and VLDL fraction, and 70% in the LDL + HDL fraction.

Major apo-LP identified in plasma HDL by the PAGE method were A I, A IV, and the C and E apo-LP groups. *Table 4* shows that, when specific activity of the apo-LPs of the whole lymph was compared to the values obtained from apo-HDL isolated from plasma, roughly 25% of the investigated apo-LPs arose from plasma, or alternatively signifies 75% of intestinal lymph apo-LPs was derived from the intestinal mucosa and incorporated into different lymph LP. These data, however, are only a semi-quantitative estimate because PAGE (19) is not a specific quantitative method for the measurement of apo-LP.

DISCUSSION

Experimentally, it has long been recognized that lacteal flow is independent of the type of fat absorbed by the intestine (4,20). In accurately measuring lymph flow, however, it is critical to maintain and replace ongoing losses of water and solutes during the experiment to avoid a reduction in the plasma colloid-osmotic pressure (6,7).

Through a constant I.V. infusion of saline and bovine albumin, the plasma protein concentration was maintained in the normal range, and the protein flux from plasma into the lymph lacteals as documented was unrelated to the passage of absorbed fat. In other words, fat absorption and transport were through a different route and bear no relation to the mechanism of intestinal lymph formation. Our results differ from those of Turner and Barrowman (4) where lymph volume with administration of MCT was 25% lower than with LCT. This discrepancy most likely derives from failure of these workers to maintain constancy of the plasma colloid-osmotic pressure. In contrast, our data support the findings of Simmonds (20). These physiologic findings are also in agreement with clinical observations in patients with lymphatic obstruction where the protein concentration in ascitic fluid did not change after MCT feeding (21).

Plasma lipoprotein transport into the lymph system, as measured by the transudation of cholesterol, behaves similarly to the plasma protein components. Since the type of fat ingested is not critical to the regulation of intestinal lymph formation, some other physiologic stimuli must control the opening of capillary gaps to allow for the egress of plasma components. Since plasma lipoproteins have a much higher molecular weight than plasma proteins, the lymph/plasma ratio of cholesterol (7.66% with LCT and 6.55% with MCT) is, as expected, considerably less than that of plasma albumin (69 and 66% respectively).

Our experiments demonstrate that the appearance of plasma cholesterol in the intestinal lacteals depends entirely on the transport of intact lipoproteins, such as plasma HDL. Furthermore, even if only 1/6 of the plasma HDL leaks into the intestinal lymph system per day, it nonetheless represents a considerable share of

Table 4

Specific activity (d.p.m./mcg) from 3 rats after I.V. infusion of ^{125}I HDL *(see Fig. 1)* of each major apo-LP pooled in the final three lymph samples collected as compared to ultracentrifugally isolated HDL from the final sample of plasma.

apo LP	A_I	E	A_{IV}	C
Rat 1				
Plasma	2.140	2.231	15.369	25.222
Lymph	605	523	694	1.643
Rat 2				
Plasma	1.185	428	932	747
Lymph	472	113	228	435
Rat 3				
Plasma	1.012	168	498	338
Lymph	98	44	99	37
Lymph/plasma x 100 (mean)				
	16.3%	25.3%	25.0%	25.2%

the lymph cholesterol mass since in the rat at least 50% of plasma cholesterol is carried by HDL. In the previous article, for example, 3.78mg/d out of 6.69 mg/d of intestinal lymph cholesterol originated from plasma during the absorption of 1900mg of cottonseed oil perfused into the duodenum.

The lymphatic lipoprotein distribution of ^{125}I-apo-HDL after a constant intravenous infusion was similar to that obtained when radioactive cholesterol or β-sitosterol was administered I.V. (see previous article). This finding signifies that whole plasma particles adhere to the lymph chylomicrons as soon as they percolate through the capillary pores to enter into the intestinal lacteals.

Although the mass of apo-LP was not precisely quantified, it is reasonable to conclude that most of apo-LPs in lymph were synthesized by intestinal mucosa in conformity with earlier reports (9,22,23). However, it should be stressed that the direct measurement of apo-LP in samples obtained from lymph as previously done (9,22,24) does not accurately quantify local intestinal protein synthesis. Thus, a large portion of apo-LP originates from plasma, as shown here, provided that the transcapillary forces responsible for the intestinal lymph output are physiologically maintained near normal.

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