Mesenteric lymph apolipoproteins in control and ethinyl estradiol-treated rats: a model for studying apolipoproteins of intestinal origin

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Abstract Rat mesenteric lymph contains all serum apolipoproteins. However, it is uncertain whether some of these apolipoproteins are derived from intestinal synthesis or are transferred from plasma. We compared lymph apolipoprotein composition, concentrations, and transport rates in normal rats and in rats treated with pharmacologic doses of ethinyl estradiol which have negligible concentrations of serum lipids and apolipoproteins. Lymph apolipoproteins were examined before and after duodenal lipid infusion. Lymph d < 1.006 and 1.006–1.21 g/ml lipoproteins were isolated and SDS-electrophoresis was performed using 10 and 3.5% polyacrylamide. During lipid absorption, lymph flow increased in control but not in treated rats. Control lymph contained all major apolipoproteins, but lymph from ethinyl estradiol-treated rats contained only apoB, A-I, and A-IV. Two apoB bands were noted on 3.5% gels in control lymph, but only the lower molecular weight protein was found in lymph from ethinyl estradiol-treated rats. In control rats, transport rates for apoA-I, A-IV, E, and C proteins increased during lipid absorption, but only in the case of A-IV was this a reflection of increased apolipoprotein concentration and not the enhanced lymph flow. In ethinyl estradiol-treated rats only the A-IV transport rate increased due to lipid infusion. It is concluded that in the ethinyl estradiol-treated rat *1*) the intestine does not synthesize apoE, C, or the high molecular weight apoB; *2*) lymphatic output of A-IV is predominantly increased during lipid absorption; and *3*) since plasma apolipoprotein concentrations are negligible, lymph lipoproteins from ethinyl estradiol-treated rats may represent a close approximation to nascent particles of intestinal origin.—**Krause, B. R., C. H. Sloop, C. K. Castle, and P. S. Roheim.** Mesenteric lymph apolipoproteins in control and ethinyl estradiol-treated rats: a model for studying apoliproteins of intestinal origin. *J. Lipid Res.* 1981. **22:** 610–619.

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Studies of mesenteric (intestinal) lymph have contributed to our overall understanding of lipoprotein metabolism. Beginning with the work of Borgström and Laurell (1) and Laurell (2), it has been known that intestinal lymph contains all of the lipoproteins found in plasma. But the origin of these lipoproteins, and in particular the apolipoproteins, remains an active field of investigation. It has been established by using mesenteric lymph-cannulated rats that the intestine can synthesize VLDL and contribute VLDL-triglyceride to the plasma pool via the mesenteric lymphatics in fasted animals (3, 4) or in animals treated with orotic acid (5). ApoA-I (3) and apoB (5) were detected immunochemically in lymph VLDL. Evidence for the intestinal production of apoA-I was also obtained by using intraperitoneal injection of radioactive amino acid precursor into normal animals (6) and later by duodenal injection into rats in which the hepatic secretion of lipoproteins was reduced by administration of 4-aminopyrazolopyrimidine (7). ApoE and most of the C proteins found in intestinal lymph are thought to be derived from the plasma (8, 9). In general, these results are consistent with those obtained using isolated intestinal perfusion (10, 11) or indirect immunofluorescence techniques (12, 13).

ApoA-IV is a major plasma apolipoprotein in the rat, found in VLDL, HDL, and the d > 1.21 g/ml lipoprotein-free fraction (14, 15). It has been identified in all rat intestinal lymph lipoproteins (9, 11) and is actively synthesized by isolated perfused intestine (11) but to a limited extent by perfused liver (16, 17). However, little data is available on the extent to which the lymphatic transport of apoA-IV is influenced by fat absorption, especially compared to apoA-I or triglyceride transport. In the present experiments, the transport of all major lymph apolipoproteins was examined as a function of time during duodenal lipid infusion in unanesthetized rats. This was conducted

Abbreviations: VLDL, very low density lipoproteins; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; EE, ethinyl estradiol; TMU, tetramethylurea; HDL, high density lipoproteins; LDL, low density lipoproteins.

in both normal control rats and in animals treated with pharmacologic doses of ethinyl estradiol (EE). Comparing lymph lipoproteins in normal and EE-treated rats provides a unique means of identifying apolipoproteins of intestinal origin since plasma apolipoproteins (18), and hence transfer of plasma apoliproteins into lymph, are negligible. The results support the concept that the intestine synthesizes primarily apoB, apoA-IV, and apoA-I, but little or no apoE and C proteins. In addition, the present experiments illustrate that apo A-IV is the most inducible of the apolipoproteins during fat absorption in both normal and EE-treated rats and therefore may be important in the formation of triglyceride-rich lipoproteins by the intestine.

