Intestinal lipoproteins in the rat with D-(+)-galactosamine hepatitis[1]

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Abstract D-(+)-galactosamine (GalN) induces severe reversible hepatocellular injury in the rat accompanied by lecithin:cholesterol acyltransferase (LCAT) deficiency, defective chylomicron (CM) catabolism, and accumulation of abnormal plasma lipoproteins (Lps), including discoidal high density lipoproteins (HDL). These abnormalities are presumed to result from hepatic injury alone, but the effect of GalN on intestinal Lps has not been studied. To assess possible effects on intestinal Lp formation and secretion, mesenteric lymph fistula rats were injected with GalN or saline. Twenty-four hours later a 2-hr fasting lymph sample was collected; this was followed by an 8-hr duodenal infusion of a lipid emulsion containing 17.7 mM [^{3}H]triolein at 3 ml/hr. Fasting lymph and fat-infused lymph flow rates, ^{3}H, triglyceride, and cholesterol output, residual ^{3}H in intestinal lumen and mucosa, total ^{3}H recovery, and d < 1.006 g/ml Lp size and lipid composition were unchanged by GalN treatment, but d < 1.006 g/ml Lps were depleted of apoE and C. Fat-infused lymph phospholipid (PL) output was higher in GalN rats due to PL-enriched d > 1.006 g/ml Lps. Electron microscopy of lymph and plasma LDL and HDL revealed spherical Lps in all samples. GalN plasma, fasting lymph, and fat-infused lymph also contained large abnormal LDL and discoidal HDL. Control lymph LDL and HDL did not differ in size from control plasma LDL and HDL. Control lymph LDL contained both apoB_{240K} and B_{335K}. However, spherical LDL and discoidal HDL in fasting lymph from GalN rats differed significantly in size from the corresponding plasma particles and became closer in size to the plasma particles with fat infusion. GalN lymph LDL contained only apoB_{240K} and had a lower PL/CE than GalN plasma LDL. GalN fasting lymph HDL, depleted of apoC and having a PL/CE of 5, became enriched in apoE and the PL/CE increased to 10 with fat infusion to closely resemble GalN plasma HDL. GalN reduces apoE and C (mainly of hepatic origin) in d < 1.006 g/ml gut Lps, which may contribute to the CM catabolic defect in GalN rats. Lymph LDL and HDL, especially in fasting lymph, may be partially gut-derived with increased filtration of plasma Lps into lymph with fat infusion. GalN fat-infused lymph HDL is enriched in apoE, but unable to transfer apoE to d < 1.006 g/ml intestinal Lps. We conclude that GalN hepatitis is a model that allows study of intestinal Lps with normal lipid digestion and absorption in the face of severe hepatic injury and LCAT deficiency.—**Black, D. D., P. Tso, S. Weidman, and S. M. Sabesin.** Intestinal lipoproteins in the rat with D-(+)-galactosamine hepatitis. *J. Lipid Res.* 1983. **24:** 977–992.

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A single intraperitoneal injection of D-(+)-galactosamine (GalN), an amino sugar, produces acute reversible hepatocellular injury in the rat. GalN hepatitis results from a depression of uracil nucleotide-dependent biosynthesis of nucleic acids, glycolipids, glycoproteins, and glycogen, accompanied by organelle injury, necrosis of hepatocytes, infiltration by inflammatory cells, and accumulation of hepatocellular fat (1–3). GalN hepatitis is accompanied by a severe deficiency of plasma lecithin:cholesterol acyltransferase (LCAT) activity (4). This enzyme, synthesized by the liver, is responsible for the esterification of the majority of cholesteryl esters in plasma lipoproteins (5, 6). The LCAT deficiency in GalN-treated rats probably is the major factor responsible for the extremely low levels of plasma cholesteryl esters and profound abnormalities of plasma lipoprotein composition and structure. Compositional studies of the plasma lipoproteins in fasting rats with GalN hepatitis reveal an increase in free cholesterol, phospholipids, and triglycerides; and a striking decrease in esterified cholesterol (7). The cholesterol, normally most abundant in the high density lipoprotein (HDL) fraction, shifts into the low density lipoprotein (LDL) density range, and the increased triglyceride remains in the very low density lipoprotein (VLDL) density range. The increased phospholipid appears in both the LDL range and in the cholesteryl ester-depleted HDL. In addition to lipid compositional changes in GalN hepatitis there is a marked alteration in the apoprotein composition of the plasma lipoproteins. Analysis of these abnormal plasma lipoproteins has revealed a decrease in VLDL

Abbreviations: CM, chylomicrons; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; GalN, D-(+)-galactosamine; PAGE, polyacrylamide gel electrophoresis; Lp, lipoprotein.

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apoE and apoC, HDL apoA and apoC, and an increase in LDL and HDL apoE (7, 8). Electron microscopy of these lipoproteins after negative staining reveals marked structural changes, most notably the appearance of discoidal HDL particles (7). The lipoprotein abnormalities in GalN-treated rats are quite similar to those that have been described in patients with alcoholic hepatitis and LCAT deficiency (9).

Recently, we have described a defect in chylomicron (CM) catabolism in fat-fed GalN rats, which is manifest by a marked postprandial hyperchylomicronemia (10). This defect is accompanied by significant deficiencies of lipoprotein lipase and hepatic lipase activities. Although deficiency of lipase activity undoubtedly contributes greatly to the CM catabolic defect, we were also interested in examining intestinal lymph lipoproteins in GalN rats to determine if any apoprotein abnormalities are present which might contribute to the defect. The first step in CM metabolism involves transfer of apoC-II from HDL to the CM particles for activation of lipoprotein lipase (11, 12). Transfer of apoE to CM from HDL also must occur during CM catabolism in order for hepatic recognition and uptake of triglyceride-depleted CM remnants to take place (13, 14). Although the transfer of these apoproteins does occur in the plasma compartment, a significant degree of transfer, especially of apoC, also occurs in mesenteric lymph (11). In the present studies we wished to determine whether the normal transfer from lymph HDL to triglyceride-rich lymph lipoproteins occurs. Although preliminary ultrastructural studies suggested that CM secretion proceeds normally in GalN animals, we also wished to determine precisely whether GalN interferes with the normal digestion and absorption of endogenous biliary lipid in the fasting state or of exogenous lipid during duodenal infusion in rats with GalN liver injury.

Previous studies of mesenteric lymph lipoproteins using models of deranged plasma lipoproteins have yielded much useful information. Windmueller (15) examined the lymph lipoproteins of rats fed orotic acid, which causes a specific defect in hepatic VLDL secretion, and confirmed the normal transport of exogenous fat as chylomicrons and endogenous lipid as VLDL-size particles from the intestine into the lymph. Krishnaiah et al. (16) used the orotic acid model to confirm that the high molecular weight apoB (B_{335K}) found in rat mesenteric lymph LDL is of hepatic origin. Glickman and Green (17) treated rats with 4-aminopyrazolopyrimidine, which inhibits hepatic lipoprotein secretion, to demonstrate that mesenteric lymph apoA-I is produced by the intestine and not simply transferred to lymph lipoproteins from plasma lipoproteins. Recently, Krause et al. (18) characterized the mesenteric lymph lipoproteins in rats treated with ethinyl estradiol which produces a profound hypolipoproteinemia. They found that the lymph lipoproteins were deficient in apoE, C and B_{335K}, confirming the hepatic production of these apoproteins. These studies and the work of others using radioisotope incorporation into apoproteins and immunochemical staining of apolipoproteins in enterocytes have shown that the intestine has the capability to synthesize low molecular weight apoB (B_{240K}), apoA-I, A-IV, and small amounts of apoC, whereas the liver synthesizes both $apoB_{240K}$ and B_{335K}, apoA-I and A-IV, most of the plasma apoC, and practically all of the apoE (11, 19–30).

The present studies provided a unique opportunity to examine intestinal lipid absorption and lipoprotein secretion and composition under the conditions of GalN hepatitis and its resultant disturbances of plasma lipoproteins and severe plasma LCAT deficiency. The results of the present studies of mesenteric lymph lipoproteins in rats with GalN hepatitis demonstrate apoprotein abnormalities of lymph lipoproteins in the face of normal intraluminal digestion and enterocyte absorption and secretion which may be explained by the derangement in plasma lipoprotein metabolism secondary to GalN-induced liver injury and reduced plasma LCAT activity. These studies also confirm the almost exclusive hepatic contribution to the $apoB_{335K}$, E, and C found in lymph lipoproteins. We have further characterized a discoidal lymph HDL that differs significantly from plasma HDL in GalN animals supporting the studies of Green, Tall, and Glickman (31) and Bearnot et al. (32) which show that the intestine synthesizes a discoidal HDL.

