The effect of chronic cholesterol feeding on intestinal lipoproteins in the rat

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Abstract Chronic cholesterol feeding has been shown to produce abnormal plasma lipoproteins in a variety of experimental animals and man. In order to explore the role of the intestine in the production of these abnormal lipoproteins, rats were chronically fed a diet containing 1% cholesterol and 10% olive oil and were compared to control animals, fed either normal chow or normal chow containing 10% olive oil. Mesenteric lymph lipoproteins from fasting lymph and from lymph obtained after acutely infusing cholesterol and olive oil were examined and compared to plasma lipoproteins from these animals. There were no differences in apoA-I output, cholesterol output, or distribution in lymph lipoproteins between the two groups of controls. The cholesterol-olive oil diet produced a mild hyperlipidemia (plasma cholesterol 81 → 95 mg/dl, plasma triglyceride 95 → 162 mg/dl). Plasma lipoprotein electrophoresis revealed an abnormal band with broad β mobility and a reduction in HDL. Lipid analysis of ultracentrifugally separated fractions demonstrated the appearance of an intermediate density (1.006–1.030 g/ml) lipoprotein in plasma markedly enriched in cholesteryl esters. Analysis of fasting mesenteric lymph from chronically cholesterol-fed animals revealed similar apoA-I, cholesterol, and triglyceride outputs when compared to controls. Although in both groups most of the cholesterol was transported in d < 1.006 g/ml lipoproteins, there was a redistribution of cholesterol transport in d > 1.006 g/ml lipoproteins. In the chronically cholesterol-fed animals, 19% of fasting lymph cholesterol was transported in a lipoprotein of density 1.006–1.030 g/ml, compared to 4% in this density in controls. During the acute infusion of cholesterol and olive oil, the output of lymph apoA-I (226 ± 20 versus 374 ± 5 μg/hr, $P < 0.025$) and lymph cholesterol (970 ± 82 ± 1774 μg/hr, $P < 0.01$) was significantly lower in the chronically cholesterol-fed group, despite no significant change in triglyceride outputs (49 ± 2 versus 58 ± 7 mg/hr). Analysis of individual lymph lipoproteins from chronically cholesterol-fed animals revealed that significantly less apoA-I and cholesterol was carried in d < 1.006 g/ml lipoproteins than in controls. There was however, both a relative and absolute increase in the cholesterol and apoA-I content of intermediate and low density lymph subfractions. Particularly prominent in lymph from chronically cholesterol-fed animals was a lipoprotein (d 1.006–1.030 g/ml) which was inconsistently found in controls. This particle was rich in cholesterol and contained apoA-I. [^{3}H]Retinol infusion studies revealed that this particle contained increased retinyl ester when compared to plasma, suggesting an intestinal origin. These results demonstrate that chronic cholesterol feeding in the rat results in altered mesenteric lymph lipoproteins which may contribute to the abnormalities found in plasma.—**Riley, J. W., R. M. Glickman, P. H. R. Green, and A. R. Tall.** The effect of chronic cholesterol feeding on intestinal lipoproteins in the rat. *J. Lipid Res.* 1980. **21:** 942–952.

Supplementary key words lymph · cholesteryl esters · apoA-I · apoB · apoE · retinyl ester

Hypercholesterolemia is well recognized as one factor that is important in the pathogenesis of atherosclerosis in man. Significant hyperlipidemia and accelerated atherosclerosis have been induced experimentally in a wide range of experimental models including the dog (1), miniature swine (2, 3), Patas monkey (4), baboon (5), rabbit (6, 7), and rat (8, 9). The rat is naturally resistant to hypercholesterolemia and atherosclerosis, and marked hypercholesterolemia has only been obtained by further manipulation, e.g., hypothyroidism (8). However, abnormal plasma lipoproteins and modest plasma cholesterol elevations in rats have resulted from feeding a diet of 1% cholesterol and 10% olive oil (9).

Characteristic changes in plasma lipoproteins have been induced by cholesterol feeding in many species. These include an increase in the VLDL fraction that has broad β mobility on lipoprotein electrophoresis and the appearance of an intermediate density lipoprotein (IDL) in the density range 1.006–1.030 g/ml that is rich in cholesterol and apoE and is not present in control plasma (9, 10). This fraction also exhibits

Abbreviations: apoB, apoA-I, etc., apolipoprotein B, apolipoprotein A-I, etc.; VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; TLC, thin-layer chromatography; SDS, sodium dodecyl sulfate.

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β mobility on lipoprotein electrophoresis. In addition, Mahley and Holcombe (8) have characterized HDL_c, a specific lipoprotein in hypercholesterolemic plasma that is rich in apoE, deficient in apoB, and contains a variable amount of apoA-I. The increase in VLDL, IDL, and LDL, as well as the appearance of HDL_c, appear to be important in the transport of the excess dietary cholesterol.

The origin and metabolism of these abnormal particles found in hypercholesterolemic plasma are largely unknown. The work of Ross and Zilversmit (11) has shown the accumulation of triglyceride-rich particles, predominantly of intestinal origin, in plasma of hypercholesterolemic rabbits. There have been no studies, however, which have directly examined the composition of mesenteric lymph lipoproteins under conditions of chronic cholesterol feeding. Therefore, the aim of the present study was to assess the role of the intestine in the pathogenesis of plasma lipoprotein changes induced by chronic cholesterol feeding in the rat by analyzing the composition of mesenteric lymph lipoproteins of animals fed a diet containing 1% cholesterol and 10% olive oil.

The present studies indicate that chronic cholesterol feeding in the rat is associated with the appearance in mesenteric lymph of lipoproteins enriched in cholesterol. In addition, intermediate density particles (d 1.006–1.030 g/ml) were prominent and carried 19% of total lymph cholesterol. These changes demonstrate that chronic cholesterol feeding is associated with changes in mesenteric lymph lipoproteins.

