# Differential transport of cholesterol and oleic acid in lymph lipoproteins: sex differences in puromycin sensitivity

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**Abstract** Adult rats of both sexes were prepared with indwelling drainage catheters in the left thoracic lymphatic duct, and with duodenal infusion catheters. Control and puromycin-treated animals were administered an aqueous test emulsion containing [7α-$^{3}$H]cholesterol and [1-$^{14}$C]oleic acid, followed two hours later, by a tracer dose of [1-$^{14}$C]-leucine. Successive 2-hr lymph samples were subjected to ultracentrifugal separations of the major lipoprotein classes. These were specifically extracted for lipids, and for DNA- and lipid-free protein. In both sexes, oleic acid absorption was largely associated with the $d < 1.006$ g/ml chylomicron fraction throughout the 6-hr experimental period. Small but consistent levels of labeled fatty acid appeared in the $1.006 < d < 1.019$ g/ml VLDL fraction. However, with both sexes 25–35% of the absorbed cholesterol appearing in lymph was recovered in the VLDL fraction. Furthermore, there were statistically greater levels of cholesterol in this lymph fraction in females than in males. Cumulative protein levels and leucine incorporation into chylomicron proteins was comparable in both sexes. However, VLDL protein in the female was significantly greater than in the male and this difference was mimicked by the greater incorporation of leucine into VLDL proteins in the female. In males, there were no significant effects of puromycin on cholesterol or oleic acid absorption, despite a marked inhibition in chylomicron protein levels and leucine incorporation into this fraction. There was also no effect of the inhibitor on VLDL protein levels or on leucine incorporation into VLDL peptides. Cholesterol but not oleic acid absorption in females was significantly depressed by administration of puromycin, and this was largely attributed to a decrease in VLDL transport of the sterol. Also, unlike males, leucine incorporation into VLDL peptides was inhibited by 75% by puromycin administration. These results emphasize the importance of non-chylomicron transport of cholesterol during absorption and suggest a hormonal influence on intestinal VLDL synthesis in female rats.—**Vahouny, G. V., E. M. Blendermann, L. L. Gallo, and C. R. Treadwell.** Differential transport of cholesterol and oleic acid in lymph lipoproteins: sex differences in puromycin sensitivity. *J. Lipid Res.* 1980. **21:** 415–424.

**Supplementary key words** very low density lipoprotein · chylomicrons

It has become increasingly apparent that a large, but variable fraction of absorbed cholesterol, of either endogenous or exogenous origin, is associated with one or more non-chylomicron lipoprotein fractions of lymph (1–6). We reported (1) in 1958 that absorbed cholesterol was largely recovered in the chylomicron-free subnatant of rat lymph, and that the extent of this transport varied with feeding conditions. Similar findings were subsequently reported in dogs (2) and rabbits (3). After fasting or feeding, rat intestinal lymph contains lipid transport particles comparable to very low density lipoproteins with respect to flotation, compositional, and electrophoretic properties (4, 5). This lipoprotein fraction contains 47% of the triglyceride and 54% of the cholesterol in fasting lymph and has been shown to be of intestinal origin (4, 7). During fat (oleate) absorption and increased secretion of chylomicrons, the distribution of cholesterol shifts toward chylomicron transport and is proportionately less in the VLDL fraction (3, 5). However, even during fat transport when 70–80% of lymph triglycerides are associated with the chylomicron fraction and only 15–25% with the VLDL fraction (5, 6), the "endogenous" cholesterol of lymph is almost equally distributed among these lipoproteins (3, 5, 6). Furthermore, this distribution appears to be dependent on the fat load (8) and on the degree of unsaturation of the dietary fat (5), both of which markedly influence chylomicron size (8, 9).

These studies, however, have been conducted only in male animals (except where not noted), and with one exception (3) have dealt with absorption and transport of endogenous rather than dietary cholesterol. We recently reported (10) a significant sex difference in the effect of puromycin on the absorption of a high dietary load of oleic acid or cholesterol into thoracic duct lymph of rats. Under the conditions of the study,

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SDS, sodium dodecyl sulfate; apo, apoprotein.

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only cholesterol absorption was inhibited by puromycin administration in male rats, while in females, lymphatic transport of both lipids was markedly reduced. The evidence indicated that these differences were not related to effects on gastric emptying or lymph flow, but were the result of differential effects of the inhibitor on the transport lipoproteins.

In the present study, sequential lymph samples were collected from rats of both sexes following intraduodenal administration of a test emulsion containing [7$\alpha$-$^3$H]cholesterol and [1-$^{14}$C]oleic acid. Following a 2-hr collection period, all animals were administered [1-$^{14}$C]leucine and all lymph samples were subjected to ultracentrifugal separation of major lipoprotein classes. Analyses of the distributions of labeled lipids and protein clearly demonstrate major differences in the transport of each lipid in lymph lipoproteins. Furthermore, the data suggest a major difference between sexes with respect to cholesterol transport in a "chylomicron-free" lipoprotein fraction.