METHODS AND MATERIALS

Male Sprague-Dawley rats (260–390 g) were injected subcutaneously with ethinyl estradiol (5 mg/kg per day) for 5 days as previously described (18); they were allowed free access to rat chow. Twenty-four hr after the last injection, rats were anesthetized with intravenous methohexital (Brevitol, Eli Lilly and Co., Indianapolis, IN). The jugular vein and duodenum (19) were cannulated with silicone rubber tubing (Silastic #135, Dow Corning Co., Midland, MI). The main mesenteric lymph duct was cannulated using siliconized (SurfaSil, Pierce Chemical Co., Rockford, IL) silicone rubber tubing. Rats were placed into restraining cages (20) during which time most animals began to recover from the short-acting anesthetic. For basal lymph collection, saline was infused intraduodenally (2 ± 0.5 ml/hr) for 90 min and lymph was collected into tubes containing EDTA. This was followed by the injection (1.0 ml) and then slow infusion (2 ± 0.5 ml/hr) for 24 hr of a sonicated lipid emulsion containing 2% olive oil, 0.1% cholesterol, 0.02% taurocholate, and 50% Intralipid triglyceride-phospholipid (Cutter Laboratories, Berkeley, CA) in saline. The constituents of this emulsion are similar to those used by Imaizumi et al. (8), except that bile acid was added to insure emulsification and absorption, as done by others (21, 22). During lipid infusion lymph samples were collected for the 24-hr period (three 2-hr and one 18-hr collection). Animals receiving intraduodenal lipid emulsion under these conditions seldom drank; nonetheless, saline was provided during lymph drainage.

Lymph lipoproteins of density less than 1.006 g/ml (chylomicrons and very low density lipoproteins) were isolated by adjusting whole lymph to d 1.010 g/ml, layering with d 1.006 g/ml solution, and spinning in the Beckman L5-50 ultracentrifuge for 24 hr at 39,000 rpm in the 40.3 rotor. The infranates were adjusted to d 1.24 g/ml, layered with d 1.21 g/ml solution, and re-centrifuged to isolate lipoproteins of d 1.006–1.21 g/ml. All lipoprotein fractions were dialyzed extensively against saline containing 0.01% EDTA and 0.01% sodium azide.

The protein content of lymph lipoprotein fractions was determined by the method of Lowry et al. (23) using albumin as standard. ApoB was estimated indirectly by the difference between the total protein and the TMU-soluble fraction (9, 24). Plasma cholesterol concentration was determined using a commercial enzymatic kit (Calbiochem, La Jolla, CA). Lymph triglyceride was determined by a modification of the automated method of Levy (25) subsequent to isopropanol-zeolite extraction (26).

Apolipoprotein composition was determined using sodium dodecyl sulfate polyacrylamide electrophoresis (27). Densitometric scanning at 550 nm (EC Apparatus Corporation, St. Petersburg, FL) of Coomassie blue-stained gels was carried out in duplicate to estimate the concentration of apolipoproteins. Peak areas were determined directly by planimetry (Ott Instruments, Kempten, Federal Republic of Germany), and concentrations were calculated from the percentage distribution of areas and the total amount of TMU-soluble protein. For each major apolipoprotein, increasing loads of protein (up to 90 μg of rat HDL) resulted in linear increases of optical density ($r = 0.97$, $n = 5$). For individual bands the coefficients of variation were less than 15%, as observed previously by others using similar methodology (9). Although absolute amounts will be influenced by the chromogenicity of individual apolipoproteins, comparison of changes in concentration of one apolipoprotein due to treatment remains valid.

Student's *t*-test was employed to determine statistical differences between means, and linear regression analysis was used to establish significant time-trends in apolipoprotein transport rates (Fig. 9) (28).

RESULTS

Rats treated with ethinyl estradiol had plasma cholesterol concentrations of less than 10 mg/dl. Plasma apolipoprotein concentrations were less than 10% of control, and when the $d < 1.006$ and $d < 1.21$ g/ml plasma fractions from EE-treated rats were subjected to SDS-PAGE, no apolipoprotein bands could be visualized, as reported previously (18). In addition to verification of the hypolipidemic effect, however, was the finding that EE-treated rats lost an average of

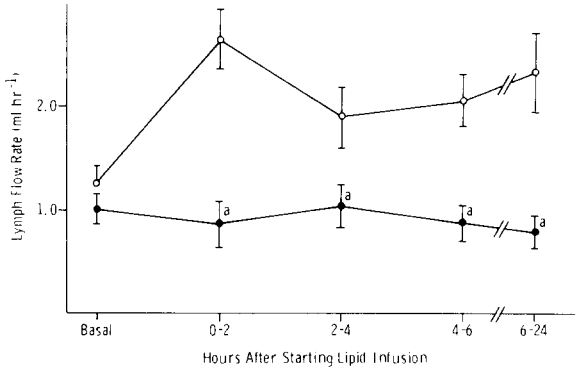

Fig. 1. Lymph flow rate as a function of time from the basal period (90 min) through 24 hr of duodenal lipid infusion. Open circles, control rats; closed circles, rats treated with ethinyl estradiol (5 mg/kg/day for 5 days). Points are the mean ± S.E. (n = 4).
[a] Significantly less than control, $P < 0.05$.

10% of their initial body weight and exhibited a significant increase in liver size relative to body weight, as noted previously (29).