METHODS

Animals and operative technique

Male rats (200–250 g) of the C.D. strain (Charles River Laboratories, Wilmington, MA) were used for all studies. Control animals were fed either commercial rat chow (Ziegler Brothers, Inc., Gardner, PA) or chow to which 10% olive oil had been added. Cholesterol-fed animals were fed standard chow containing 1% cholesterol and 10% olive oil. A solution of olive oil (with or without added cholesterol) was poured over the chow and it was allowed to stand for several days before feeding. The olive oil and cholesterol-fed animals were maintained on the diet for 4–6 weeks. Blood was taken via tail vein puncture under light ether anesthesia before commencing the diet and after 4–6 weeks for measurement of plasma cholesterol, triglyceride, apoA-I, and for lipoprotein electrophoresis. Mesenteric lymph lipoproteins were then analyzed in cholesterol-fed animals and controls. Cannulation of the main mesenteric lymphatic duct and duodenum and subsequent maintenance of the animals have been previously described (12). Animals were operated upon in the early morning, and were allowed to recover from surgery and anesthesia during the day. Fasting lymph was collected from the evening following surgery to the following morning, a period of approximately 16 hr, during which time the animals had free access to a solution containing 0.9% (w/v) saline and 5% (w/v) dextrose. They were lightly restrained in cages during the experiments.

Infusion studies

In order to compare the effects of an acute infusion of cholesterol and olive oil on the composition of mesenteric lymph lipoproteins, control animals and chronic cholesterol-fed animals were infused with a mixture of 40 mM sodium taurocholate, olive oil, and 0.9% saline (in a volume ratio 25:10:65), containing 1 g% cholesterol. This solution was prepared by homogenization in a Willems Polytron (Brinkman Instruments Inc., Westbury, NY). All infusions were delivered intraduodenally by a constant infusion pump at a rate of 1 ml/hr.

Fatty lymph was collected in hourly aliquots for up to 8 hr. Both fasting and fatty lymph were collected on ice and contained 5,5′dithionitrobenzoic acid (DTNB) in 0.7 M phosphate buffer, pH 7.4, in a final concentration of 1 mM, to inhibit the action of the enzyme lecithin:cholesterol acyltransferase (LCAT).

Isolation of lipoproteins

Lymph samples were defibrinated and lipoproteins were separated by sequential ultracentrifugation in a Beckman L5-75 ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA). Chylomicrons were prepared by ultracentrifugation at 3×10^6 *g-min*, using the SW 27 swinging bucket rotor, and were removed from the subnatant by means of a tube slicer. They were then purified by agarose column chromatography as previously described (12). Lymph lipoproteins of d < 1.006 g/ml were isolated by further ultracentrifugation at 1×10^8 *g-min* and were also purified by passage through agarose columns. The density of the subnatant was raised by the addition of solid KBr. The d 1.006–1.030 and d 1.030–1.063 g/ml fractions were isolated by ultracentrifugation for 1×10^8 *g-min* and were washed by an additional centrifugation at the upper density limit. The d 1.063–1.21 g/ml fraction was centrifuged in a 40.3 Ti rotor at 39,000 rpm for 44 hr and was washed by recentrifugation for another 44 hr. The lipoproteins were dialyzed for 24–48 hr at 4°C against deionized water,

pH 8, (buffered with EDTA) and were then lyophilized (Virtis Automatic Freeze Dryer, Gardiner, NY). The dried samples were delipidated by the addition of 5 to 10 ml chloroform–methanol 2:1 and the apoprotein precipitates were washed twice with anhydrous diethyl ether at 4°C. The apoproteins were solubilized in 0.2 M Tris buffer, pH 7.2, containing 0.1 M decyl sulphate, and were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Lipid analysis

Lipoprotein lipids were extracted in chloroform–methanol 2:1 (v/v), according to the method of Folch, Lees, and Sloane Stanley (13). Lipid composition was determined by the method of Downing (14) as modified by Katz, Shipley, and Small (15), as previously described (16). Cholesterol and triglyceride values on whole serum and lymph were determined using the Autoanalyzer technique (Autoanalyzer I, Technicon, Inc., Tarrytown, NY).

Lipoprotein electrophoresis

Lipoprotein electrophoresis of whole plasma and lymph lipoproteins was performed on 1% agarose (Seakem Bio. Products, Rockland, MA) containing 12% bovine serum albumin (17), in a counter immunoelectrophoresis apparatus (M.R.A., Corp., Boston, MA). A current of 30 mA was applied with a dye marker in BSA and electrophoresis was stopped when the dye had progressed two thirds of the plate. Samples were fixed in 5% acetic acid, washed with water, dried, and stained for lipid with Oil Red O.

Apoprotein analysis

Apoproteins were analyzed by SDS-polyacrylamide gel electrophoresis in 5.6% polyacrylamide gels and stained for protein with Coomassie blue. The protein bands were identified by comparison of their R_f values with those of purified rat apoA-IV, apoE, and apoA-I. Apoproteins were quantitated by densitometric scanning of stained gels at 550 nM with a Gilford linear gel scanner (Gilford Instrument Laboratories Inc., Oberlin, OH). When varying amounts of apoproteins were applied to a series of gels, densitometry showed that over the range of applied protein (5–15 μg) each band retained its relationship (percent of total), indicating the linearity of the densitometric methods employed (12). Protein concentration was determined using the method of Lowry et al. (18).