MATERIALS AND METHODS

Female Sprague-Dawley rats, 250–300 g, were used in all experiments. Animals were maintained on standard laboratory chow until the day before surgery when an 18-hr fast was instituted allowing free access to water. Under ether anesthesia, the main mesenteric lymphatic was cannulated by the method of Bollman, Cain, and Grindlay (33) with a clear vinyl tubing (i.d., 0.50 mm; o.d., 0.80 mm) pretreated with 7% TDMAC-Heparin (Polysciences, Inc., Warrington, PA) to retard clotting. Silicone tubing was introduced approximately 2 cm into the duodenum through an incision in the gastric fundus. The tubing was secured by a transmural suture and the fundal incision was closed by a purse-string suture. Silicone tubing was placed free in the peritoneal cavity for injection of GalN and saline. The animals were then placed in metal restraining cages and allowed to recover in warm boxes maintained at 30°C. A saline solution

(145 mM NaCl, 4 mM KCl) was infused via the duodenal cannula at a rate of 3 ml/hr. Glucose was not added since it could minimize the GalN effect (1). After 12 hr of recovery, GalN animals were injected via the peritoneal tubing with D-(+)-galactosamine-HCl (Sigma Chemical Co., St. Louis, MO) dissolved in physiological saline (250 mg/ml) at a dosage of 1000 mg/kg. Control animals were injected with an equal volume of saline.

Lipid infusion

Twenty-four hours after injection (i.e., 36 hr post-surgery) of GalN in experimental animals and saline in the controls, an 8-hr duodenal lipid infusion was started after collection of a 2-hr fasting lymph sample. The lipid emulsion was prepared as follows: sodium taurocholate (96% pure, Calbiochem-Behring Corp., La Jolla, CA) was prepared at a concentration of 19 mM in a phosphate buffer (6.75 mM Na_2HPO_4, 16.5 mM NaH_2PO_4) containing 145 mM NaCl and 4 mM KCl. In a separate container triolein and egg yolk lecithin (Sigma Chemical Co., St. Louis, MO) and glycerol tri[9,10-^{3}H(N)]oleate (New England Nuclear, Boston, MA) were dissolved in chloroform and mixed, and the solvent was then evaporated under a N_2 stream. Next, the taurocholate-buffer was added and the emulsion was prepared by sonication using twelve 30-sec bursts from a probe of a Bronwill Biosonik II (150 W) ultrasonic generator with the intensity setting at 80%. The emulsion contained 17.7 mM triolein, 0.125 μCi/ml of ^{3}H-labeled triolein, 3.3 mM lecithin, and 19 mM taurocholate. Just prior to use, aliquots were taken from the top, middle, and bottom of the emulsion and the radioactivity was measured to ensure homogeneity of the emulsion. Triglyceride and radioactivity determinations on aliquots from each batch of emulsion taken at the beginning and end of each infusion revealed variation of less than 5%.

Lipid infusion and collection of lymph

The emulsion was infused into the duodenal cannula at a rate of 3.0 ml/hr for 8 hr. Samples of mesenteric lymph were collected for 2 hr prior to the lipid infusion and then at 2, 4, 5, 6, 7, and 8 hr after beginning the infusion. Rats used expressly for isolation of lymph LDL and HDL for compositional analysis were infused with nonradioactive lipid after collection of a 4-hr fasting lymph sample. Timed samples were collected at 2, 4, 6, and 12 hr after starting the infusion. Samples were defibrinated using wooden applicator sticks and a preservative solution (1 ml per 100 ml of lymph) containing 1% EDTA, 2% azide, 0.01% chloramphenicol, and 0.005% gentamicin was added. Samples were refrigerated immediately after collection. At the end of the lipid infusion animals were bled by abdominal aortic puncture. Blood was collected in EDTA tubes (1.5 mg/ml blood) and immediately centrifuged for 20 min at 4°C at 3,000 rpm. The plasma samples were preserved as described above. The proximal and distal halves of the small intestine were tied off separately and the lumen of each was washed three times with 5-ml aliquots of 5 mM sodium taurocholate. Aliquots from these washings were taken for radioactivity determination and lipid extraction. Proximal and distal small intestinal mucosa was scraped and homogenized for lipid extraction. The stomach and colon were tied off and excised separately and saponified, acidified, and extracted with petroleum ether for radioactivity measurement (34).

Lipoprotein isolation

Sequential density ultracentrifugation of lymph and plasma samples was accomplished using a Beckman SW 41 Ti rotor at 17°C (35). The density classes separated were chylomicrons ($d < 1.006$ g/ml, 1.06×10^6 *g-min*), VLDL ($d < 1.006$ g/ml, 230×10^6 *g-min*), LDL (1.006 g/ml $< d < 1.05$ g/ml, 268×10^6 *g-min*), and HDL (1.05 g/ml $< d < 1.21$ g/ml, 469×10^6 *g-min*). VLDL samples were washed once in a Beckman SW 50.1 Ti rotor at 228×10^6 *g-min*. Contaminating proteins were removed from the CM fraction by gel filtration chromatography using 2% agarose (Biogel A-50m, 50–100 mesh, Bio-Rad Laboratories, Richmond, CA) in 0.8 × 20 cm columns (36). Columns were eluted with 0.9% NaCl containing 0.01% EDTA, 0.02% azide, 0.0001% chloramphenicol, and 0.00005% gentamicin, and the CM fraction was eluted in the void volume. All lipoprotein fractions were dialyzed at 4°C against 0.01% EDTA, 0.02% azide, 0.0001% chloramphenicol, and 0.00005% gentamicin in deionized water with two changes of dialysate prior to polyacrylamide gel electrophoresis or electron microscopy. Only the 2-hr fasting lymph sample and the lymph from the final 2 hr of the lipid infusion were fractionated into various lipoprotein classes. These lymph samples will henceforth be referred to as fasting lymph and fat-infused lymph, respectively.

Lipid extraction

Whole plasma and lymph and lymph lipoprotein fractions were extracted before lipid analysis, and the procedures are described under the lipid analysis section. Acidified samples of intestinal lumen contents and mucosa were extracted using toluene–ethanol 2:1 (v/v) (37).

Quantitative lipid and protein analysis

Plasma and lymph lipoprotein total and esterified cholesterol were determined by gas–liquid chromatographic analysis of extracts of saponified and nonsa-

ponified samples, and cholesteryl esters were estimated by difference (38). Cholesteryl ester values were calculated from cholesterol determinations using a correction factor of 1.67. An internal standard of β-sitosterol was added to each sample prior to extraction and was used as the basis for calculation of results. Plasma and lymph lipoprotein phospholipids were determined by extraction of samples by sequential addition of 50 volumes of methanol and 50 volumes of chloroform. Extracts were dried under N_2, digested with 70% perchloric acid, and phosphorus was determined by the method of Rouser, Fleischer, and Yamamoto (39). For plasma and lymph phospholipid mass determinations, phosphorus values were converted to phospholipid values using a factor of 25. The phosphorus values were assumed to be the molar equivalents of lymph and plasma phospholipids. Plasma and lymph lipoprotein triglycerides were determined by the method of Biggs, Erickson, and Moorehead (40). Protein determinations were made by the modified Lowry method (41).

Polyacrylamide gel electrophoresis (PAGE)

Lipid was extracted from dialyzed lipoprotein fractions using several volumes of cold anhydrous ether. Samples were lyophilized overnight before the addition of a reducing sample buffer containing sodium dodecyl sulfate (SDS) and β-mercaptoethanol. Samples were then heated at 90°C for 4 min before application to gels. Discontinuous SDS polyacrylamide slab gel electrophoresis was carried out according to the method of Laemmli (42). Stacking gel (pH 6.8) was 3% polyacrylamide, and the running gel (pH 8.8) was a linear gradient from 3.5–27% polyacrylamide. Electrophoresis was performed immediately after formation of the stacking gel in either an LKB (LKB Instruments, Inc., Rockville, MD) or Bio-Rad (Bio-Rad Laboratories, Richmond, CA) vertical slab gel apparatus using a constant current of 25 mamp per slab. Gels were fixed, stained, and destained with solutions described by Weber and Osborn (43). Lipoprotein apoproteins were identified on slab gels by comparison to molecular weight standards (myosin, phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme) and also by comparison to published SDS PAGE electrophoretograms of rat apoproteins.

Separation of lipids by thin-layer chromatography

Proximal intestinal luminal contents and mucosal extracts were applied to silica gel G plates. Plates were developed in a solvent system consisting of petroleum ether–diethyl ether–acetic acid 150:30:1.5 (v/v/v). Lipid standards were co-chromatographed with samples. Lipid spots were identified by exposure to iodine vapor and by comparing migration of standards. Spots were then scraped after iodine evaporation into scintillation vials followed by addition of 1 ml of ethanol and 10 ml of a toluene-based scintillant.