Mesenteric lymph flow rates during the basal period

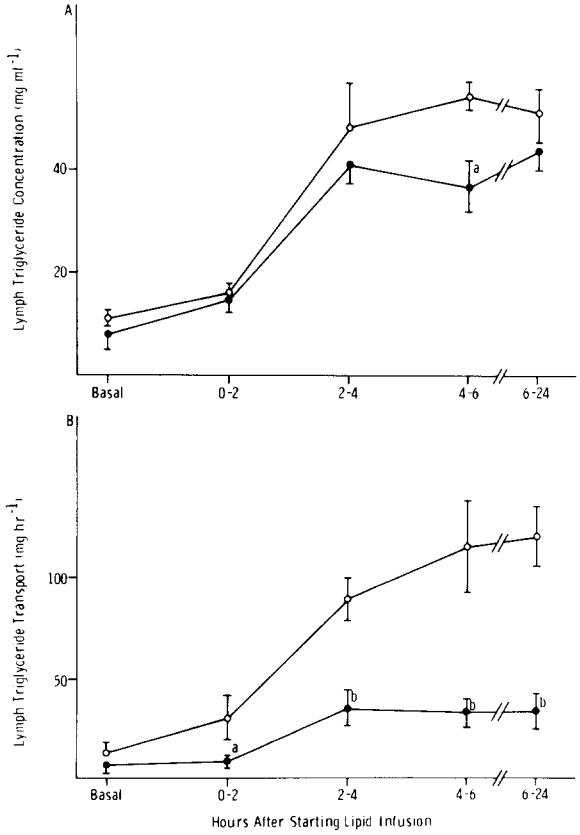

Fig. 2. Lymph triglyceride concentration (A) and transport rate (B) during the basal period and during 24 hr of duodenal lipid infusion. Open circles, control rats; closed circles, rats treated with ethinyl estradiol. Mean ± S.E. (n = 4).
[a] Significantly less than control, $P < 0.05$.
[b] Significantly less than control, $P < 0.01$.

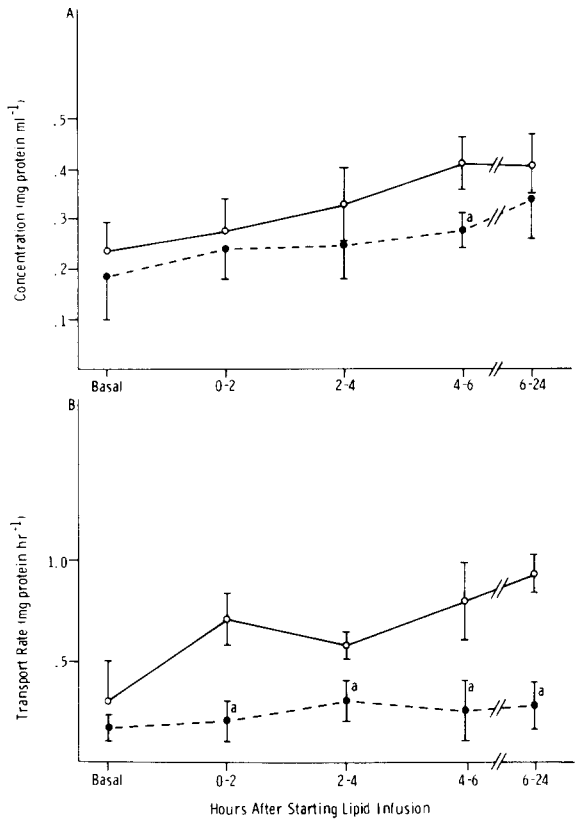

Fig. 3. Lymph lipoprotein (d < 1.006 g/ml) protein concentration (A) and transport rates (B) during basal and lipid infusion periods. Open circles, control rats; closed circles, rats treated with ethinyl estradiol. Mean ± S.E. (n = 4).
[a] Significantly less than control, $P < 0.05$.

were not different between control and ethinyl estradiol-treated groups (**Fig. 1**). However, at all times during duodenal lipid infusion (fat absorption), lymph flow did not increase in EE-treated rats as it did in control animals. Lymph triglyceride concentration was similarly increased in both groups except during the 4 to 6-hr collection (**Fig. 2A**). The transport rate of lymph triglyceride was significantly lower than control due to ethinyl estradiol during the entire lipid infusion period (Fig. 2B). The concentration of lymph d < 1.006 g/ml lipoproteins increased gradually in control and EE-treated rats (**Fig. 3A**). As with triglyceride concentrations, lymph chylomicron and VLDL protein concentration was significantly lower in the ethinyl estradiol group in the 4 to 6-hr period. However, the lymphatic transport of d < 1.006 g/ml lipoproteins was always significantly lower in EE-treated rats (Fig. 3B). The higher density lipoprotein lymph fractions (LDL and HDL) had protein concentrations (**Fig. 4A**) and transport rates (Fig. 4B) several-fold lower than d < 1.006 lipoproteins. In control animals the concentration of d < 1.006 g/ml lipoprotein-protein increased from 240 μg/ml during the basal

period to an average of 355 μg/ml during the lipid infusion period (Fig. 3A), but the mean concentration of d 1.006–1.21 g/ml lipoproteins in control animals did not change significantly during lipid infusion and averaged 57 μg/ml for all time periods (Fig. 4A). In EE-treated rats, the concentration of d < 1.006 g/ml lipoproteins increased from a basal value of 180 μg/ml to a mean of 220 μg/ml during lipid absorption, but higher density lipoproteins in treated animals averaged only 24 μg/ml for basal and lipid infusion periods (Fig. 4A). As with d < 1.006 g/ml lipoproteins, the transport rates for lipoproteins of d 1.006–1.21 g/ml were lower after ethinyl estradiol treatment, and increased from 56 to 171 μg/hr in control animals when the lipid infusion was started (Fig. 4B).