Immunological studies

ApoA-I was purified by preparative electrophoresis of rat serum apoHDL using a Polyprep 100 (Buchler Instruments, Fort Lee, NJ). Twelve rats were bled from the aorta under light ether anesthesia and the plasma was pooled. HDL was prepared by ultracentrifugation (as above) at density 1.21 g/ml, solid KBr being used for raising the density. It was removed by aspiration, dialyzed exhaustively for 48 hr at 4°C against 0.9% saline containing 0.04% EDTA, pH 7.4. It was then delipidated using ethanol–ether 3:2 (v/v) (19) and the apoprotein precipitate was washed twice with anhydrous ether, dried under nitrogen, and redissolved in 0.2 M Tris buffer, pH 7.4, containing 0.1 M decyl sulfate. The electrophoresis gel contained 5.6% polyacrylamide in 20% SDS and the running buffer also contained 20% SDS. Electrophoresis was continued for 14–18 hr and the fractions were collected at 10°C in a Fractomette 200 (Buchler Instruments, Fort Lee, NJ). Protein peaks in the elution fractions were monitored using ultraviolet densitometry in a Gilford 250 Spectrophotometer (Gilford Instruments, Oberlin, OH). This technique revealed complete separation of apoC, apoA-I, apoE, and apoA-IV.

Antiserum to apoA-I prepared in this way was made by injecting small amounts (15–20 μg) intramuscularly into New Zealand white male rabbits as previously described (20), and the resulting antisera were characterized by immunodiffusion and immunoelectophoresis in agarose by standard methods.

Quantitative electrophoresis of apoA-I

Quantitation of apoA-I was performed by the rocket immunoelectrophoresis technique of Laurell (21) as previously described (20). The value for male rat plasma apoA-I in this assay was 42.9 ± 1.6 mg/dl (n = 12 animals). The intraassay and interassay coefficients of variation were 2.0% and 3.8%, respectively.

[^{3}H]Leucine incorporation studies

The incorporation of [^{3}H]leucine into lymph chylomicron apoproteins in chronic cholesterol-fed rats was studied. Twelve hours after cannulation of the main mesenteric lymph duct, a constant intraduodenal infusion of the cholesterol-olive oil mixture was commenced. After 1 hr, a single dose of 100 μCi of [^{3}H]leucine was given intraduodenally. Lymph was collected for 2 hr and the chylomicrons were purified and delipidated, and the apoproteins were subjected to electrophoresis on SDS-polyacrylamide gels. Radioactivity incorporated into individual proteins on polyacrylamide gels was determined by cutting the gels in 1mm-slices with a lateral gel slicer, dissolving them in 10 ml Aquasure (New England Nuclear, Boston, MA), and counting them in a Beck-

man liquid scintillation counter (L5-350, Beckman Instruments, Fullerton, CA). Individual slices were related to specific protein bands by comparison with duplicate gels that were stained and scanned densitometrically.

[^{3}H]Retinol transport studies

Esterified retinol has been shown to be a specific marker for triglyceride-rich lipoproteins of intestinal origin (22, 23). To determine whether the lymph lipoprotein particles of density 1.006–1.030 g/ml were of intestinal origin, [^{3}H]retinol was infused intraduodenally during cholesterol-olive oil infusion into chronic cholesterol-fed animals. The lipoproteins of d < 1.006 and d 1.006–1.030 g/ml were isolated from lymph by ultracentrifugation and dialyzed against 0.9% saline at 4°C. To determine whether the serum d 1.006–1.030 g/ml fraction contained esterified retinol, one animal was killed 2 hr after [^{3}H]retinol, bled from the aorta under light ether anesthesia, and the serum lipoproteins were isolated in parallel with the lymph fractions from the same animal. Separation of retinol and retinyl ester was performed by column chromatography on alumina (24). The two fractions were evaporated to dryness under nitrogen at 50°C, taken up in 10 ml of Aquasure, and counted in a Beckman liquid scintillation counter.

RESULTS

Changes in plasma lipoproteins induced by cholesterol feeding

In order to document that the 1% cholesterol—10% olive oil diet induced changes in plasma lipoproteins as have been previously described (8, 9) we examined plasma lipids and lipoproteins in the chronic cholesterol-fed animals. After 4–6 weeks, the cholesterol-olive oil diet produced a mild hyperlipidemia with an increase in plasma cholesterol from 81 ± 3 mg/dl to 95 ± 7 mg/dl which was not statistically significant ($P = 0.1$, n = 8). Plasma triglycerides increased from 95 ± 15 mg/dl to 162 ± 12 mg/dl ($P < 0.01$, n = 8). The olive oil diet resulted in no change in plasma cholesterol (86 ± 5 mg/dl) and an increase in plasma triglyceride to 176 ± 17.3 mg/dl, ($P < 0.05$, n = 3 animals). **Fig. 1,** panels *1*) and *2*), shows a representative lipoprotein electrophoresis of whole serum from a control chow-fed rat and a cholesterol-olive oil-fed animal. A lipoprotein band with broad β mobility and a reduction in the α migrating or HDL band can be seen in the serum of the cholesterol-fed animal. These changes are similar to those previously described in cholesterol-fed rats (10). No changes were produced by chronic olive oil feeding.

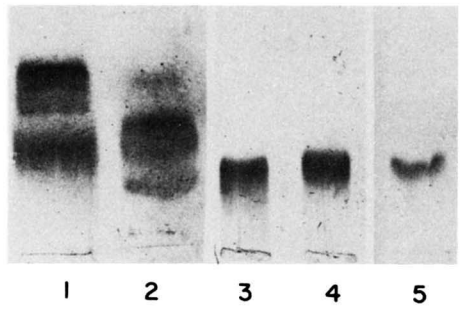

Fig. 1. Agarose gel lipoprotein electrophoresis of whole plasma from a control *1*) and chronically cholesterol-fed rat *2*), demonstrating an abnormal band with broad β migration and reduction in the α (HDL) band, in the plasma from the chronically cholesterol-fed animal. Lanes *3*), *4*), and *5*) are lymph lipoprotein subfractions from a chronically cholesterol-fed animal demonstrating an abnormal band with slightly slower migration; *3*) d < 1.006, *4*) d 1.006–1.030, and *5*) d 1.030–1.063 g/ml.