We have also presented evidence that this lipoprotein ($1.006 < d < 1.019$ g/ml), which is more prominent in female rats, is also highly sensitive to puromycin inhibition of protein synthesis and lipid transport. A preliminary report of these studies was presented earlier (11).

## MATERIALS AND METHODS

### Materials

Radioactive lipids were purchased from Amersham-Searle Corporation, Arlington Heights, IL and [1-$^{14}$C]-leucine was obtained from New England Nuclear Corporation. The lipids were from Supelco, Bellefonte, PA. The purity of oleic acid (99%) was determined by gas–liquid chromatography of the methyl ester, and the purity of [1-$^{14}$C]oleic acid was determined by stream splitting and counting split fractions from gas–liquid chromatography. Cholesterol and [7$\alpha$-$^3$H]cholesterol were purified through the dibromide, and purity was determined by gas–liquid radiochromatography. Puromycin was purchased from Nutritional Biochemical Corporation, Cleveland, OH.

### Experimental procedure

Male and female rats of the Wistar strain (Charles River), weighing 200–300 g, were maintained on laboratory chow and water ad libitum prior to use. The left thoracic lymphatic duct was cannulated with polyethylene tubing (PE 25 Intramedic) cephalad to the cysterna chyli (12) and an indwelling infusion catheter was secured into the duodenum 1.5 cm from the pyloric sphincter. The animals were placed in restraining cages and infused with 0.9% saline-5% glucose (3 ml/hr) via the duodenal catheter.

Following an overnight fast, experimental animals were given a total of 15 mg puromycin in nine hourly injections (10, 13). The first four intraperitoneal injections of 2.5 mg of puromycin were followed by five hourly injections of 1 mg puromycin. Control animals received similar injections of saline alone. At the time of the fifth injection, each rat was given, by intraduodenal administration, 1 ml of the lipid test emulsion over a 1-min period, and the saline–glucose infusion was continued. The aqueous test emulsion was prepared as previously described (14) and contained the following components per ml of physiological saline: 25 mg of [7$\alpha$-$^3$H]cholesterol (25 $\mu$Ci), 110 mg of [1-$^{14}$C]oleic acid (5 $\mu$Ci), 144 mg of sodium taurocholate, and 25 mg of albumin. Two hours after the test emulsion, [1-$^{14}$C]leucine (15 $\mu$Ci in 1 ml of saline–glucose) was injected via the duodenal infusion catheter and the saline–glucose infusion was continued for the remaining four hours of study.

Lymph was collected in 2-hr periods in heparinized tubes in ice. Volumes were recorded and aliquots of each sample were immediately subjected to preparative ultracentrifugation at 12°C (15) in a 40.3 Beckman rotor. Lipoprotein fractions were obtained by sequential centrifugation: $d < 1.006$ g/ml, 60 min at 16,100 $g$; $d < 1.019$ g/ml, 14 hr at 114,600 $g$; $d < 1.063$ g/ml, 14 hr at 114,600 $g$; and $d < 1.21$ g/ml, 22 hr at 114,600 $g$. All lipoprotein fractions were purified by recentrifugation under the same conditions. Under these centrifugal conditions, the $d < 1.006$ g/ml fraction is designated chylomicrons. By SDS-polyacrylamide disc gel electrophoresis (16, 17), this fraction contained apolipoproteins B, A-1, A-4, E, and the C apolipoproteins. The $1.006 < d < 1.019$ g/ml lipoprotein fraction, hereafter referred to as VLDL, had a similar apoprotein composition to the chylomicrons in the present study, and to the small chylomicrons, or VLDL described by others (17–20). The $1.019 < d < 1.063$ g/ml lipoproteins contained primarily apo B typical of LDL (21), and the $1.063 < d < 1.21$ g/ml lipoproteins contained A-1 and a faint band of apo E characteristic of nascent lymph HDL (22).

### Protein analysis

Aliquots of each lymph sample and each ultracentrifugal fraction were delipidated in ten volumes ethanol–diethyl ether 3:1 (v/v). These were stored at 2°C overnight. The protein precipitate was sedimented by centrifugation, and the solvent was retained for isotope and mass analyses. The protein was washed once with 10 ml cold ethanol–ether and twice with 5 ml hot 10% trichloroacetic acid to remove

labeled free leucine. The precipitate was reisolated by centrifugation, allowed to dry overnight, and dissolved in 1 ml 0.2 M SDS (23). Aliquots were taken for protein determination (24) using 0.2 M SDS as blanks and serum albumin, fraction V, in SDS as the standard. Aliquots were also taken for determination of radioactivity using 10 ml Bray liquid scintillant (25). All counts were corrected for quenching by external standardization in a Beckman LS-250 liquid scintillation spectrometer.