The apolipoprotein composition of lymph d < 1.006 g/ml lipoproteins of control (**Fig. 5,** top) and ethinyl estradiol-treated rats (Fig. 5, bottom) is shown both during the basal period and as a function of time during duodenal lipid infusion. A striking feature of these apolipoprotein patterns is the apparent absence of apoE and the C proteins in lymph from EE-treated animals, whereas control lymph contains all of the major apolipoproteins found in rat plasma, both before and during lipid infusion. Similar patterns are seen in the gels from the d 1.006–1.21 g/ml lymph lipoproteins (**Fig. 6**), except that in control lymph, apoB consistently appeared in the form of two high molecular weight bands and no C proteins were apparent. In EE-treated animals only one apoB band was present in this lipoprotein fraction.

In order to separate the high molecular weight apoB components better, SDS electrophoresis was performed using 3.5% polyacrylamide. In the control d

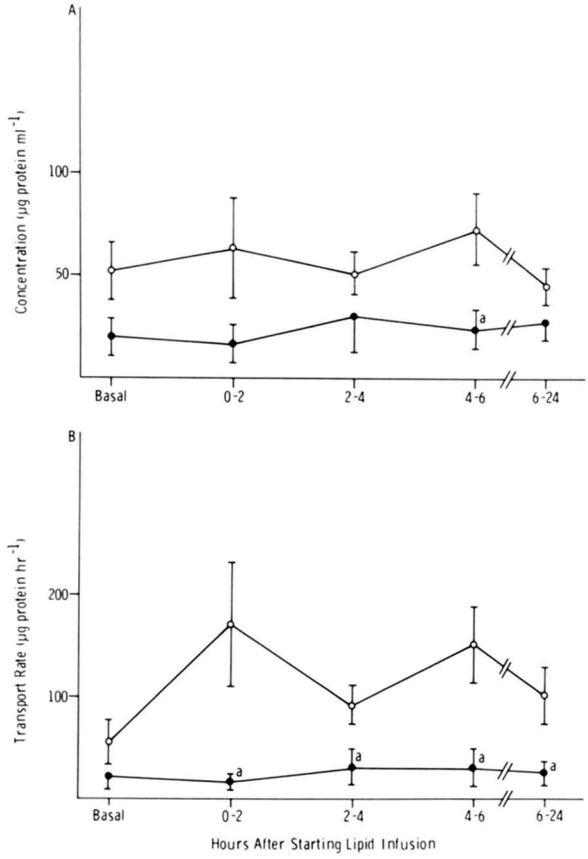

Fig. 4. Lymph lipoprotein (d 1.006–1.21 g/ml) protein concentration (A) and transport rates (B) during basal and lipid infusion periods. Open circles, control rats; closed circles, rats treated with ethinyl estradiol. Mean ± S.E. (n = 4).
[a] Significantly different from control, $P < 0.05$.

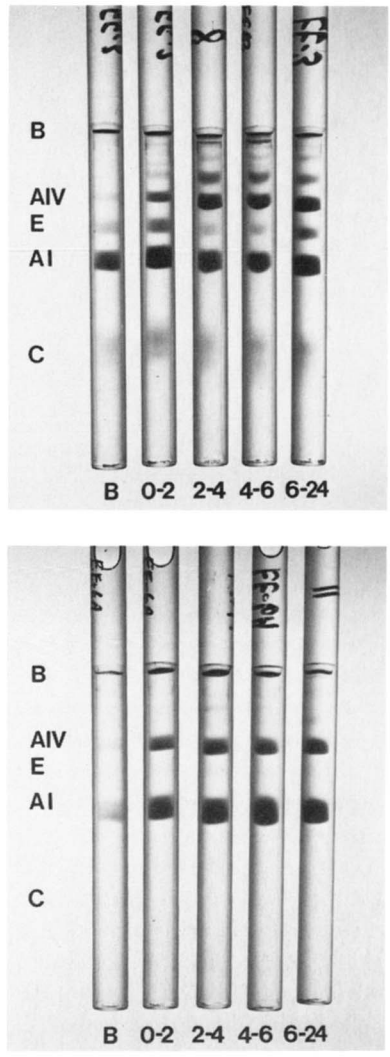

Fig. 5. Typical pattern of d < 1.006 g/ml lymph apolipoproteins, from a control animal (top) and an ethinyl estradiol-treated animal (bottom), separated by SDS-PAGE using 10% polyacrylamide. Apolipoprotein composition is shown for the basal period (B) and after 24 hr of lipid infusion. The amounts of total protein applied to the gels were, from left to right, 39, 52, 46, 68, and 56 μg for control, and 10, 37, 44, 54, and 49 μg for the treated animal.