Liquid scintillation counting

Aqueous samples were added to a toluene-based butyl PBD-PBBO scintillant containing Biosolv 3 (Beckman Instruments, Inc., Fullerton, CA). Samples extracted into solvent were counted after evaporation of the solvent under N_2 and addition of the above scintillant without Biosolv. Scintillation counting was performed in a Packard Tri-Carb liquid scintillation counter (Packard Instruments Co., Downers Grove, IL). Correction for quenching was accomplished using the channels ratio method.

Electron microscopy

Dialyzed lipoproteins were negatively stained for electron microscopy with 2% phosphotungstate solution (pH 5.9) as described by Forte and Nichols (44). Spherical particle size was determined using a magnifying micrometer to measure the diameters of 100 free-standing particles in each preparation. In those preparations containing discoidal particles, the edge thickness and long axis of 100 particles were measured.

Statistical Analysis

Results were statistically evaluated using Student's two-tailed t test. The null hypothesis was rejected at $P < 0.05$.

RESULTS

Effect of GalN on plasma lipids in lymph fistula rats

Previous studies from this laboratory have established that GalN achieves its maximum effect, as measured by depression of plasma LCAT and cholesteryl esters, approximately 24 hr after a single intraperitoneal injection (4, 7). In the present study the fasting lymph collection was started 24 hr after GalN injection. In pilot studies we maintained control (n = 3) and GalN (n = 3) lymph fistula rats for a comparable period of time and sampled their venous blood 24 hr after GalN or saline injection and found plasma cholesteryl esters in GalN-treated rats to be less than 20% of total plasma cholesterol. Control animals had normal values (60–70% of total cholesterol as esters). Thus, even with lymph drainage, GalN produced a marked decline in plasma cholesteryl ester concentration 24 hr after injection. Plasma lipids were analyzed at the end of the 8-hr intraduodenal infusion (34 hr after GalN injection). A reduction in total plasma

cholesterol with a concomitant significant reduction of cholesteryl esters was noted in GalN-treated animals (total cholesterol: control, 60.0 ± 13.0 mg/dl; GalN, 34.0 ± 6.6 mg/dl; $\bar{x}$ ± SEM) with the percent of total plasma cholesterol as cholesteryl ester falling to 21% in GalN animals compared to 68% in controls ($P < 0.001$). In previous studies it has been shown that the extent of reduction of plasma cholesteryl esters is a very sensitive indicator of GalN-induced liver injury and also reflects the extent of plasma lipoprotein abnormalities (4, 7).

Digestion, absorption, and recovery of infused [^{3}H]triolein in control and GalN-treated rats

There was no significant difference between the GalN-treated and control rats in regard to recovery of the ^{3}H from lymph, intestinal lumen, intestinal mucosa, stomach, cecum, and colon. Approximately 40% of the infused ^{3}H was recovered in the mesenteric lymph in both groups. The very small amount of radioactivity (<0.5% in both groups) in the gastric contents demonstrates the absence of significant duodenogastric reflux, and the small amount (<1%) recovered from the cecum and colon indicates that almost complete absorption of the infused lipid occurred in the small intestine.

There was also no significant difference between GalN-treated and control rats in the distribution of the infused [^{3}H]oleate among various lipid classes in the proximal luminal contents and proximal intestinal mucosa (**Table 1**). In both groups of animals it was apparent that intraluminal hydrolysis of triolein was complete with approximately 50% of the radioactivity as free fatty acid and the rest as partial glycerides. Examination of values obtained from the proximal intestinal mucosa showed that intracellular re-esterification of the absorbed fatty acid and monoglyceride into triglyceride was the same for both control and GalN animals with almost half of the [^{3}H]oleate incorporated into triglyceride (Table 1). Thus, intraluminal digestion of the infused triolein and re-esterification of the absorbed fatty acid and monoglyceride appear to proceed normally despite treatment with GalN. Previous studies have obtained higher mucosal content of labeled triglyceride after lipid absorption (45). However, those studies were performed in male rats, whereas the present study used female rats. Hormonal factors may therefore account for the difference in degree of mucosal re-esterification.

Total recovery of ^{3}H from all measured sources (approximately 57%) was the same in both groups of animals. The unaccounted [^{3}H]oleate presumably was transported from the intestine by routes other than the main mesenteric lymph duct, such as secretion into accessory lymphatic channels or directly into the portal circulation (46). A portion of the oleate was also probably catabolized by the gut.

Effects of GalN on lymph flow and lipid transport

Fasting lymph flowed at approximately 2 ml/hr in both groups with no statistically significant difference. With duodenal lipid infusion, the flow rate increased by 1.5–2.0-fold in both groups. At no time was there a statistically significant difference between control and GalN animals.

Both groups demonstrated a lymph triglyceride fatty acid transport rate of approximately 15 μmol/hr in fasting lymph. With lipid infusion, triglyceride output rose to a steady state level of 100–110 μmol/hr by 5–6 hr in both groups of rats. Analysis of triglyceride fatty acid output as measured by ^{3}H radioactivity output revealed that 70–80% of the [^{3}H]oleic acid was in the form of triglyceride in both control and GalN animals.

The total cholesterol transport in fasting lymph was approximately 1.0 μmol/hr for both groups. Lymph cholesterol transport increased slightly with lipid infusion and then declined to fasting values by the end of the experiment. Most of the cholesterol in fasting lymph is esterified (60–70%), and GalN had no effect on the cholesteryl ester content of fasting lymph. Cholesteryl

TABLE 1. Distribution of radioactivity in various lipid classes in proximal intestinal lumen and mucosa in control and GalN-treated rats[a]

	MG + PL	DG	FA	TG	CE
Proximal lumen					
Control (4)	48.6 ± 6.2	2.6 ± 1.3	48.8 ± 6.3	0	0
GalN (5)	37.5 ± 3.0	10.5 ± 3.1	51.1 ± 2.6	0.9 ± 0.9	0
Proximal mucosa					
Control (4)	15.7 ± 7.1	14.5 ± 1.6	25.3 ± 5.8	44.6 ± 13.7	0
GalN (5)	6.8 ± 2.3	18.1 ± 0.6	32.7 ± 5.5	42.3 ± 7.2	0

[a] Numbers are expressed as mean ± SEM of percent of total luminal or mucosal radioactivity. Numbers in parentheses are numbers of animals used. MG, monoglyceride; PL, phospholipid; DG, diglyceride; FA, fatty acid; TG, triglyceride; CE, cholesteryl ester. None of the GalN values were significantly different from control values at $P < 0.05$.

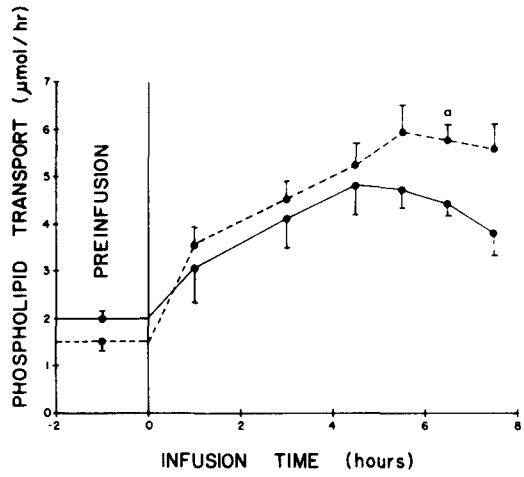

Fig. 1. Lymph phospholipid transport in control (● —— ●) and GalN (● – – – ●) rats before and during intraduodenal lipid infusion. Individual points represent mean ± SEM for each group of animals. a, Significant difference at $P < 0.05$.

ester transport declined slightly during lipid infusion in both groups.

Phospholipid transport in fasting lymph was approximately 2.0 μmol/hr in control animals and 1.5 μmol/hr in GalN rats, differences that were not significantly different (**Fig. 1**). However, with lipid infusion, phospholipid transport in lymph was higher in GalN animals than in controls by the time steady state triglyceride transport was achieved (Fig. 1). A statistically significant difference ($P < 0.05$) was achieved between the two groups between 6 and 7 hr. As will be shown below, the increased phospholipid output in fat-infused lymph from GalN animals was present in the higher density (d > 1.006 g/ml) lymph fraction.