### Lipid extraction and analysis

The protein-free ethanol–ether extracts, were evaporated to dryness at room temperature under nitrogen. The lipid residue was extracted with 10 ml of chloroform–methanol 2:1 (v/v) according to Folch, Lees and Sloane Stanley (26). Following separation of the methanol–water phase, the chloroform phase was washed (26) and evaporated to dryness under nitrogen. The lipids were taken up in 1 ml hexane for isotope analysis and thin-layer chromatographic separation of lipids (10). All isotope data were corrected to dpm. Apparent differences between groups were analyzed for significance by Student's $t$ test. All values are presented as means ± SEM. Differences of $P < 0.05$ were considered significant.

## RESULTS

### Lymph flow and lymphatic absorption of lipids

The total lymph volumes for the 6-hr experimental period was comparable for control females and males (**Table 1**). In females, this was 16.3 ± 0.3 ml, or a flow rate of 2.7 ± 0.1 ml/hr. In males, total lymph volume was 15.9 ± 0.1 ml, or a flow rate of 2.7 ± 0.4 ml/hr. Puromycin administration to either sex resulted in a significant decrease in lymph production despite the infusions (Table 1) and this effect was consistent for each lymph collection period (data not shown).

The data on the cumulative lymphatic absorption of [7$\alpha$-$^3$H]cholesterol and [1-$^{14}$C]oleic acid in female and male rats are summarized in **Fig. 1.** Under the conditions of the present study, the rates and extent of absorption of either cholesterol or oleic acid in female and male rats were essentially the same. Also, except for oleic acid absorption in males, the variation in lipid absorption among rats of either sex was small in contrast to the earlier studies (10) in which larger doses of lipids were administered intragastrically. Thus, for all rats of both sexes, 12.0 ± 0.5% of the 25-mg dose of cholesterol was absorbed during the 6-hr test period, with a maximal rate of absorption of 1.3 ± 0.15 mg/hr occurring during the 2 to 6-hr period after administration. With oleic acid, 48.5 ± 3.9% of the 110-mg dose was absorbed, with a maximal rate of absorption of 11.6 ± 1.3 mg/hr occurring during the 2 to 4-hr period after administration. The percentages of total cholesterol or total fatty acid absorption appearing in lymph during each 2-hr collection period were comparable to data from rats administered larger doses of these lipids via the intragastric route (10).

### Differential transport in chylomicron and VLDL fractions

Comparative data on the cumulative appearance of labeled cholesterol and oleic acid in the chylomicron and VLDL fractions of lymph are summarized in **Fig. 2.** In female rats, transport of [7$\alpha$-$^3$H]cholesterol in the chylomicrons appeared somewhat greater than in the VLDL fraction although these differences were not statistically significant. However, in males, chylomicron transport of cholesterol was significantly greater than VLDL transport of the sterol; also, by 6 hr, cholesterol transport in the chylomicron fraction of males exceeded that in females. In contrast to cholesterol transport, oleic acid transport in both sexes was largely associated with chylomicron fraction. Transport of the labeled fatty acid in the VLDL fraction was small and was reasonably consistent within each time period throughout the study.

As shown in **Fig. 3,** the percentage distribution of the total absorbed cholesterol between the chylomicron and VLDL fraction was not consistent during each successive lymph collection period, and this was true for both sexes. Thus, during the first 2 hr after lipid administration, when the rate of cholesterol absorption was still low, and oleic acid absorption was high, chylomicron transport of absorbed cholesterol markedly exceeded transport in the VLDL fraction. From 4–6 hr, when the rate of cholesterol absorption was still maximal and the rate of fatty acid absorption had diminished, the percentages of absorbed cholesterol in the two lipoprotein fractions were essentially the same, i.e. approximately 35–45% in each lipoprotein fraction. Furthermore, there were statistical differences between the female and male rats when comparing the distribution of cholesterol among lipoproteins. In addition to the transport of cholesterol in chylomicrons and VLDL, approximately 20% was associated with lipoproteins of $1.019 < d < 1.21$ g/ml (LDL and HDL), but this level was consistent for all periods and both sexes.