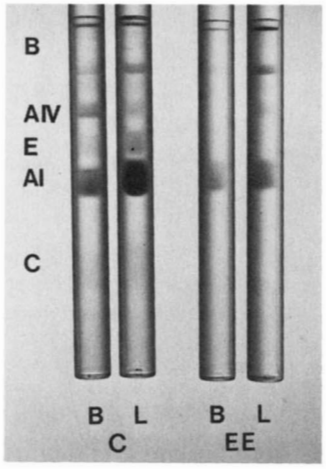

Fig. 6. Typical pattern of d 1.006–1.21 g/ml lymph apolipoproteins from control (C) and ethinyl estradiol-treated (EE) rats during the basal period (B) and after 24 hr of duodenal lipid infusion (L). SDS-PAGE was performed using 10% polyacrylamide. The amounts of total protein applied to the gels were, from left to right, 8, 17, 8, and 7 μg.

< 1.006 g/ml lymph fraction, one major apoB band was present together with a minor higher molecular weight band which corresponded in size to the major apoB band found in plasma from control animals (**Fig. 7**). This high molecular weight apoB was usually absent from basal lymph samples in control animals and appeared in increasing amounts during fat absorption (not shown). EE-lymph lipoproteins of d < 1.006 g/ml contained only the lower molecular weight apoB during all collection periods. In control lymph, the high molecular weight apoB was found al-

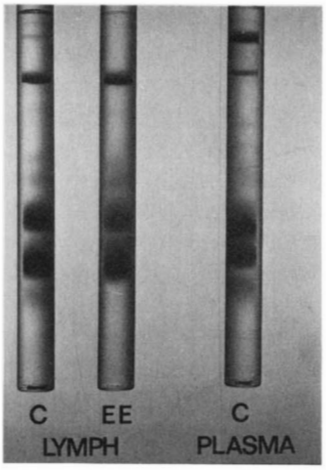

Fig. 7. Typical pattern of d < 1.006 g/ml apolipoproteins from control and ethinyl estradiol-treated rats using SDS-PAGE with 3.5% polyacrylamide. Gels are from lymph samples collected during the overnight duodenal lipid infusion (6–24 hr) and compared to the pattern observed in control plasma from intact (nonfistula) rats (d < 1.21 g/ml). The amounts of protein applied are, from left to right, 43, 40, and 50 μg.

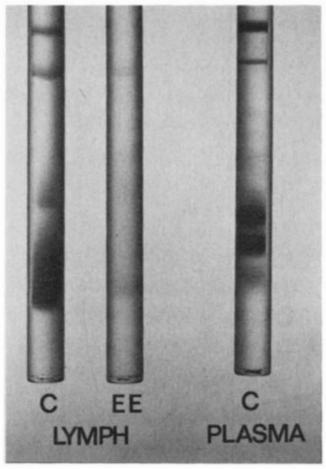

Fig. 8. Typical pattern of d 1.006–1.21 g/ml apolipoproteins from control and ethinyl estradiol-treated rats using SDS-PAGE with 3.5% polyacrylamide. Gels are from lymph samples collected from the overnight duodenal lipid infusion (6–24 hr) and compared to the pattern observed in control plasma from intact (nonfistula) rats (d < 1.21 g/ml). The amounts of protein applied are, from left to right, 18, 8, and 50 μg.

most exclusively in the higher density lipoproteins (d 1.006–1.21 g/ml), but again EE-lymph contained only the low molecular weight apoB (**Fig. 8**).

The gels in Fig. 5 illustrate the apolipoprotein composition in the major lymph lipoprotein fraction (i.e., which proteins are present), and suggest that in both control and ethinyl estradiol-treated rats the concentrations of certain apolipoproteins may be altered due to lipid absorption. To verify these apparent changes in apolipoprotein concentrations, the 10% SDS gels were analyzed by densitometric scanning and planimetry, and concentrations were calculated from the percentages of total TMU-soluble protein. ApoB was not scanned but quantitated indirectly using TMU precipitation.

In both groups of animals, only apoA-IV concentration increased due to lipid absorption. In controls the concentration of apoA-IV increased from a basal value of 17.1 μg/ml to an average of 54.5 μg/ml during the 24-hr lipid infusion period (**Table 1**). In EE-treated rats, A-IV concentration increased from 12.8 to 42.6 μg/ml. During basal lymph collection, the concentrations of apoA-I and apoC were lower in EE-treated rats. But during lipid infusion, apoE, apoC, and apoB were significantly lower due to treatment, especially apoE and C, which were present at very low concentrations in lymph from EE-treated rats.