Effect of GalN on lymph lipoprotein composition and distribution of lipids among various lymph lipoprotein classes

Table 2 shows the distribution of lipids and radioactivity in lymph lipoprotein density fractions. Values for the CM and VLDL fractions were determined directly; those for the d > 1.006 g/ml fraction were calculated by subtracting the total of the values for the d < 1.006 g/ml fraction from those determined from whole lymph. The VLDL fraction of fasting control lymph contains approximately two-thirds of the lipid of each class. Twenty percent of the total cholesterol and 31% and 25% of the triglycerides and phospholipid, respectively, were found in the CM fraction. The d > 1.006 g/ml fraction contained 14% of the free cholesterol, 18% of the cholesteryl ester, 8% of the triglyceride, and 23% of the phospholipid. These values agree closely with those from previous studies (47). Fasting GalN lymph differs significantly from control lymph only in the distribution of free cholesterol, which is present in a greater proportion in the d > 1.006 g/ml fraction at the expense of that in the CM fraction. In fat-infused control lymph, there is a shift of all lipid classes into the CM fraction, most notably in the case of triglyceride (Table 2). GalN fat-infused lymph values differ from those of control animals in that significantly more phospholipid is transported in the d > 1.006 g/

TABLE 2. Distribution of lymph lipid and radioactivity in control and GalN-treated rats[a]

	CH	CE	TG	PL	^{3}H
Fasting control (4)					
CM	19.6 ± 0.6	19.5 ± 0.7	31.0 ± 5.3	25.1 ± 5.3	
VLDL	66.8 ± 16.0	62.4 ± 15.0	60.7 ± 16.0	51.8 ± 5.7	
d > 1.006	13.6 ± 4.4	18.1 ± 5.3	8.4 ± 7.2	23.1 ± 8.0	
Fasting GalN (5)					
CM	10.9 ± 1.3[b]	13.7 ± 1.7	28.6 ± 6.3	23.1 ± 1.0	
VLDL	37.6 ± 10.0	37.5 ± 10.1	62.8 ± 7.4	43.8 ± 3.9	
d > 1.006	50.9 ± 8.4[c]	48.1 ± 8.5[d]	8.6 ± 4.7	33.1 ± 4.9	
Fat-infused control (4)					
CM	48.2 ± 8.7	38.8 ± 7.0	71.4 ± 16.1	48.5 ± 2.8	72.8 ± 3.8
VLDL	39.3 ± 9.0	37.2 ± 8.6	21.9 ± 5.0	36.3 ± 1.6	10.0 ± 0.3
d > 1.006	12.5 ± 5.3	24.6 ± 8.7	7.0 ± 7.0	15.2 ± 4.4	17.4 ± 3.5
Fat-infused GalN (5)					
CM	39.4 ± 4.7	30.6 ± 3.6	49.7 ± 9.1	25.5 ± 2.7[b]	52.7 ± 6.5[d]
VLDL	25.4 ± 7.7	33.3 ± 10.4	20.5 ± 1.4	19.7 ± 5.8	11.2 ± 0.9
d > 1.006	35.2 ± 7.1	36.1 ± 7.2	30.1 ± 6.0	54.8 ± 8.4[e]	36.1 ± 6.0[d]

[a] Values are expressed as mean ± SEM of percent of total lipid mass or radioactivity of lymph. Numbers in parentheses are numbers of animals used. Lipid and radioactivity values for the d > 1.006 g/ml fraction were determined for each animal by subtraction of total lipid and radioactivity in the d < 1.006 g/ml fraction from the values for whole lymph. CH, free cholesterol; CE, esterified cholesterol; TG, triglyceride; PL, phospholipid; ^{3}H, radioactivity as tritium.

[b,c,d,e] GalN values were not significantly different from corresponding control values except at [b] ($P < 0.005$); [c] ($P < 0.05$); and [e] ($P < 0.025$). At [d], $P < 0.10$.

TABLE 3. Lymph d < 1.006 g/ml lipoprotein composition in control and GalN-treated rats[a]

	CH	CE	TG	PL	Protein
Fasting VLDL					
Control (4)	1.93 ± 0.03	4.32 ± 0.27	68.4 ± 1.6	20.0 ± 1.5	5.40 ± 0.21
GalN (5)	1.23 ± 0.27	2.80 ± 0.74	75.1 ± 1.7	16.3 ± 0.9	4.60 ± 0.12[b]
Fat-infused VLDL					
Control (4)	0.79 ± 0.07	1.1 ± 0.14	79.4 ± 1.7	14.9 ± 1.4	3.80 ± 0.24
GalN (5)	0.51 ± 0.13	0.76 ± 0.22	85.4 ± 2.5[b]	9.90 ± 2.10	3.40 ± 0.26
Fasting CM					
Control (4)	1.24 ± 0.22	2.90 ± 0.55	73.4 ± 4.0	17.6 ± 3.3	4.90 ± 0.41
GalN (5)	0.80 ± 0.08	2.29 ± 0.32	74.1 ± 2.8	20.4 ± 2.6	2.32 ± 0.24[c]
Fat-infused CM					
Control (4)	0.36 ± 0.07	0.46 ± 0.11	91.0 ± 1.0	7.1 ± 0.7	1.08 ± 0.17
GalN (5)	0.40 ± 0.04	0.35 ± 0.07	90.9 ± 1.8	7.2 ± 1.5	1.13 ± 0.15

[a] Values are expressed as mean ± SEM of percent of total mass of CM or VLDL. Numbers in parentheses are numbers of animals used. CH, free cholesterol; CE, esterified cholesterol; TG, triglyceride; PL, phospholipid.
[b,c] GalN values were not significantly different from corresponding control values except at [b] ($P < 0.05$) and [c] ($P < 0.005$).

ml fraction. The additional phospholipid transported in lymph obtained from fat-infused GalN rats is apparently accounted for in this fraction (Fig. 1).

Table 3 shows the composition of d < 1.006 g/ml lymph lipoproteins in control and GalN rats. Control and GalN fasting lymph CM and VLDL did not differ significantly in lipid composition. Fasting lymph CM from both groups closely resembled VLDL in composition; however, GalN fasting lymph CM and VLDL contained significantly less protein per particle than control CM and VLDL. As will be shown, this difference may be explained by differences in apoprotein composition. Compositional analysis of fat-infused lymph CM and VLDL from both groups showed an enrichment of these lipoproteins in triglyceride, especially the CM fraction, with a relative decrease in the percentage of other components; however, no significant differences were found between GalN animals and controls (Table 3). These values are in close agreement with those previously published for rat lymph CM and VLDL during lipid infusion (48, 49); moreover, no significant differences in protein composition between control and GalN animals were found in fat-infused lymph.

Effect of GalN on d < 1.006 g/ml lymph lipoprotein apoproteins as determined by PAGE

Control lymph CM apoprotein composition as visualized by PAGE is shown in **Fig. 2.** In both fasting and fat-infused control lymph CM, apoA-I, A-IV, and B_{240K} appear to be the major apoproteins. The GalN CM apoprotein pattern was similar to the control pattern with the exception of greatly diminished amounts of apoE and C, noted in both fasting and fat-infused CM. In both groups there was a relative increase in apoA-IV content with fat infusion. The control apoprotein patterns were similar to those reported by others for mesenteric lymph CM, including the finding of only $apoB_{240K}$ (16, 18, 27, 29, 50). The corresponding gels of lymph VLDL showed patterns very similar to those noted for the CM, including greatly diminished apoE and C bands in VLDL from GalN animals (**Fig. 3**).

Effect of GalN on lipoprotein size and morphology

Because of technical difficulties encountered in negative staining such large particles as CM, including the significant particle deformation that can occur and the

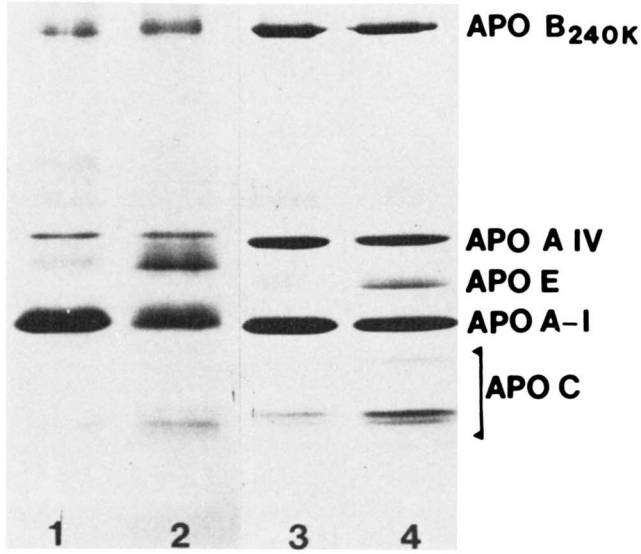

Fig. 2. Representative PAGE gels of lymph CM apoproteins from control and GalN rats. 1, GalN fasting lymph; 2, control fasting lymph; 3, GalN fat-infused lymph; 4, control fat-infused lymph. Equal amounts of protein (50 μg) were applied to each lane of the slab gel to allow comparison of the relative contribution of each apoprotein to the total apoprotein complement of the particle.