Of the total absorbed oleic acid, 89.0 ± 1.6% in males, and 81.9 ± 0.4% in females was associated with lymph lipoproteins of density < 1.019 g/ml, and this

TABLE 1. Effect of puromycin in lymph flow and cholesterol and oleic acid absorption in female and male rats[a]

| Measurement | Female | | Male | |
|---|---|---|---|---|
| | Control | Puromycin | Control | Puromycin |
| Lymph flow, ml/hr | 2.7 ± 0.1 | 1.3[b] ± 0.02 | 2.7 ± 0.4 | 1.7[b] ± 0.1 |
| Cholesterol absorption, % of fed dose | | | | |
| Total lymph | 11.6 ± 0.6 | 8.7[b] ± 1.1 | 12.8 ± 1.1 | 11.2 ± 2.2 |
| Chylomicrons (d < 1.006 g/ml) | 4.9 ± 0.2 | 4.3 ± 0.6 | 6.5[c] ± 0.8 | 4.5 ± 1.2 |
| VLDL (1.006 < d < 1.019 g/ml) | 4.1 ± 0.2 | 2.4[b] ± 0.3 | 3.2 ± 1.5 | 3.9 ± 0.6 |
| Oleic acid absorption, % of fed dose | | | | |
| Total lymph | 46.3 ± 2.0 | 41.2 ± 2.8 | 52.9 ± 13.3 | 36.6 ± 6.3 |
| Chylomicrons (d < 1.006 g/ml) | 31.3 ± 1.7 | 30.0 ± 2.1 | 42.4 ± 13.4 | 26.6 ± 5.2 |
| VLDL (1.006 < d < 1.019 g/ml) | 6.7 ± 0.5 | 4.5[b] ± 0.1 | 5.1 ± 0.1 | 4.1 ± 0.5 |

[a] All values are means ± SEM.
[b] $P < 0.05$ between puromycin-treated and control animals.
[c] $P < 0.05$ between female and male animals.

difference was statistically significant. Although there appeared to be a tendency toward greater transport of oleic acid (and cholesterol) in the chylomicron fraction in males, this was not statistically significant.

As shown in Fig. 3, the relative distribution of total absorbed oleic acid between the chylomicron and VLDL fractions remained more consistent for each time period, particularly in the male, than was observed with the distribution of cholesterol. In the female, there was a relative increase in the percentage of oleic acid associated with the VLDL fraction from 4–6 hr after feeding, when total oleic acid absorption was diminishing.

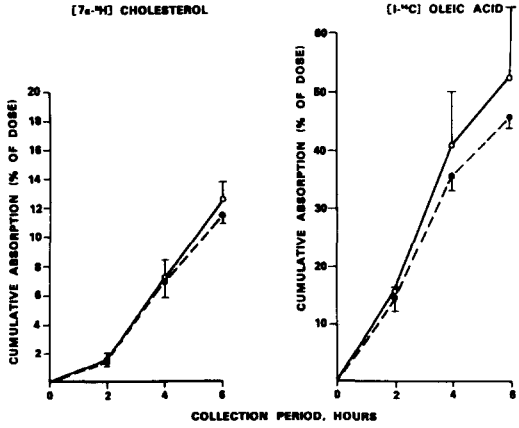


**Fig. 1.** Cumulative appearance of [7α-$^3$H]cholesterol and [1-$^{14}$C]-oleic acid into thoracic duct lymph of male and female rats. All animals received an intraduodenal infusion of an aqueous emulsion containing 25 mg of albumin, 25 mg of [7α-$^3$H]cholesterol, 110 mg of [1-$^{14}$C]oleic acid and 144 mg of sodium taurocholate in 1 ml saline. Open circles represent means ± SEM from four female rats. Closed circles represent means ± SEM from four male rats.

### Lipoprotein protein and labeling patterns

Data on chylomicron and VLDL protein and incorporation of labeled leucine into these lipoprotein fractions are shown in **Fig. 4.** The cumulative protein levels in the chylomicron fraction were comparable in males and females, and this similarity was reflected in the comparable levels of leucine incorporation into this lipoprotein fraction. However, VLDL protein in the female increased dramatically over the 6-hr period, and greatly exceeded the levels seen in the same lipoprotein fraction in the male. This major difference between sexes was also evident in the levels of leucine incorporation into the VLDL protein (Fig. 4, bottom panels).

### Effect of puromycin on lipid transport and protein synthesis

As shown in Table 1, puromycin treatment of female rats resulted in a significant depression of cholesterol absorption (to 75% of control) and this was due largely to a significant decrease in cholesterol transport in the VLDL fraction. There were no significant changes in chylomicron cholesterol transport, or in the amount of labeled sterol associated with LDL and HDL (data not shown). Puromycin had no effect on net oleic transport into lymph of females,

nor was there an effect on the transport of the labeled fatty acid in the chylomicron fraction. However, puromycin treatment of female rats resulted in a significant reduction (33%, $P < 0.05$) of fatty acid transport in the VLDL fraction. Since this fraction transports only about 14% of the total fatty acid absorbed, the puromycin-induced decrease in VLDL transport of absorbed oleic acid was not reflected as a significant effect on total absorption.