Plasma apoproteins

Plasma levels of apoA-I were measured in twelve rats by quantitative immunoelectrophoresis before commencement of the diet and after 4 weeks of cholesterol feeding. There was no significant change in apoA-I levels (43 ± 1.6 mg/dl versus 38 ± 1.7 mg/dl, $P = 0.1$). **Table 1** shows the lipid composition of individual lipoproteins from control (chow-fed) and cholesterol-fed animals as determined by thin-layer chromatography. The cholesteryl ester content of VLDL and IDL was increased and the cholesteryl ester content of LDL was decreased in plasma from cholesterol-fed animals. The cholesterol distribution in the plasma of cholesterol-fed animals (not shown in Table 1) reflects the increased cholesteryl ester content of the VLDL and IDL fractions. In cholesterol-fed animals, 33 ± 3% of total cholesterol was carried by VLDL, 26 ± 5% by IDL, 11 ± 3% by LDL, and 30 ± 6% by HDL, the values for controls being 15%, 6%, 25%, and 55%, respectively.

Lipoprotein electrophoresis

Panels *3*), *4*), and *5*) of Fig. 1 show the migration on agarose electrophoresis of lymph lipoprotein subfractions from a chronically cholesterol-fed animal. The d < 1.006 g/ml fraction (Fig. 1, panel *3*) has a band with mobility slower than the broad β lipoprotein demonstrated in the plasma of the cholesterol-fed animals. A similarly migrating band was seen in the lymph d 1.006–1.030 g/ml fraction (Fig. 1, panel *4*). In lymph from both sets of animals, (chow or olive oil) a faintly staining lipoprotein with similar migration was inconsistently seen at this density (not shown). The

TABLE 1. Lipid distribution in serum lipoproteins from control and chronic cholesterol-olive oil-fed animals

Density (g/ml)	Lipid Composition[a]			
	Cholesterol	Cholesteryl Ester	Triglyceride	Phospholipid
d < 1.006 (VLDL)				
Control	4 ± 0.4	4 ± 0.9	76 ± 5.8	15 ± 3.5
Chol-fed	3 ± 0.5	8 ± 1.9	75 ± 1.6	13 ± 1.9
P[b]	NS	<0.05		
d 1.006–1.030 (IDL)				
Control				
Chol-fed	9 ± 0.8	26 ± 8.9	44 ± 10.4	18 ± 1.2
d 1.030–1.063 (LDL)				
Control	4 ± 0.3	43 ± 2.4	22 ± 0.9	30 ± 2.6
Chol-fed	10 ± 1.3	36 ± 2.9	27 ± 6.9	32 ± 3.9
P	<0.05	<0.05		
d 1.063–1.21 (HDL)				
Control	9 ± 0.8	39 ± 3.8	5 ± 1.2	49 ± 3.8
Chol-fed	8 ± 0.7	41 ± 0.8	3 ± 0.1	46 ± 2.7
P	NS	NS		

[a] Percentage of composition estimated by quantitative TLC. Values given are mean ± SEM.
[b] Difference by chi-square test; NS, not significant.
There were four animals in each group.

lymph d 1.030–1.063 g/ml fraction (Fig. 1, panel *5*), from cholesterol-fed animals exhibited β mobility. There was little staining in the HDL density range (not shown).

Output and distribution of cholesterol in lymph

The output of cholesterol was determined in both sets of controls and chronically cholesterol-fed animals in fasting lymph and during the acute infusion of the cholesterol-olive oil mixture. As shown in **Table 2** the cholesterol output was similar in fasting lymph from control, (541 ± 62, 421 ± 26 μg/hr) and chronic cholesterol-fed animals (492 ± 34 μg/hr). However, during the infusion of cholesterol and olive oil, control animals put out significantly more cholesterol than those chronically fed cholesterol (1774 ± 2.5, 1661 ± 62 versus 970 ± 82 μg/hr, $P < 0.01$).

TABLE 2. Output of cholesterol (μg/h) in mesenteric lymph in control and chronically cholesterol-fed animals

	Controls		Chronic Chol-fed
	Chow-fed	10% Olive Oil	
Fasting lymph	541 ± 62 (n = 8)	421 ± 26 (n = 3)	492 ± 34 (n = 8)
Fatty lymph	1714 ± 215 (n = 3)	1661 ± 62 (n = 3)	970 ± 82[a] (n = 3)
d < 1.006	1681 ± 203	1460 ± 216	677 ± 61[b]
d 1.006–1.030	72 ± 9	67 ± 18	147 ± 48[b]
d 1.030–1.063	32 ± 4	52 ± 7	73 ± 24[b]
d 1.063–1.21	31 ± 10	64 ± 13	67 ± 41

[a] $P < 0.01$ compared to both sets of control animals.
[b] $P < 0.05$ compared to both sets of control animals.
Cholesterol was determined in mesenteric lymph and lipoprotein subfractions in fasting lymph and after the acute infusion of cholesterol-olive oil (fatty lymph) to both sets of controls and chronically cholesterol-olive oil-fed rats. Values for fatty lymph are the means of eight hourly collections.