The transport rates for all apolipoproteins in control rats increased due to lipid absorption. ApoA-IV increased 400%; apoE, 195%; apoA-I, 86%; apoC, 74%; and apoB, 59%; although changes in apoB were

not significant. The large increase in the transport rates for apoA-IV reflects changes in protein concentration (Table 1) as well as increases in lymph flow (Fig. 1). In EE-treated rats, only apoA-IV transport increased with lipid infusion, from 11.4 to 34.1 μg/hr. During basal collection in EE-treated rats, transport rates for apoE and C were much lower than control values, but during lipid infusion the transport of all apolipoproteins was lower in EE-treated rats.

The major apolipoprotein transport rates were also plotted as a function of time during lipid infusion. In control animals, apoB transport rates did not change consistently during lipid infusion in these experiments (**Fig. 9A**). ApoA-I transport rates increased 2- to 3-fold (Fig. 9B), whereas the transport of apoA-IV (Fig. 9C) increased to the greatest extent due to lipid absorption (approximately 7-fold). In EE-treated rats, only the transport of apoA-IV increased due to lipid absorption (Fig. 9C). At all time intervals the transport rates of these apolipoproteins were lower in treated animals (Fig. 9).

DISCUSSION

Numerous authors (6–11, 30) have reported the apolipoprotein composition of rat mesenteric lymph. A consistent finding is the presence of apoE and C together with apoA-I, A-IV, and B. However, it is uncertain whether these proteins are present as the result of de novo intestinal synthesis or transfer from plasma. Indirect immunofluorescent localization of apoB and apoA-I in the intestine of fasted versus fat-fed rats provides strong evidence in favor of intestinal synthesis for these proteins (12, 13). However, since fat feeding increases the transfer of plasma proteins into intestinal lymph (31), it is difficult to rule out the plasma as a source of these apolipoproteins. Direct support for de novo synthesis comes from intestinal perfusions (10, 11) or from in vivo experiments in which hepatic lipoprotein secretion is minimized (5, 7). All of these approaches combined suggest that apoA-I is a major intestinal apolipoprotein, together with apoB and apoA-IV, but that apoC and E in lymph are primarily of plasma (hepatic) origin.

The presence of apoB, A-I and A-IV in mesenteric lymph of ethinyl estradiol-treated rats provides further evidence for the intestinal synthesis of these apolipoproteins, since practically no lymph apolipoproteins could have been derived from plasma in this model. Therefore, lymph lipoproteins of the ethinyl estradiol-treated rat may represent nascent particles which have not been altered by contact with plasma lipoproteins. It appears that in EE-treated rats the

TABLE 1. Lymph apolipoprotein concentrations and transport rates of d < 1.006 g/ml lipoproteins in control and ethinyl estradiol-treated rats

	ApoA-I	ApoA-IV	ApoE	ApoC[a]	ApoB[b]
			μg/ml		
Concentration					
Control					
B	102.1 (20.7)	17.1 (12.7)	13.9 (9.6)	57.1 (26.2)	111.5 (37.8)
L	115.9 (12.3)	54.5 (9.5)[c]	22.8 (3.6)	64.6 (7.1)	105.6 (10.9)
EE-treated					
B	63.2 (12.7)[d]	12.8 (5.6)	7.4 (2.9)	3.2 (2.5)[d]	65.1 (17.2)
L	88.1 (15.2)	42.6 (10.5)[c]	9.1 (2.8)[e]	5.3 (1.4)[e]	79.0 (8.1)[e]
			μg/hr		
Transport Rate					
Control					
B	128.8 (48.0)	22.2 (3.5)	16.9 (5.0)	77.3 (15.1)	133.9 (61.9)
L	239.6 (19.5)[c]	111.1 (19.1)[c]	49.9 (7.8)[c]	134.7 (12.7)[c]	212.5 (19.4)
EE-treated					
B	65.0 (18.3)	11.4 (5.6)	6.4 (3.6)[d]	3.0 (2.1)[d]	62.5 (19.6)
L	75.8 (10.6)[e]	34.1 (7.1)[c,e]	6.8 (1.9)[e]	3.8 (0.9)[e]	67.3 (5.2)[e]

[a] The designation apoC is given to the entire group of apoproteins with molecular weights less than apoA-I.
[b] Calculated as the mass of TMU-insoluble material (n = 4).
[c] Significantly different from (B) value in same group, $P < 0.05$.
[d] Significantly different from (B) value in control group, $P < 0.05$.
[e] Significantly different from (L) value in control group, $P < 0.05$.
Values are the mean ± SE (in parentheses) of 4 gels from four animals during the basal period (B) and 16 gels from four animals during 24 hr of duodenal lipid infusion (L). Calculations are based on percentages of TMU-soluble protein.