In male rats, there was no significant effect of puromycin on total lymphatic absorption of cholesterol, nor were there significant differences in the chylomicron or VLDL transport of the sterol. However, as indicated earlier, the level of absorbed labeled cholesterol associated with the chylomicron fraction in males was significantly higher than that in the female. Although it appeared the puromycin had some effect on chylomicron transport of the fatty acid in males, these differences from the controls were not significant. Oleic acid transport in the VLDL fraction in male rats was more constant, but, unlike that in females, was unaffected by puromycin treatment of the animals.

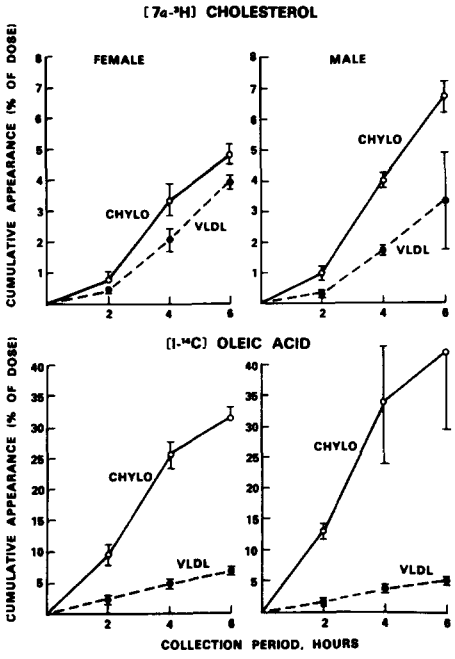


**Fig. 2.** Cumulative appearance of [7α-³H]cholesterol and [1-¹⁴C]-oleic acid into chylomicrons (d < 1.006 g/ml) and very low density lipoproteins (1.006 < d < 1.019 g/ml) lymph lipoproteins of female and male rats. All animals received the test emulsion via an indwelling intraduodenal catheter. Open circles represent means ± SEM for four samples of d < 1.006 g/ml lipoproteins at each time period. Closed circles represent means ± SEM for four samples of 1.006 < d < 1.019 g/ml lipoproteins at each time period. In addition, approximately 20% of the cholesterol and 10–18% of the oleic acid were recovered in lipoproteins 1.019 < d < 1.21 g/ml (LDL + HDL).

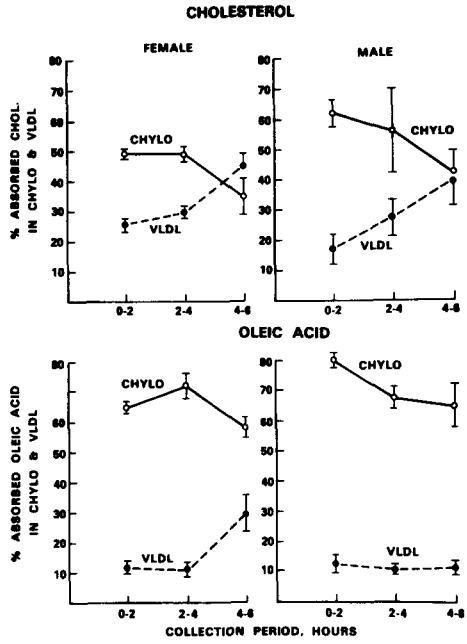


**Fig. 3.** Percentage distribution of total absorbed cholesterol and oleic acid between lymph chylomicrons and VLDL at each lymph collection period after lipid administration. The composition of the lipid emulsion and separation and analyses of lipoprotein fractions are described in the text. Open circles represent means ± SEM for four chylomicron samples at each time period. Closed circles represent means ± SEM for four samples of VLDL at each time period.

The data in **Table 2** summarize total lymph protein, protein of individual lipoprotein fractions, and incorporation of [1-¹⁴C]leucine into these fractions in male and female control and puromycin-treated rats. In both sexes, the effect of puromycin was evident in terms of depression of total lymph protein, with levels ranging from 45% of control in males to 55% of control in females. This depression was reflected by the inhibition of leucine incorporation induced by puromycin in both sexes, e.g., 36% of control in males and 57% of control in females.

Chylomicron protein levels were markedly depressed by puromycin treatment and this effect was comparable in both sexes (to 43% of control in males and to 40% in females). This however, was not directly reflected by inhibition of leucine incorporation into this lipoprotein fraction. Thus, in females, puromycin treatment resulted in a significant reduction of leucine incorporation with chylomicron protein, but was only 35% (compared to 57% in total chylomicron protein). In males, a similar level of inhibition was observed but this was not significantly different from controls.