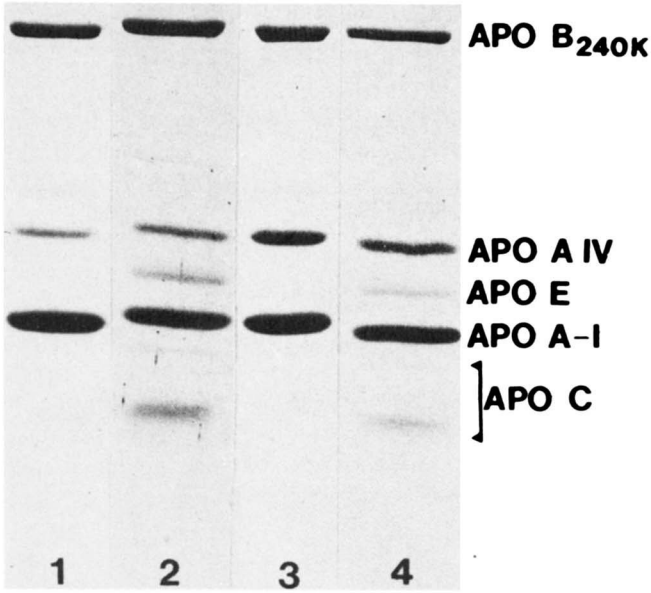

Fig. 3. Representative PAGE gels of lymph VLDL apoproteins from control and GalN rats. Otherwise labeled the same as Fig. 2.

prohibitive number of particles that would have to be measured because of the tremendous variation in size, the sizes of lymph CM of both groups were indirectly compared by determination of the triglyceride (core lipid) to phospholipid (surface lipid) mass ratio for these particles. All other lipoproteins were measured directly from electron micrographs. Control and GalN fasting lymph CM ratios were 4.2 and 3.6, respectively. With duodenal fat infusion, the ratios increased to 12.8 and 12.6, respectively. These results agree closely with those published by Glickman, Kirsch, and Isselbacher for lymph CM during lipid absorption and no significant differences are noted between control and GalN animals.

Electron micrographs of plasma VLDL from control and GalN animals are illustrated in **Fig. 4.** Control plasma VLDL consists of smooth-edged spherical particles, whereas GalN plasma VLDL consists of both smooth-edged spherical particles and large irregularly shaped particles that appear to have excess surface material. Plasma VLDL of similar morphology in fasting GalN rats have been described before by this laboratory (7). Fasting lymph VLDL from control and GalN animals appear as smooth-edged spheres (Fig. 4). With lipid absorption these particles enlarged but maintained their spherical shape (Fig. 4). At no time were any of the large irregularly shaped VLDL particles of GalN plasma found in the lymph. Examination of the measured diameters of spherical particles only shows that the GalN plasma spherical VLDL are significantly larger in diameter than controls (**Table 4**). Lymph VLDL from both groups increased in size with fat absorption, but no difference is noted between control and GalN sizes in either fasting or fat-infused lymph (Table 4).

Plasma LDL from control animals appear as smooth-edged spherical particles compared to GalN plasma LDL which shows more size variation and several large irregularly shaped structures which may be vesicular (**Fig. 5**). Spherical LDL from the plasma of GalN-treated rats are significantly larger than control plasma LDL (Table 4); however, spherical LDL in control and GalN fasting lymph are of approximately the same diameter (300 Å) (Table 4). Both control and GalN lymph

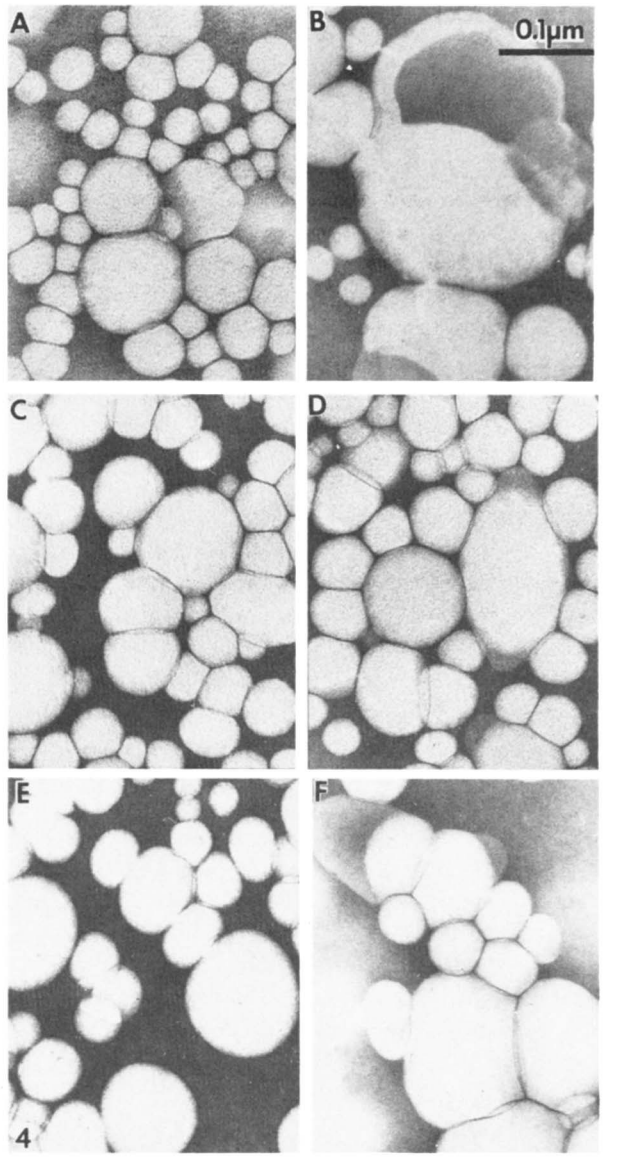

Fig. 4. Electron micrographs of negatively stained control and GalN plasma and lymph VLDL. A, control plasma; B, GalN plasma; C, control fasting lymph; D, GalN fasting lymph; E, control fat-infused lymph; F, GalN fat-infused lymph.

TABLE 4. Spherical lipoprotein particle sizes[a]

	Fasting Lymph	P[b]	Fat-infused Lymph	P	Plasma
VLDL					
Control	601 ± 24	<0.05	839 ± 47	<0.001	516 ± 15
GalN	632 ± 27	NS	850 ± 44	<0.02	660 ± 33
P[c]	NS		NS		<0.01
LDL					
Control	311 ± 15	NS	282 ± 15	NS	268 ± 4
GalN	302 ± 14	<0.001	361 ± 18	NS	422 ± 25
P	NS		<0.02		<0.001
HDL					
Control	139 ± 5	NS	110 ± 3	NS	122 ± 4
GalN	161 ± 7	<0.001	211 ± 5	<0.001	112 ± 4
P	NS		<0.001		NS

[a] Particle diameter expressed as Å ± SEM. One hundred free standing particles were measured in each preparation.
[b] *P* value for comparison of lymph particle size to corresponding plasma particle size.
[c] *P* value for comparison of GalN particle size to corresponding control particle size.

contain spherical LDL particles (Fig. 5). However, both fasting and fat-infused GalN lymph contain some of the large abnormal particles found in plasma (Fig. 5). With fat infusion the mean diameters of the spherical lymph LDL from both groups of rats changed to more closely resemble plasma particle sizes (Table 4) and retained their spherical shape (Fig. 5). Control lymph LDL particles became smaller in size with fat infusion, although the sizes in fasting and fat-infused lymph were not statistically significantly different from plasma values. GalN fasting spherical lymph LDL were significantly smaller than spherical plasma LDL, but with fat infusion, the spherical lymph LDL were larger and no longer significantly different in size from GalN spherical plasma LDL (Table 4).

Control plasma HDL consist of a homogeneous population of spherical particles (**Fig. 6**). GalN plasma HDL contains, in addition to spherical particles, discoidal particles, most of which on negative staining are stacked in rouleaux (Fig. 6); however, some of the particles that appear to be spherical may represent discoidal particles viewed *en face*. The size of the spherical plasma HDL particles is not significantly different in control and GalN animals (Table 4).

Lymph HDL consist of only spherical particles in both fasting and fat-infused control lymph that do not significantly differ in size from plasma HDL (Fig. 6, Table 4). However, GalN lymph HDL consists of both spherical and discoidal particles (Fig. 6). Again, it should be noted that some of the apparent spherical particles may be discs viewed *en face*. The spherical HDL in GalN fasting lymph are not significantly larger than control fasting lymph HDL but are significantly larger than the GalN plasma spherical HDL and become even larger with fat infusion (Table 4). The discoidal HDL in GalN fasting lymph are significantly larger in thickness and diameter than the plasma discoidal HDL; however, with fat infusion these dimensions decrease and resemble more closely those of the plasma discoidal HDL (**Table 5**).

Effects of GalN on plasma and lymph LDL and HDL phospholipid to cholesteryl ester ratio (PL/CE) and apoprotein composition

Additional studies were performed to more fully characterize the plasma and lymph LDL and HDL particles in order to determine how much filtration of plasma particles into lymph occurred. Control and GalN lymph fistula rats (two animals in each group) were prepared as described previously. In order to obtain sufficient lymph to isolate adequate quantities of lymph LDL and HDL for analysis, a 4-hr fasting lymph sample was obtained from each animal followed by a 12-hr duodenal lipid infusion. Lymph from the final 6 hr of fat infusion was used for isolation of lymph LDL and HDL. The lymph samples and plasma from the animals in each group were pooled and the LDL and HDL were isolated by sequential centrifugation. The plasma lipid values obtained from these animals at the end of the lipid infusion were similar to those found in the previous groups of animals. Thus, the percentage of total plasma cholesterol as cholesteryl esters decreased to <15% in the GalN animals compared to the control values of >65%.