Having established the cholesterol outputs in whole lymph, we next wished to characterize the distribution of cholesterol within the lipoprotein fractions, with special attention to the intermediate density (d 1.006–1.030 g/ml) range. **Fig. 2** shows the cholesterol distribution within the lipoprotein subfractions of fasting and fatty lymph expressed as a percentage of total lymph cholesterol. Since the percentage distribution was similar for both control groups (Table 2), only chow fed controls are shown in this figure. In fasting lymph (Fig. 2a) most of the cholesterol was present in d < 1.006 g/ml lipoproteins in both groups. However, in the chronically cholesterol-fed animals, the d 1.006–1.030 g/ml fraction carried significantly more cholesterol (10% versus 4%) whereas the cholesterol carried in the d 1.030–1.063 g/ml fraction was reduced (6% versus 14%). Since the hourly transport of cholesterol in fasting lymph was similar in both groups, the intermediate density fractions quantitatively carried more cholesterol than in controls.

With the acute infusion of cholesterol and olive oil to both groups, there was further cholesterol redistribution (Fig. 2b). Significantly less cholesterol was transported in d < 1.006 g/ml lipoproteins in the chronically cholesterol-fed animals compared to controls at this density. However, in the intermediate and low

density subfractions, significantly more cholesterol was transported in the chronically cholesterol-fed animals compared to controls. It can be seen from Table 2 that cholesterol distribution in the fatty lymph was different in absolute as well as relative amounts. These results show that, although chylomicrons and VLDL remained the main transport form for cholesterol in both groups, with chronic cholesterol feeding there was a redistribution of cholesterol transport that was associated with lipoproteins in the intermediate density fractions of mesenteric lymph.

Lipid composition of mesenteric lymph lipoproteins

In view of the altered lymph cholesterol distribution in chronically cholesterol-fed animals, the lipid composition of each lipoprotein fraction was determined in fasting lymph (Table 3) and after fat feeding (Table 4). As shown in **Table 3,** no difference in lipid composition was seen in the d < 1.006 g/ml lipoproteins prepared from fasting lymph of chronically cholesterol-fed animals when compared to chow-fed controls. In the d > 1.006 g/ml lipoproteins from fasting lymph, there was an increase in free cholesterol content in the chronically cholesterol-fed animals. In addition, the esterified cholesterol content of the 1.006–1.030 and 1.030–1.063 g/ml fractions in the cholesterol-fed animals was significantly elevated.

The lipid composition of lymph lipoproteins in chow-fed control and chronically cholesterol-fed animals after the infusion of cholesterol and olive oil is shown in **Table 4.** The relative lipid composition of the d < 1.006 g/ml lipoproteins (chylomicrons and VLDL) was comparable. In contrast, chronically cholesterol-fed animals infused acutely with cholesterol-olive oil showed a marked increase in the cholesteryl ester content of the 1.006–1.030 and 1.030–1.063 g/ml fractions compared to control animals. The HDL fraction of the chronically cholesterol-fed animals was also enriched in cholesterol. These data are in accord with

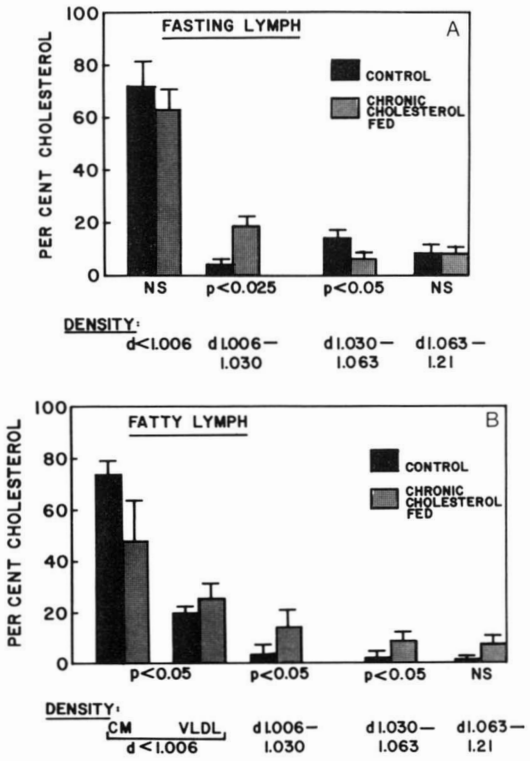

Fig. 2. Distribution of cholesterol in fasting A) and fatty B) lymph lipoproteins from chow-fed controls and chronically cholestrol-fed animals. Lipoproteins were isolated from lymph as described in Methods. Percentage cholesterol was calculated by the lipoprotein cholesterol distribution (TLC) expressed as a percentage of total lymph cholesterol (Autoanalyser technique).

TABLE 3. Lipid composition[a] of fasting lymph lipoproteins

Density	Cholesterol	Chol Ester	Triglyceride	Fatty Acid	Phospholipid
d < 1.006					
Control (n = 3)	3 ± 0.4	6 ± 0.7	72 ± 3.5	5 ± 1.3	21 ± 1.8
Chol-fed (n = 6)	4 ± 0.8	9 ± 3.6	68 ± 3.1	2 ± 0.3	18 ± 0.8
P[b]	NS	NS			
d 1.006–1.030					
Control	4 ± 0.4	12 ± 1.2	54 ± 5.8		32 ± 0.9
Chol-fed	6 ± 0.4	23 ± 4.0	45 ± 4.4	3 ± 0.9	23 ± 0.9
P	<0.005	<0.005			
d 1.030–1.063					
Control	6 ± 1.5	17 ± 1.3	25 ± 1.2	6 ± 1.7	28 ± 2.5
Chol-fed	10 ± 0.9	36 ± 4.6	21 ± 4.9	5 ± 1.8	30 ± 1.9
P	<0.01	<0.025			
d 1.063–1.21					
Control	5.8 ± 1.4	14 ± 1.7	7 ± 1.1	4 ± 1.2	72 ± 4.3
Chol-fed	11.1 ± 1.4	18 ± 1.9	4 ± 1.9	2 ± 0.3	68 ± 3.7
P	<0.025	NS			

[a] Percentage of composition determined by quantitative TLC. Values given are mean ± SEM.
[b] Difference by chi-square test; NS, not significant.