The most dramatic difference between females and males was observed in the VLDL fraction. The two-fold higher level of VLDL protein in the female was reduced by 73% with puromycin treatment, while

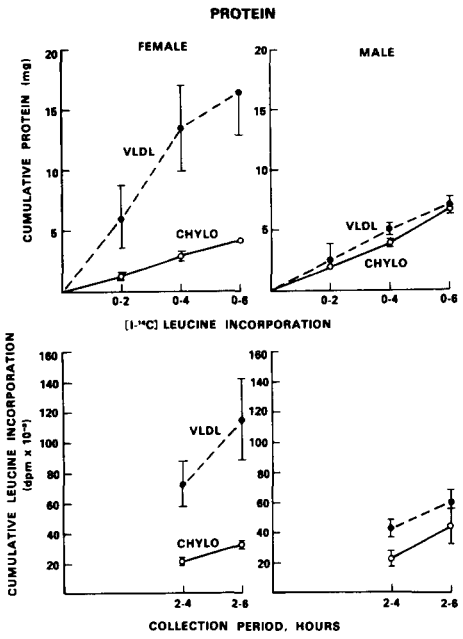


**Fig. 4.** Cumulative values for protein and leucine incorporation into chylomicrons and VLDL protein in female and male rats. Upper panels show lipoprotein values for each time period following administration of the lipid test emulsion. Lower panels represent incorporation of the [1-$^{14}$C]leucine, administered 2 hr after the lipid dose, into chylomicron and VLDL protein. Open circles represent means ± SEM for four analyses of chylomicrons at each time period. Closed circles represent means ± SEM for four analyses of VLDL at each time period.

this same fraction was completely unaffected in the male. These differences were closely correlated to differences in leucine incorporation into VLDL protein. Leucine incorporation into VLDL of female rats was almost twice that in males, and was inhibited by 71% with puromycin treatment. In contrast, puromycin had no effect on amino acid incorporation into VLDL proteins in the male ($62 \times 10^3$ dpm in controls versus $66 \times 10^3$ dpm in puromycin-treated males).

Differences between the effects of puromycin on the proteins of the VLDL fractions, and between males and females is even further exaggerated when comparing these parameters in successive 2-hr lymph collections. As shown in **Fig. 5** (upper panels), cumulative chylomicron protein levels in the male were significantly different than in females by 6 hr, and in both sexes these levels were markedly inhibited by puromycin. These differences were largely reflected in the effects of puromycin on cumulative leucine incorporation (Fig. 5, lower panels), although the variations in male controls precluded differentiating between sexes.

The data in **Fig. 6,** (upper panels), clearly show the cumulative difference between the sexes with respect to VLDL protein, and emphasize the contrast in the effect of puromycin on this fraction between sexes. Thus, puromycin treatment of female rats was associated with a continued depression of VLDL protein appearance in lymph, while in males, there was no effect of puromycin on the already lower levels of VLDL protein. These marked differences were exactly paralleled by data on incorporation of labeled leucine into VLDL proteins (Fig. 6, lower panels).

## DISCUSSION

In the present study, and that reported earlier (10), we have been largely concerned with elucidating differential lipoprotein transport of exogenous cholesterol and oleic acid in lymph, and sex differences in sensitivity of individual lymph lipoproteins to the protein antagonist, puromycin. The approach in the present study was modified as follows: animals were provided a constant infusion of saline–5% glucose intraduodenally to avoid major differences in lymph flow, particularly during puromycin administration (27); the dose of oleic acid was reduced from 292 mg to 110 mg and that of cholesterol from 50 mg to 25 mg; the lipids were infused as an aqueous emulsion intraduodenally to circumvent differences in gastric emptying which is also markedly affected by puromycin administration (28); labeled leucine was infused intraduodenally 2 hr after the lipid dose to obtain lymph lipoprotein labeling patterns; and 2-hr fractions of lymph were subjected to ultracentrifugal separations of lipoproteins of density $< 1.006$ g/ml and lipoproteins of density $1.006 < d < 1.019$ g/ml.

The isolation of a "VLDL" fraction of thoracic duct lymph at $1.006 < d < 1.019$ g/ml for $10^8$ *g*-av min, rather than at $d < 1.006$ g/ml (e.g., 17–20) was based on several criteria. In preliminary studies, it was determined that 80–90% of the absorbed oleic acid, but only half of the absorbed cholesterol was associated with the chylomicron fraction of lymph. The lipoprotein fraction isolated at $1.006 < d < 1.019$ g/ml contained only 10–17% of the absorbed oleic acid and almost half of the absorbed cholesterol found in the two lowest density lipoprotein fractions, and had an apoprotein composition comparable to VLDL, or "small chylomicrons" described previously (17, 20). Furthermore, the lipid distribution between the two lipoprotein fractions obtained from fed male animals in the present study is comparable to that reported by others (5, 6).