LDL and HDL in plasma from normal rats consist of a core of predominantly hydrophobic cholesteryl ester with a surface coat that is mostly phospholipid. In rats with GalN liver injury, these particles become depleted of cholesteryl ester, probably secondary to the plasma LCAT deficiency, and become enriched in phos-

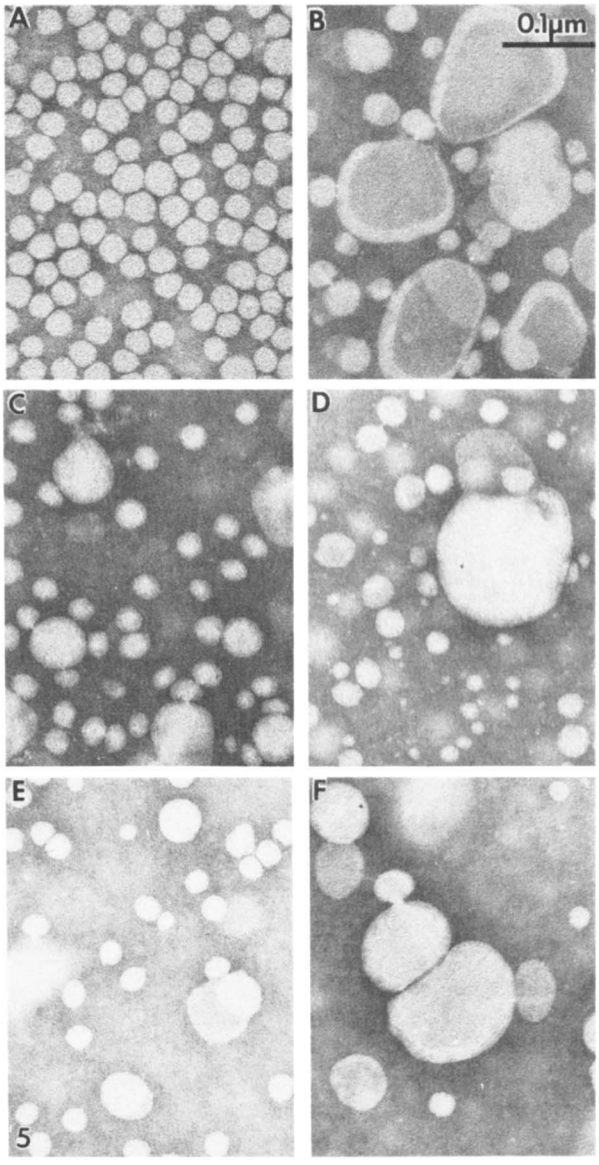

Fig. 5. Electron micrographs of negatively stained control and GalN plasma and lymph LDL. Otherwise labeled the same as Fig. 4.

pholipid (7). These compositional changes account for the abnormal LDL and discoidal HDL structures observed in GalN plasma. Phospholipid and cholesterol determinations from plasma and lymph LDL and HDL were used to calculate phospholipid to cholesteryl ester ratios (**Table 6**). Since the phospholipid to cholesteryl ester ratio is several-fold higher in GalN plasma LDL and HDL than in controls, this ratio is useful in comparing plasma LDL and HDL to lymph particles in control and GalN rats in order to estimate the degree of filtration of LDL and HDL into lymph. However, alteration of plasma lipoproteins after filtration into lymph cannot be ruled out.

Control fasting lymph LDL has a PL/CE ratio almost the same as that of control plasma LDL (Table 6); however, with fat infusion, this ratio increases to a value three times higher than the plasma ratio, suggesting production of an LDL distinctly different from the plasma particle (Table 6). The PL/CE ratio of GalN LDL in fasting lymph is much lower than the plasma value (Table 6). With lipid infusion the GalN lymph LDL value increases to a value slightly higher than control, but still much lower than the GalN plasma ratio (Table 6).

The PL/CE ratio in control fasting lymph HDL is 3.4 compared to 1.8 in plasma HDL, and the ratio in control fat-infused lymph increases to a value even

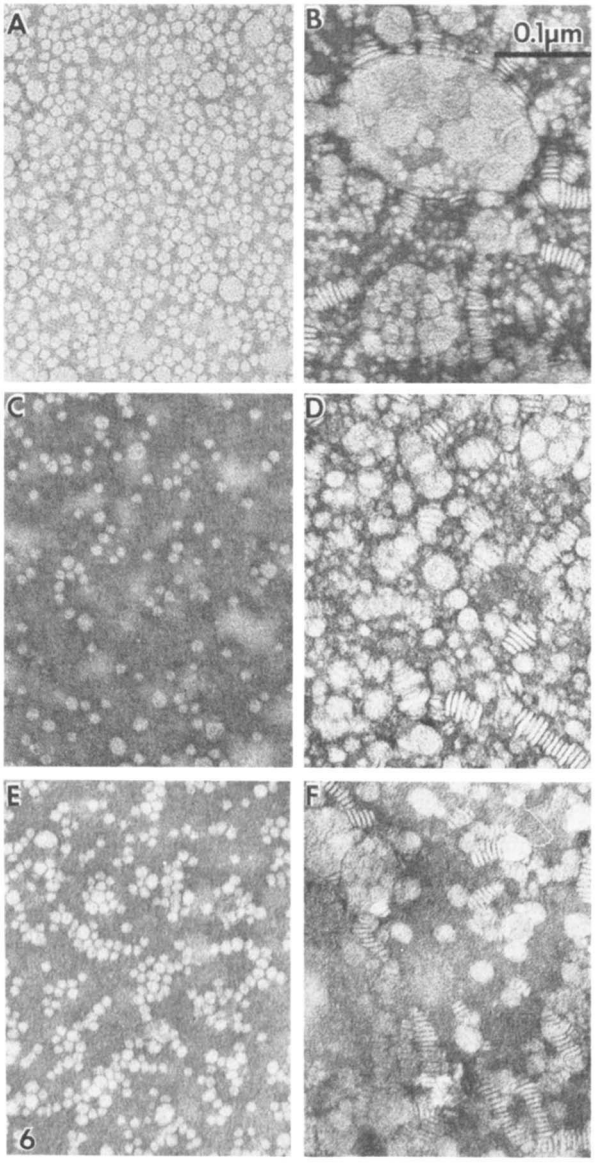

Fig. 6. Electron micrographs of negatively stained control and GalN plasma and lymph HDL. Otherwise labeled the same as Fig. 4.

TABLE 5. GalN discoidal HDL particle sizes[a]

	Fasting Lymph	P[b]	Fat-infused Lymph	P	Plasma
Thickness	47 ± 0.7	<0.005	44 ± 0.8	NS	42 ± 0.8
Diameter	235 ± 6	<0.001	178 ± 3	NS	178 ± 5

[a] Discoidal particle thickness and diameter expressed as Å ± SEM. One hundred stacked particles in rouleaux were measured in each preparation.
[b] *P* value for comparison of lymph particle value to the corresponding plasma value.

higher (4.9) than control plasma HDL (Table 6). The PL/CE ratio of 5 for GalN fasting lymph HDL is higher than the corresponding control value and half of the GalN plasma HDL ratio, and the GalN fat-infused lymph HDL ratio (10) is identical to the GalN plasma HDL ratio (Table 6).

Effect of GalN on lymph LDL and HDL apoproteins as assessed by PAGE

There were no marked differences in the apoprotein composition of control fasting and fat-infused lymph LDL samples by inspection of PAGE gel patterns (**Fig. 7**). Both apoB_{335K} and B_{240K} were present as the predominant apoproteins with smaller amounts of apoA-IV, E, A-I, and C. In contrast to controls, GalN lymph LDL differed by the striking absence of apoB_{335K} and increased levels of apoA-IV and A-I (Fig. 7). The fasting and fat-infused GalN gel patterns did not appear to differ significantly.

Gels of control lymph HDL showed that apoA-I is the major apoprotein band with smaller amounts of apoA-IV, E, C, and B_{240K} (**Fig. 8**). The only observed difference between control fasting and fat-infused lymph HDL appeared to be the slight increase in the apoA-IV band and decrease in the apoC band. GalN lymph HDL gels also had apoA-I as the major apoprotein band and smaller amounts of apoA-IV, E, C, and B_{240K}; however, there was much less apoC present in both the fasting and fat-infused GalN lymph HDL as compared to the corresponding control patterns (Fig. 8). The intensity of the GalN HDL apoE band increased greatly with fat infusion and more closely resembled the HDL particle found in GalN plasma, which has previously been shown to be deficient in apoC and enriched in apoE (7).