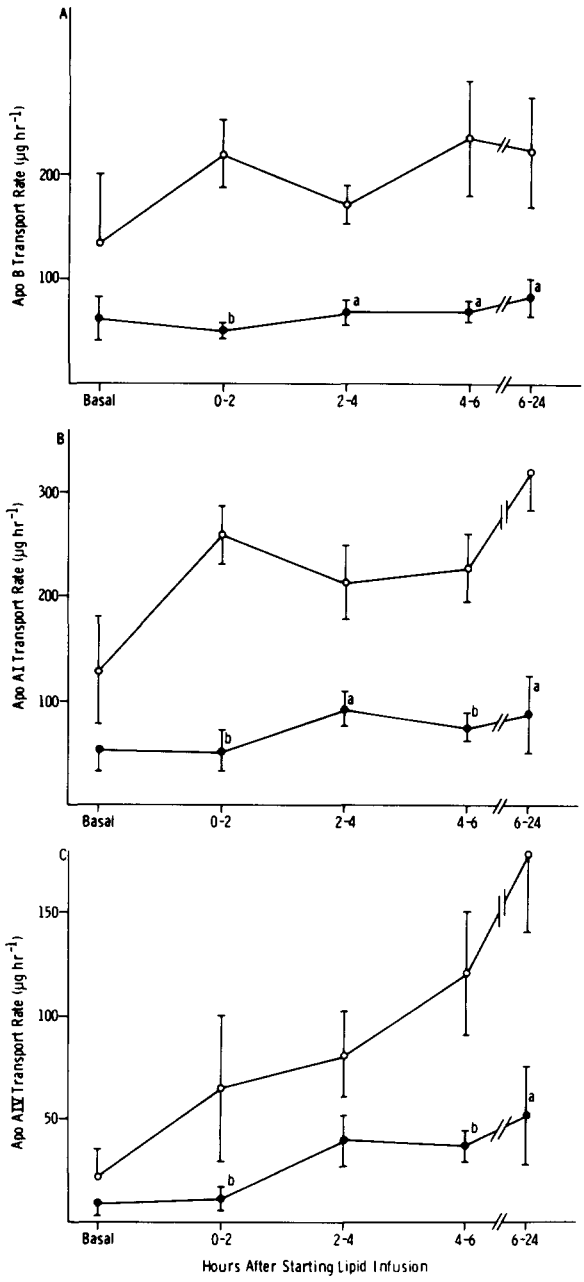

Fig. 9. Lymphatic transport of apoB (A), apoA-I (B), and apoA-IV (C) in d < 1.006 g/ml lipoproteins during basal and lipid infusion periods. Values on the ordinate are derived from densitometric scanning and planimetry and therefore represent "apparent" rates, since the chromogenicity of individual apolipoproteins was not determined. Open circles, control rats; closed circles, rats treated with ethinyl estradiol. Mean ± S.E. (n = 4). Linear regression analysis for apoA-IV transport rates reveals a significant increase with time for control ($r = 0.68$, n = 20, $P < 0.01$) and treated groups ($r = 0.53$, n = 20, $P < 0.05$). Slopes between control and EE groups are significantly different ($P < 0.01$) for apoA-IV. For apoA-I, only control transport rates were positively correlated with time ($r = 0.66$, n = 20, $P < 0.01$), but differences between slopes were significant ($P < 0.01$). No significant linear trends could be established for apoB in either group. Significant differences between groups at each collection period were determined using Student's *t*-test.

[a] Significantly less than control, $P < 0.05$.

[b] Significantly less than control, $P < 0.01$.

intestine synthesizes little or no apoE or C proteins, as reported by others for normal animals (8, 9, 11). From a comparison of the apolipoprotein composition of control and EE-treated rat mesenteric lymph, evidence is also presented for the intestinal secretion of one form of apoB (low molecular weight). This apoB appeared in all lymph lipoproteins, but the higher molecular weight apoB was most evident in the control lymph d > 1.006 g/ml fraction, which, unlike the triglyceride-rich lipoproteins, contained considerable amounts of both apoB proteins. The high molecular weight apoB was the major species found in control plasma from rats without lymph fistula. Its presence in lymph lipoproteins undoubtedly reflects transfer from plasma, especially since it appears to a greater degree in lymph during absorption, when generally more plasma proteins are transferred to intestinal lymph, than in lymph during a basal period (31). Our interpretation as to the origin of these apoB proteins using the EE rat model is consistent with the recent findings of Krishnaiah et al. (32), who used 3.5% polyacrylamide gels to separate the low molecular weight component ("intestinal" apoB) from the high molecular weight "hepatic" apoB. These authors have determined that the molecular weights of the large and small apoB proteins were 335,000 and 240,000 daltons, respectively. Emphasis in the present study has been directed toward the origin of these proteins based on differences between control and EE lymph composition, and no attempt has been made to characterize these proteins.

The concentration and flux of apolipoproteins into lymph in the ethinyl estradiol-treated rat are lower, compared to control, even for apolipoproteins that are presumed to be primarily of intestinal origin. This is consistent with a 30–50% decrease in lipid absorption in EE-treated rats, as determined by the appearance of radioactivity in plasma after duodenal administration of radiolabeled lipid in unanesthetized, unrestrained rats.[1] It has been proposed that the low levels of plasma apolipoproteins in the EE-treated rat are the result of enhanced catabolism of circulating lipoproteins (33) via an induced, receptor-mediated process (34). The lower intestinal apolipoprotein production (lymph transport rates) found in the present study represents an additional mechanism responsible for the low levels of plasma apolipoproteins in intact animals treated with ethinyl estradiol.