In the present study, there were no significant differences in the cumulative absorption of either lipid into lymph of female and male rats or in the relative proportions of oleic acid absorption into the chylomicron and VLDL fractions of females and

TABLE 2. Effect of puromycin on protein content and on [1-$^{14}$C]leucine incorporation into total lymph proteins and lipoprotein fractions[a]

| Measurement | Female | | Male | |
|---|---|---|---|---|
| | Control | Puromycin | Control | Puromycin |
| Total lymph protein, 2–6 hr | | | | |
| mg (2–6 hr) | 96.5 ± 6.6 | 44.2[b] ± 4.3 | 105.6 ± 17.8 | 58.0[b] ± 7.8 |
| dpm × $10^{-3}$ | 886 ± 33 | 380[b] ± 42 | 824 ± 82 | 535[b] ± 69 |
| Chylomicron protein | | | | |
| mg (2–6 hr) | 2.9 ± 0.2 | 1.2 ± 0.05 | 4.7 ± 1.0 | 2.0[b,c] ± 0.2 |
| dpm × $10^{-3}$ | 32 ± 2 | 21[b] ± 2 | 45 ± 13 | 29 ± 3 |
| VLDL protein | | | | |
| mg (2–6 hr) | 10.2 ± 1.7 | 2.7 ± 0.9 | 4.5 ± 0.1 | 3.4 0.1 |
| dpm × $10^{-3}$ | 118 ± 31 | 33[b] ± 6 | 62[c] ± 10 | 66[c] ± 2 |

[a] All values are means ± SEM.
[b] $P < 0.05$ between puromycin-treated and control animals.
[c] $P < 0.05$ between female and male animals.

males. Thus between the two fractions, 58–70% of total oleic acid transport in females and 64–80% in males was associated with chylomicrons. In females the proportion of labeled oleic acid increased in the VLDL fraction of lymph, but this occurred only in the later time period when oleic acid absorption was no longer maximal.

In contrast, there was an entirely different pattern with respect to cholesterol absorption. During the initial slower phase of cholesterol absorption (0–2 hr), a greater percentage of the absorbed cholesterol was associated with the chylomicron fraction in both sexes. However, the percentage of cholesterol transported in the chylomicron fraction was significantly higher, and that in the VLDL fraction was significantly lower in the male than in the female. As the rate of cholesterol absorption increased during the later time periods (2–6 hr), the distribution of cholesterol in the two transport particles was comparable in both sexes. These data strongly emphasize both the difference in transport forms of cholesterol and oleic acid in rat lymph and the relative importance of the non-chylomicron transport of cholesterol in each sex, particularly in the female.

The most compelling evidence for a sex difference in this lipoprotein fraction is derived from data on the protein levels and leucine incorporation into the VLDL fraction. In the male, the absolute amount of protein in the chylomicron and VLDL fractions did not change through the sequential lymph collection period, and this was largely mimicked by data on leucine incorporation into the protein of these fractions (Fig. 4). In the female, the cumulative level of protein and of leucine incorporation into the chylomicron proteins was comparable to that seen in the male. However, there was a major difference in these

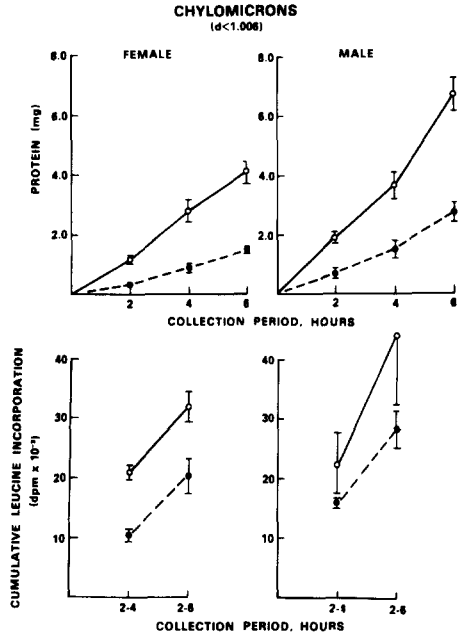


**Fig. 5.** Cumulative protein (upper panels) and [1-$^{14}$C]leucine incorporation (lower panels) in lymph chylomicrons ($d < 1.006$ g/ml) of control and puromycin-treated rats. Labeled leucine was administered 2 hr after the lipid dose. Open circles represent means ± SEM for four samples of chylomicrons from control rats at each time period. Closed circles represent means ± SEM for four samples of chylomicrons from puromycin-treated rats at each time period.