TABLE 4. Lipid composition[a] of lymph lipoproteins after infusion of 1% cholesterol and 10% olive oil

Density	Cholesterol	Chol. Ester	Triglyceride	Fatty Acid	Phospholipid
Chylomicrons					
Control (n = 3)	2 ± 0.11	3 ± 0.3	86 ± 1.0	1 ± 0.3	7 ± 0.1
Chol-fed (n = 4)	1 ± 0.4	2 ± 0.7	88 ± 1.9	2 ± 0.3	9 ± 1.4
P[b]	NS	NS			
d 1.006					
Control	2 ± 0.3	5 ± 0.1	69 ± 3.9	2 ± 0.1	22 ± 2.6
Chol-fed	4 ± 0.8	6 ± 1.0	71 ± 2.9	2 ± 0.6	18 ± 0.9
P	NS	NS			
d 1.006–1.030					
Control	6 ± 1.9	5 ± 0.8	53 ± 5.7	8 ± 0.8	25 ± 1.8
Chol-fed	5 ± 0.6	24 ± 6.6	36 ± 9.4	2 ± 0.8	35 ± 4.6
P	NS	<0.05			
d 1.030–1.063					
Control	8 ± 1.9	9 ± 2.6	19 ± 9.4	6 ± 1.2	61 ± 2.7
Chol-fed	9 ± 1.5	42 ± 8.3	9 ± 1.9	2 ± 0.2	41 ± 7.4
P	NS	<0.005			
d 1.063–1.21					
Control	8 ± 0.7	10 ± 3.4	2 ± 0.0	6 ± 1.1	76 ± 3.2
Chol-fed	13 ± 1.8	15 ± 1.8	2 ± 0.2	4 ± 0.2	66 ± 3.3
P	<0.025	NS			

[a] Percentage of composition determined by quantitative TLC. Values given are mean ± SEM.
[b] Difference by chi-square test; NS, not significant.

the distribution data (Fig. 2b, Table 2) which demonstrated that the more dense fractions transported significantly more total cholesterol in fatty lymph, in chronically cholesterol-fed animals.

ApoA-I/apoE ratios of mesenteric lymph lipoproteins

As shown in **Fig. 3,** there was a marked difference in the apoprotein composition of the 1.006–1.030 g/ml fraction in mesenteric lymph compared to the same fraction isolated from the plasma of a chronic cholesterol-fed animal. In lymph this lipoprotein subfraction was apoA-I-rich (**Table 5**) compared to plasma where apoE was the major apoprotein. In plasma from cholesterol-fed animals, the apo AI/apoE ratio was <1 in all lipoprotein subfractions except HDL. This is consistent with previous studies which have shown an increase in apoE in plasma lipoproteins of chronic cholesterol-fed animals (10).

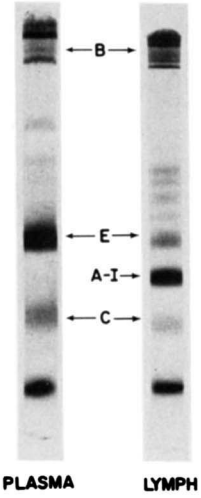

Fig. 3. SDS-Polyacrylamide gels of the d 1.006–1.030 g/ml lipoproteins from plasma and lymph of a chronically cholesterol-fed animal. Major apoprotein bands are labeled. The ink marker indicating the electrophoresis front is seen at the bottom of the gel.

Isotopic-labeling studies

Fig. 4 shows the [^{3}H]leucine incorporation into major apoproteins of lymph chylomicrons in a chronic cholesterol-fed animal 1 and 2 hr after [^{3}H]leucine. Radioactivity was found in the major apoproteins with R_f values 0.1, 0.53, and 0.69, corresponding to apoB, apoA-IV, and apoA-I, respectively. There was no labeling of apoE, indicating that this apoprotein was not actively synthesized by the intestine of chronically cholesterol-fed rats in response to acute cholesterol olive oil infusion.

[^{3}H]Retinol incorporation studies

To determine whether the d 1.006–1.030 g/ml fractions from lymph of chronic cholesterol-fed animals was secreted by the intestine or filtered from plasma, [^{3}H]retinol, mixed with the cholesterol-olive oil emulsion, was acutely infused and lymph was collected in three chronically cholesterol-fed animals. The d < 1.006 g/ml fraction of the lymph was re-

TABLE 5. ApoA-I/Apo E ratio[a] in lipoproteins from control and chronically cholesterol-fed animals[b]

Density (g/ml)	Control Mesenteric Lymph	Chronic Chol-fed	
		Mesenteric Lymph	Plasma
d < 1.006	5.9 ± 1.6	5.0 ± 1.1	0.14 ± 0.05[c]
d 1.006–1.030	1.0 ± 0.3	1.9 ± 0.6	0.26 ± 0.04[c]
d 1.030–1.063	0.8 ± 0.2	0.85 ± 0.12	0.44 ± 0.12[c]
d 1.063–1.21	1.8 ± 1.1	2.5 ± 0.8	3.9 ± 1.2

[a] Determined by scanning SDS-polyacrylamide gels (see Methods).
[b] Four animals in each group.
[c] $P < 0.05$ by chi-square test compared to mesenteric lymph from chronically cholesterol-fed animals. Control animals were chow-fed.