In the present experiments, intestinal lymph flow was lower in EE-treated rats during lipid absorption. In normal animals, both enhanced blood flow to the intestine and enhanced intestinal capillary perme-

[1] Krause, B. R., and P. S. Roheim. Unpublished results.

ability are probably responsible for the rise in lymph flow following fat feeding (1, 35). Lymph flow usually increases before lymph lipid (1), as in the present experiments. Administration of pharmacologic doses of ethinyl estradiol also decreases bile flow in rats (29) due to alterations in hepatic membrane lipid structure (36) and Na^+, K^+-ATPase activity (37). Whether a similar mechanism is involved in the decreased lymph flow during lipid absorption in the EE-treated rats remains to be determined. It is also possible that the decreased absorption might be causally related to the decreased bile secretion.

ApoA-IV is a major plasma apolipoprotein in the rat (14) and is synthesized mainly by the intestine (38). It appears to be unique among the apolipoproteins in that its concentration if not affected by the sex or age of the animal (39) or thyroid status (40), but is profoundly sensitive to the nutritional state of the animal, since fed rats have much higher plasma A-IV concentrations than fasted animals.[2] In the present experiments, only apoA-IV concentration increased during lipid absorption in control animals and to a lesser extent in EE-treated rats. In addition, apoA-IV lymphatic transport rates increased to the greatest extent in both control and treated groups. These findings add further support to the suggestions of Beisiegel and Utermann (41) and Green et al. (42), based on human studies, that apoA-IV may be of major significance in the metabolism of triglyceride-rich intestinal lipoproteins.

From labeling experiments in vivo, it has been suggested that apoA-IV is produced independently from apoA-I (9). In the present study, apoA-IV appeared to be more responsive to lipid infusion than apoA-I, both in normal and EE-treated rats. However, one cannot rule out the possibility that ethinyl estradiol inhibits the intestinal synthesis of apolipoproteins, especially apoA-I, during lipid absorption. Therefore, any extrapolation of the present data to the normal animal requires further demonstration of normal intestinal function in EE-treated rats.

In the present experiments, lymph was collected for only 24 hr, without an initial overnight basal period, in an attempt to minimize the loss of plasma proteins. Mesenteric lymph-cannulated rats infused intraduodenally with lipid lose approximately 25 mg/hr of total protein (assuming a lymph protein concentration of 12 mg/ml (8) and a lymph flow rate of 2 ml/hr (Fig. 1)). After 24 hr, about 600 mg of protein would have to be replaced, representing well over two-thirds of the total plasma protein pool, since more than 90% of the lymph protein is plasma protein derived from the circulation. It is possible that the loss of plasma proteins would be a stimulus for increased concentration of apolipoproteins in intestinal lymph during prolonged lymph drainage (8), especially in view of the fact that enhanced apolipoprotein production occurs in livers from plasma protein-depleted nephrotic animals (43).

Only the apolipoprotein concentrations of d < 1.006 g/ml lipoproteins were measured in the present study, since this fraction contained several-fold more apolipoprotein-protein than the d > 1.006 g/ml fraction. Although most apoA-I is found in the triglyceride-rich particles during fat absorption (8), it has been suggested recently that increased amounts of intestinally produced apolipoproteins may appear in the lymph d > 1.21 g/ml fraction and in the portal venous blood when no lipid is being absorbed (44). It is possible that in the present experiments the lymphatic transport of apolipoproteins could be a reflection of shifts in the distribution between various lipoprotein fractions, the d > 1.21 g/ml lymph fraction, or portal blood.

In the present study the values for apoE concentration (22.8 μg/ml) and transport rate (49.9 μg/hr) in control rats after 24 hr of lipid infusion agree very well with values obtained by radioimmunoassay (8) from similarly treated animals after the same time interval of infusion (26.9 μg/ml and 37 μg/hr). ApoA-I concentration (119.2 μg/ml) and transport rate (239.6 μg/hr) are also in reasonable agreement with radioimmunoassay data (225 μg/ml, 292 μg/hr (8)).

In conclusion, the ethinyl estradiol-treated animal exhibits lower lymphatic transport rates of lipoproteins and apolipoproteins during lipid absorption, compared to control animals. This represents an additional mechanism for the hypolipidemic effect observed earlier in these animals (18). The ethinyl estradiol model provides a unique opportunity to examine lymph apolipoproteins synthesized primarily by the intestine, since practically no plasma "contamination" of lymph apolipoproteins occurs. It is also possible that mesenteric lymph lipoproteins from EE-treated rats could be considered as intestinally produced nascent lipoprotein particles and therefore might be useful in further studies dealing with the structure and metabolism of nascent particles. It appears that the EE-treated rat intestine synthesizes primarily apoB (low molecular weight), A-I, and A-IV but little or no C proteins. Although this appears to be the case in normal animals based on other approaches (7–13, 32), further experiments are required to demonstrate normal intestinal function in EE-treated rats. Compared to other apolipoproteins, lymphatic transport of apoA-IV is stimulated to the greatest

[2] Chuang, M., and P. S. Roheim. Unpublished results.

extent by lipid absorption in normal and treated rats, suggesting a possibly unique role for apoA-IV in the formation of triglyceride-rich lipoproteins in the intestine.

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