TABLE 6. Phospholipid/cholesteryl ester ratios for control and GalN plasma and lymph LDL and HDL[a]

	Fasting Lymph	Fat-infused Lymph	Plasma
LDL			
Control	2.0	6.1	1.7
GalN	2.5	7.9	26
HDL			
Control	3.4	4.9	1.8
GalN	5.0	10	10

[a] Ratios were calculated from phospholipid and cholesterol values from samples pooled from two GalN and two control animals.

DISCUSSION

The present studies show that in rats with GalN-induced hepatitis and mesenteric lymph diversion, changes in plasma lipid levels occur similar to those seen in intact animals. Most notably a marked decrease in plasma cholesteryl esters was observed, presumably secondary to a plasma LCAT deficiency. Morphologic and compositional analysis of the plasma lipoproteins in GalN animals in the present study revealed abnormalities similar to those described previously in intact animals, including abnormal phospholipid-enriched, cholesteryl ester-poor LDL and discoidal HDL.

Intraluminal digestion and absorption of both endogenous and exogenous lipid occur normally in GalN-treated rats, producing mesenteric lymph CM and VLDL of normal lipid composition. VLDL-size particles predominate in fasting lymph in both control and GalN

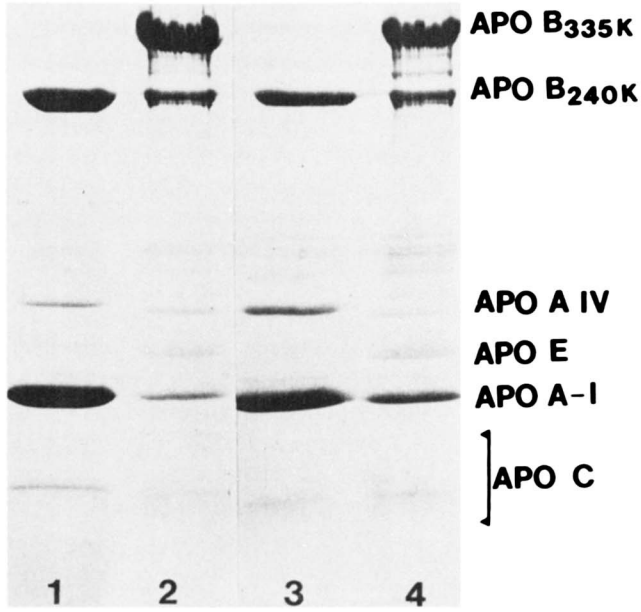

Fig. 7. PAGE gels of lymph LDL apoproteins from pooled samples from control and GalN rats. Otherwise labeled the same as Fig. 2.

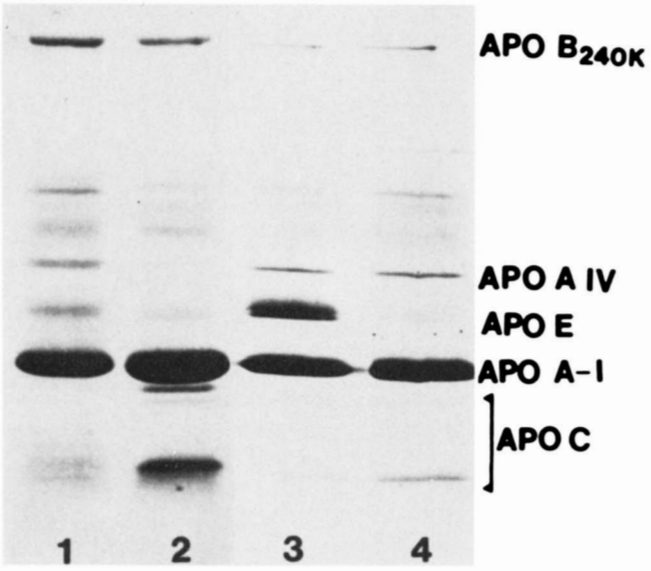

Fig. 8. PAGE gels of lymph HDL apoproteins from pooled samples from control and GalN rats. Otherwise labeled the same as Fig. 2.

animals and, as shown by others, appear to reflect the absorption of endogenous lipid, mainly of biliary origin (47). Since GalN animals produced normal amounts of fasting lymph VLDL, it can be assumed that biliary lipid flow is preserved even in the face of hepatic injury. The enrichment of the d > 1.006 g/ml lipoproteins with free cholesterol in GalN fasting lymph most likely reflects the presence of LDL and HDL enriched with free cholesterol.

With constant intraduodenal infusion of lipid in control and GalN animals there was a shift of the majority of the lymph lipid into triglyceride-rich CM as lymph triglyceride reached a constant output rate by 5–6 hr. Transport rates of the other lymph lipids changed accordingly with lipid infusion except in the case of phospholipid, which achieved a higher output in GalN lymph, due to an enrichment of the fat-infused lymph d > 1.006 g/ml lipoproteins with phospholipid.

The sizes of the d < 1.006 g/ml lymph lipoproteins in GalN-treated animals were normal. With lipid infusion, CM from both groups increased appropriately in size as assessed by the phospholipid to triglyceride ratio as an indirect measure of particle size. Lymph VLDL size, as assessed by direct measurement of electron micrographs, increased slightly in both groups with fat infusion. The most interesting aspect of GalN d < 1.006 g/ml lymph lipoproteins was noted in the PAGE gels of the apoproteins of these particles. The major apoproteins of the d < 1.006 g/ml lipoproteins in control animals were apoA-I, A-IV, B_{240K}, known to be synthesized by the intestine, and minor apoprotein constituents apoE and C, which are mainly of hepatic origin (11, 23, 24, 26–30). Both fasting and fat-infused GalN lymph CM and VLDL were greatly depleted in apoE and C when compared to controls. This depletion in the GalN fasting lymph d < 1.006 g/ml lipoproteins was reflected in the statistically significant decrease in the protein content of these particles as noted in Table 3. It has been shown that significant amounts of apoE dissociate from lipoproteins during ultracentrifugation (52); however, in the present study, control and GalN samples were treated in identical fashion, and CM were isolated with one spin followed by purification using agarose column chromatography to remove albumin and other protein contamination. Therefore, it is doubtful that loss of apoE could account for the differences noted. Both control and GalN lymph CM and VLDL appeared to have a relative increase in apoA-IV after fat infusion. This increase in apoA-IV probably decreases the contribution of apoE and C to the overall apoprotein complement in the fat-infused lymph CM and VLDL, accounting for the loss of statistical significance in the protein content (Table 3). These apoprotein changes in GalN lymph d < 1.006 g/ml lipoproteins are similar to those found by Krause et al. (18) in rats treated with ethinyl estradiol. The hypolipoproteinemia produced by ethinyl estradiol results in decreased filtration into lymph of hepatic lipoproteins, especially HDL, thus decreasing the availability of apoE and C for transfer to newly secreted CM and VLDL. However, after ethinyl estradiol treatment, the output of lymph triglyceride-rich lipoproteins is greatly decreased, suggesting an adverse effect on the intraluminal digestion and/or intestinal absorption and secretion of infused lipid. This was clearly not the case in GalN-treated rats, providing the opportunity to isolate large quantities of nascent-like CM and VLDL while apparently preserving normal intestinal function.

The deficiencies of apoE and C of GalN lymph d < 1.006 g/ml lipoproteins may have important metabolic implications, if these deficiencies persist in the plasma compartment. We have previously documented a profound catabolic defect for CM in fat-fed GalN animals accompanied by a significant decrease in lipoprotein lipase and hepatic lipase activities (10). Although the lipase deficiency probably accounts for the defect, the lack of sufficient amounts of apoC on the CM particle for activation of lipoprotein lipase may also be important (12). Since apoE is important for hepatic recognition of CM remnants prior to uptake, the apoE deficiency in GalN CM may contribute to a remnant uptake defect (13, 14).

LDL and HDL have been isolated from mesenteric lymph by others; however, studies of the incorporation of radiolabeled amino acids into the apoproteins of these particles to determine their site of origin are hampered by the exchange of the labeled apoproteins among dif-

ferent lipoprotein classes (50). Studies of lipoprotein synthesis by the isolated perfused intestine have suggested an intestinal origin for at least some LDL and HDL (19, 20, 23). The size of control lymph LDL in the present study does not differentiate it from plasma LDL, since the sizes are comparable. In the GalN animals, fasting spherical lymph LDL is significantly smaller than spherical plasma LDL, suggesting a different site of origin; however, selective filtration of smaller plasma LDL into the lymph cannot be excluded. With fat infusion, the GalN spherical lymph LDL become larger and are no longer significantly different in size from the spherical plasma particle, suggesting increased filtration of plasma LDL into the lymph with increased lymph flow. In addition to spherical LDL particles, both fasting and fat-infused GalN lymph were noted to contain several large irregularly shaped particles in the LDL density range similar to those found in the plasma (7).