moved by ultracentrifugation and 85% of the recovered radioactivity was found in this fraction. The d > 1.006 g/ml bottom, containing 15% of the total counts, was then centrifuged at d 1.030 g/ml and 50% of the counts originally in the d > 1.006 g/ml bottom were found in the d 1.006–1.030 g/ml fraction. After separation of retinyl ester and retinol from the d 1.006–1.030 g/ml fraction by alumina column chromatography, 90% of these counts were found in the esterified retinol fraction. In one animal where lymph and plasma lipoproteins were isolated in parallel, the lymph d 1.006–1.030 g/ml retinyl ester fraction contained more than ten times the radioactivity compared to the similar plasma fraction. This suggests that the d 1.006–1.030 g/ml particle isolated in lymph from the cholesterol-fed animals is secreted by the intestine into mesenteric lymph and not filtered from the plasma.

Output of ApoA-I and triglyceride in mesenteric lymph

Table 6 shows the apoA-I and triglyceride output of fasting and fatty lymph in controls and chronically cholesterol-fed animals. In fasting lymph there was no difference in all groups in either apoA-I or triglyceride output. However, after the infusion of cholesterol and olive oil, there was a negligible increase in apoA-I output in the chronically cholesterol fed animals (210 ± 26 → 226 ± 20 μg/hr) despite an increase in triglyceride output. Control animals, acutely infused with cholesterol and olive oil, had a significant increase in apoA-I as shown. In the chronically cholesterol-fed animals, there was a significant reduction in apoA-I output in d < 1.006 g/ml lipoproteins compared to controls and this was also associated with a reduction in cholesterol output in this density range (Table 2). These findings suggest that chronic cholesterol feeding is associated with an alteration in the formation of d < 1.006 g/ml lipoproteins in response to an acute cholesterol load.

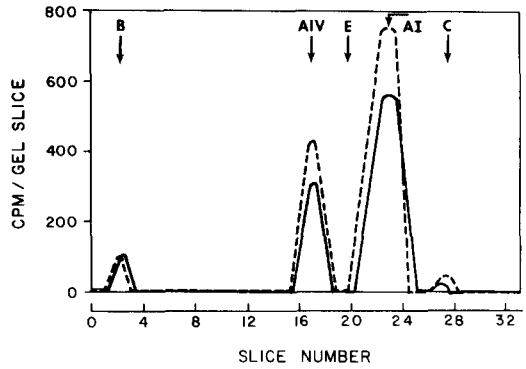

Fig. 4. Time course of [^{3}H]leucine incorporation into lymph chylomicron apoproteins in a chronically cholesterol-fed animal after a constant intraduodenal infusion of the cholesterol-olive oil emulsion had been established. Chylomicrons were collected 1 h (solid line) and 2 h (broken line) after administration of radioactive tracer. Radioactivity was present in gel slices corresponding to apoB, apoA-IV, apoA-I, and apoC, but not in apoE.

DISCUSSION

The present studies confirm that feeding a 1% cholesterol, 10% olive oil diet to rats results in abnormalities in plasma lipoproteins similar to those reported with more extensive diets (8) and avoided other variables such as hypothyroidism or bile salt feeding. Olive oil was added to the diet to enhance cholesterol absorption. Control animals chronically fed 10% olive oil had similar apoA-I, cholesterol, and triglyceride outputs in mesenteric lymph, and similar apoA-I and cholesterol distribution in lipoprotein subfractions, compared to the chow-fed controls. The present

TABLE 6. Output of ApoA-I (μg/h) and triglyceride (mg/h) in mesenteric lymph of control and chronically cholesterol-fed animals

	Controls		
	Chow-fed (n = 3)	10% Olive Oil (n = 3)	Chronic Chol-fed (n = 3)
Fasting lymph			
Triglyceride	6 ± 1	6 ± 2	5.5 ± 2
ApoA-I	213 ± 29	186 ± 13	210 ± 26
Fatty lymph			
Triglyceride	58 ± 7	61 ± 9	49 ± 2
ApoA-I	374 ± 5	361 ± 15	226 ± 20[a]
d < 1.006	192 ± 3	172 ± 6	111 ± 10[a]
d 1.006–1.030	26 ± 2	28 ± 7	38 ± 4[a]
d 1.030–1.063	30 ± 3	34 ± 2	27 ± 1
d 1.063–1.21	112 ± 7	110 ± 16	65 ± 16[a]

[a] $P < 0.05$ compared to both sets of control animals.
Triglyceride and apoA-I were determined in mesenteric lymph and lipoprotein subfractions in fasting lymph and after the infusion of cholesterol-olive oil to both sets of control and chronically cholesterol-fed animals.

studies confirm that chronic cholesterol feeding results in alterations of plasma lipoproteins that consist of a modification of the VLDL and IDL fractions of plasma and a reduction in plasma HDL. Both qualitatively and quantitatively, the VLDL and IDL fractions of plasma from chronically cholesterol-fed rats were enriched in cholesteryl ester and carried a larger proportion of serum cholesterol than in control chow fed animals. Accompanying these changes was a reduction in plasma HDL. There was no significant change in plasma apoA-I in the animals fed cholesterol, and [^{3}H]leucine incorporation into chylomicron apoproteins failed to demonstrate any labeling of apoE to suggest intestinal synthesis of this apoprotein after chronic cholesterol feeding (Fig. 4).

A major objective of the present study was to examine whether cholesterol feeding was associated with alterations in mesenteric lymph lipoproteins. The present studies provide evidence that this is the case. In fasting lymph, as well as after the acute infusion of cholesterol and olive oil, chronically cholesterol-fed animals showed an alteration in the distribution of lymph cholesterol when compared with control animals. In fasting lymph from chronically cholesterol-fed animals, all lymph lipoprotein fractions, with the exception of the d < 1.006 g/ml fraction, contained increased free cholesterol (Table 3), while after acute cholesterol-olive oil infusion, these fractions contained more cholesteryl ester (Table 4).