The observed fasting lymph LDL PL/CE ratios for both the control and GalN animals (2.0 and 2.5, respectively) are in very close agreement with the ratio of approximately 2 that can be calculated from normal fasting rat lymph d 1.006–1.063 g/ml lipoprotein compositional data collected by Riley et al. (53). With lipid infusion their ratio increased to approximately 12, which is higher than the fat-infused control and GalN lymph LDL values in the present study. However, their infusate differed from ours including the presence of 1% cholesterol, and our density cut was slightly different from theirs (d 1.006–1.05 g/ml in our study versus d 1.006–1.063 g/ml in theirs). The PL/CE ratio of control fasting lymph LDL in our study did not differ from that of plasma, but with fat infusion this ratio increased to three times that of control plasma LDL, suggesting intestinal production of a different LDL particle. The GalN fasting lymph LDL had a ratio almost the same as the corresponding control value, and the GalN fasting lymph ratio increased to slightly higher than the control value and nearer the GalN plasma LDL value with fat infusion. Thus, increased filtration of GalN plasma LDL into the lymph is suggested. However, since the GalN lymph LDL PL/CE ratio was much lower than the GalN plasma LDL ratio, especially in fasting lymph, a significant amount of LDL is probably gut-derived. The PAGE gels of lymph LDL after GalN do not have $apoB_{335K}$ bands. The absence of $apoB_{335K}$, which is exclusively produced by the liver, has been noted previously by Krishnaiah et al. (16) in thoracic duct lymph LDL isolated from rats treated with orotic acid, whereas the lymph LDL from untreated animals contains both apoB species. Krause et al. (18) also noted the absence of $apoB_{335K}$ in d > 1.006 g/ml lymph lipoproteins from rats treated with ethinyl estradiol (18). Although apoA-I is a minor constituent of plasma LDL from both normal and GalN-treated rats, it is present in lymph LDL from both groups of animals, with much heavier bands noted in the GalN lymph LDL as compared to control lymph LDL, both fasting and fat-infused (7). Riley et al. (53) have noted a prominent apoA-I band in gels of lymph LDL from rats chronically fed cholesterol and from normal rats (53). Intestinal production of LDL is further supported by findings of Riley et al. (53) of a significant incorporation of radiolabeled retinol, a marker for intestine-derived lipoproteins, into lymph LDL in their cholesterol-fed rats. Imaizumi et al. (11) have also detected apoA-I in lymph LDL by radioimmunoassay in glucose- and fat-fed rats. These data taken as a whole suggest that, although filtration of plasma LDL into lymph does occur, at least a portion of the lymph LDL is synthesized by the intestine. However, alteration of lipoprotein composition in lymph after filtration from plasma, although doubtful, cannot be excluded.

Plasma and lymph HDL (d 1.05–1.21 g/ml) from control and GalN rats were characterized in the present study. Control plasma and lymph HDL consist of only spherical particles that do not differ significantly in size. Green et al. (31) have described both spherical and discoidal HDL particles in lymph from normal rats that was collected with an LCAT inhibitor, which was not used in the present studies. Presumably, addition of the LCAT inhibitor prevents the action of LCAT on the nascent HDL particles, thereby preserving the phospholipid-enriched, cholesteryl ester-poor discoidal structure. The control fasting lymph PL/CE ratio in our study is 3.4 compared to the control plasma HDL ratio of 1.8, suggesting the presence of a distinctly different HDL in control fasting lymph. The ratio of 3.4 is somewhat higher than the value of 2.3 determined by Green et al. (31) for fasting lymph HDL to which no LCAT inhibitor was added. In the present study the PL/CE ratio of control fat-infused lymph HDL is 4.9, even higher than the fasting lymph value. This observation supports the concept of intestinal production of a particle even more different from plasma HDL with increased lipid absorption. However, alteration of filtered plasma HDL in lymph is possible. This finding is in disagreement with Green et al. (31) who found a decrease in the PL/CE ratio under these conditions to a value identical to that of plasma. However, their infusate (Intralipid) was different and their HDL density range (d 1.063–1.21 g/ml) differed slightly from ours (d 1.05–1.21 g/ml).

Most interesting in the present study was the finding of discoidal HDL (d 1.05–1.21 g/ml) particles in the lymph from GalN animals. These particles are similar in structure to discoidal HDL isolated from liver perfusions and the plasma of individuals with alcoholic liver

disease or familial LCAT deficiency (9, 54, 55). The fact that the GalN fasting lymph discoidal HDL are larger than the plasma discs strongly suggests that the lymph particle is synthesized by the intestine, since it is doubtful that larger particles would be preferentially filtered from the plasma. However, alteration of filtered HDL in the lymph to produce a larger particle cannot be ruled out. The PL/CE ratio of GalN fasting lymph HDL is 5, or half the fat-infused lymph and plasma ratios which are both 10. This number agrees closely with the ratios calculated from the data of Green et al. (31) (4.5) and Riley et al. (53) (approximately 5) from fasting lymph HDL from normal rats that was collected with an LCAT inhibitor. PAGE gels show that GalN lymph HDL becomes enriched in apoE with lipid absorption to more closely resemble the electrophoretic pattern we have previously reported for GalN plasma HDL (7). These data suggest production by the intestine of an HDL different in composition from the plasma HDL, and with duodenal lipid infusion and increased lymph flow there is increased filtration of the plasma HDL into the intestinal lymph. Similar observations suggesting increased filtration of plasma HDL into lymph with lipid absorption were made by Green et al. (31) in their study of discoidal lymph HDL from normal rats that was collected with an LCAT inhibitor. Bearnot et al. (32) have recently shown that intestinal discoidal HDL and apoA-I production is unaffected by bile diversion in fasting animals, which suggests that discoidal HDL is not derived from triglyceride-rich lipoproteins in lymph (32). Moreover, radiolabeled HDL infused into the plasma of bile-diverted rats did not appear in the lymph (32). These findings further support the intestinal production of discoidal HDL.

It is possible that GalN not only induces a plasma LCAT deficiency but also decreases lymph LCAT activity, which may be derived from plasma. Clark and Norum (56) have found LCAT activity in rat mesenteric lymph to be only 2–3% of plasma activity in fasting rats, but there was an increase in lymph LCAT activity with lipid absorption. Although the lymph LCAT activity was found to be low, the ratio of LCAT to HDL at times exceeded the ratio found in plasma. If lymph LCAT activity is important in the alteration of newly secreted intestinal LDL and HDL, its deficiency in GalN animals may be partly responsible for the abnormal lymph LDL and HDL observed in the present study. Moreover, after undergoing compositional and morphological changes in the plasma compartment, intestinal lipoproteins may be a major source of plasma lipoproteins in GalN animals, since a pronounced lipoprotein secretory defect has been observed in isolated perfused GalN livers.[3]

The apoC deficiency in the d < 1.006 g/ml GalN lymph lipoproteins probably reflects the presence of lymph HDL deficient in apoC which therefore can only transfer limited amounts of apoC to newly secreted lymph CM and VLDL. The minor synthesis of apoC by the intestine, as demonstrated by immunochemical and radiolabeling methods, probably accounts for the presence of small amounts of apoC in the GalN lymph CM and VLDL PAGE gels (23–25, 30). The reason for the deficiency of apoE in GalN lymph CM and VLDL is more complicated. As can be seen, especially with fat infusion, GalN lymph HDL is enriched in apoE, as has previously been shown for GalN plasma HDL (7). ApoE has been shown to transfer rapidly from normal HDL to triglyceride-rich lymph lipoproteins, and this transfer from normal HDL is not dependent upon the presence of LCAT activity (50, 57). Therefore, some factor such as increased affinity of the GalN HDL for apoE may be preventing this transfer. Glomset et al. (58) observed defective transfer of HDL apoE to triglyceride-rich lipoproteins in patients with hereditary LCAT deficiency and abnormal discoidal plasma HDL enriched in apoE. It is known that GalN interferes with hepatic protein glycosylation (1). Therefore, it is possible that glycosylated apoproteins of hepatic origin have abnormal or deficient carbohydrate moieties in GalN animals (59). The apoE in GalN HDL may not transfer to other lipoproteins because of abnormal or deficient glycosylation. Although we cannot state with certainty from the present studies that the apoproteins produced by the intestine and associated with intestinal lipoproteins in GalN animals are normally glycosylated, they probably are not affected since lipid absorption proceeds normally.

The present study represents the first time lymph lipoproteins have been carefully studied in animals with severe hepatitis and LCAT deficiency using a pharmacologic model that apparently has no direct effects on intestinal lipid digestion and absorption. GalN hepatitis provides not only an opportunity to observe changes in lymph lipoproteins in the face of severe liver injury, but also is a useful in vivo model for producing intestinal triglyceride-rich lipoproteins, uncontaminated by hepatic apoproteins, and a discoidal lymph HDL that is probably produced by the intestine.

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