In fasting lymph, as well as after acute cholesterol-olive oil feeding, intermediate density fractions of lymph, which in control animals were inconsistently present, carried significantly more cholesterol in the chronically cholesterol-fed rat. Particularly prominent in lymph was the density fraction 1.006–1.030 g/ml. This lymph lipoprotein was a triglyceride- and cholesteryl ester-rich particle which contained apoB and apoA-I as major apoproteins. Although its lipid composition was similar to plasma particles in this density range, its apoproteins suggested that it might be of intestinal origin. The finding that fed-[^{3}H]retinol accumulated in this lymph particle as retinyl ester strongly suggests an intestinal origin for this particle, rather than filtration from plasma into mesenteric lymph.

While the studies of Ross and Zilversmit (11) clearly show an altered catabolism of intestinal triglyceride-rich lipoproteins with a resultant accumulation of remnant particles in plasma, the present studies also indicate that such particles may originate directly from the intestinal mucosa. While we have not directly examined the catabolism of these intermediate density lymph particles, it is probable that their catabolism is also impaired and that they contribute to the altered plasma lipoproteins observed in chronic cholesterol-fed animals. It is of interest that a recent report describes secretion of such particles from perfused livers of chronically cholesterol-fed animals as well (25).

It is of interest to compare the effect of acute cholesterol-olive oil infusion in control rats versus chronically cholesterol-fed animals. Previous studies have shown that acute cholesterol infusion in control animals results in an increase in the cholesterol content (mainly as ester) in lymph d < 1.006 g/ml lipoproteins (26). Whereas the cholesterol content of lymph chylomicrons is normally about 1%, cholesterol infusion can increase this to 6%, a value found in the present study as well. There have been no previous studies however, on the cholesterol content of other lymph lipoproteins, comparing acute cholesterol feeding to control or chronically cholesterol-fed rats. Our results show marked differences between the response of control animals when compared to chronically cholesterol-fed rats, when both groups are infused acutely with cholesterol-olive oil infusions.

While all groups elevated the lymph triglyceride to similar levels with lipid feeding (Table 6), there was a marked reduction in hourly lymph cholesterol (Table 2) and apoA-I secretion (Table 6) in chronically cholesterol-fed animals compared to both set of controls. This decrease in cholesterol and apoA-I secretion was not evenly distributed throughout the lipoprotein density classes of mesenteric lymph. Rather there was a marked reduction in the cholesterol and apoA-I content of the d < 1.006 g/ml lymph lipoproteins, with a relative as well as an absolute increase of these components in the intermediate density fractions of lymph. The interpretation of these findings is unclear. It might be argued that, faced with the requirement to transport a large amount of exogenous cholesterol, the capacity of the preferential transport vehicle, namely the d < 1.006 g/ml lymph lipoproteins, may be exceeded with additional cholesterol therefore transported in more dense lymph lipoprotein fractions. Several pieces of evidence argue against this hypothesis. Control animals acutely infused with cholesterol predominantly carry this cholesterol in d < 1.006 g/ml lymph lipoproteins (Table 2). In chronically cholesterol-fed animals, the cholesterol carried in this lipoprotein fraction is markedly reduced. Furthermore, basal lymph cholesterol values from both groups of animals were similar (Table 2) yet chronically cholesterol-fed animals also had reduced d < 1.006 g/ml cholesterol, with relative and absolute increases in the cholesterol content of other lymph lipoproteins. These findings suggest rather that there is some impairment in the capacity of the d < 1.006 g/ml lipoproteins to carry dietary cholesterol in the chronically cholesterol-fed rat. There are preliminary

data from primate studies which suggest that the intestinal response to chronic cholesterol feeding may involve a reduction in endogenous cholesterol transport when increased amounts of dietary cholesterol are absorbed (27). While we did not carry out similar studies it would seem that, if such processes occurred in the cholesterol-fed rat, they could not immediately explain the prominence of intermediate density lymph fractions in cholesterol transport. Studies of chylomicron formation within the intestinal mucosa will be required to determine whether chronic cholesterol feeding affects this process.

Finally, it is possible that the changes observed in mesenteric lymph lipoproteins after chronic cholesterol feeding may have important consequences on systemic lipoprotein metabolism. It has recently been shown that catabolism of triglyceride-rich lipoproteins contributes importantly to circulating high density lipoproteins (16, 28). In man as well as the rat, the intestine is a major source of apoA-I for circulating HDL, which it provides through catabolism of intestinal d < 1.006 g/ml lipoproteins. The findings of decreased apoA-I secretion in the lymph d < 1.006 g/ml lipoproteins, as well as a marked decrease in total lymph apoA-I secretion in the chronically cholesterol-fed rat, may in part be responsible for the decreased HDL levels observed in the chronically cholesterol-fed rat. Furthermore, studies in the rat suggest that a portion of mesenteric lymph apoA-I is carried in the HDL fraction of lymph (29). In the chronically cholesterol-fed rat, this fraction is enriched in cholesterol and carries less apoA-I than in control rats (Table 6). While the importance of these changes is unknown, it is possible that they are also contributory factors to the observed changes in systemic HDL.

In summary, the present studies have shown that chronic cholesterol feeding in the rat results in significant changes in apoprotein and lipoprotein secretion by the intestine. The effects of chronic cholesterol feeding in the intestinal mucosa and the quantitative importance of these changes to systemic lipoprotein metabolism remain to be determined.

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