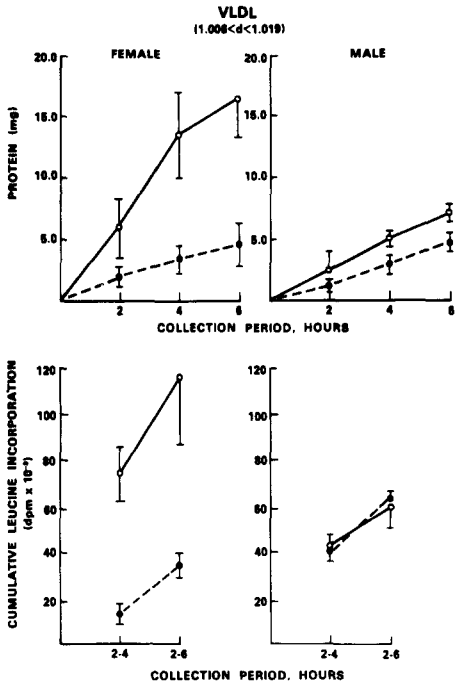


**Fig. 6.** Cumulative protein (upper panels) and [1-$^{14}$C]leucine incorporation (lower panels) in lymph VLDL ($1.006 < d < 1.019$ g/ml) of control and puromycin-treated rats. Labeled leucine was administered 2 hr after the lipid dose. Open circles represent means ± SEM for four samples from control rats at each time period. Closed circles represent means ± SEM for four samples from puromycin-treated rats.

same parameters with respect to the VLDL fraction. In the female, protein level in this fraction was consistently three to four times the protein level in the chylomicron fraction, and this difference was paralleled by the extent of leucine incorporation into the proteins of each lipoprotein fraction.

Additional evidence for the importance of the VLDL fraction in transport of absorbed exogenous cholesterol, particularly in the female, is provided by the data showing specific puromycin inhibition of leucine incorporation into this lipoprotein fraction accompanied by decreased cholesterol transport in VLDL.

The present studies do not address the apoprotein composition of the chylomicron and VLDL fractions in male and female rats. However, the work of others suggests that there is little qualitative difference in the apolipoprotein patterns of native chylomicrons and VLDL (small chylomicrons) of rat intestine or of mesenteric lymph (19, 20). The major apoproteins associated with these particles are apo B, apo A-I, apo A-IV, apo E and the C-apolipoproteins. Of these, apo B, apo A-I and apo A-IV are synthesized by the intestine in significant amounts (29–33), while apo E and apo C appear to be primarily of hepatic origin and are acquired upon secretion of the nascent transport particles into lymph (20, 33, 34).

It is generally held that apo B is a major determinant of chylomicron and VLDL transport by the intestine (7, 30, 35, 36). Recent studies (7, 17, 21, 36) suggest that the pool of apo B in the intestine is adequate to sustain maximal fat absorption unless excess amounts of lipid are infused into isolated intestinal loops (30). Thus, in our earlier study, the high intragastric lipid load may be predicted to have depleted apo B. Under this condition, puromycin administration to female rats, which are more sensitive to protein synthesis antagonists (10), resulted in significant inhibitions of both cholesterol and fatty acid absorption. In male rats, however, only cholesterol absorption was affected. In contrast, in the present study, the potential effect of the protein antagonist on gastric emptying and lymph flow has been circumvented, and the use of lower lipid doses has allowed a more precise differentiation of the effects of puromycin on individual transport particles, particularly in the female. Under these conditions, it may be predicted (30) that apo B was not a major limitation to the formation of transport particles. The continued secretion of chylomicrons and VLDL in males, and of chylomicrons in females under conditions of depressed intestinal protein synthesis supports the concept of a preformed pool of apo B, and suggests that the effect of puromycin in females may be related either to other apoproteins such as apo A-I or apo A-IV, or to phospholipids.

Apo A-I is a major constituent of intestinal chylomicrons and VLDL (31, 32), is synthesized in the intestine (31, 32, 37), and turns over rapidly (31). Apo A-IV is also synthesized in intestine (32, 33). These apoproteins, in our study, are likely candidates for the more rapid synthesis demonstrated in the VLDL fraction of the female relative to the male and for the puromycin sensitivity of the VLDL fraction in the female. It seems possible that, in an analogous manner to the estrogen effect on hepatic apo VLDL-II synthesis (38), apo A-I or apo A-IV synthesis in the intestine has a hormonal component, and that one or more of these apoproteins are essential for assembly or secretion of the cholesterol-transporting "VLDL" particle. There is, as yet, little evidence for direct hormonal regulation of intestinal apolipoprotein synthesis.

Finally, the effect of puromycin on phospholipid synthesis must be considered. In isolated mucosal cells, puromycin results in a marked depression of both protein and phosphatidylcholine synthesis, under conditions where triglyceride biosynthesis is unaffected (39). Furthermore, the synthesis of phos-

phatidylcholine in the intestine has been reported to affect polysome profiles (40). In bile duct-cannulated rats, phosphatidylcholine and polysome formation are both decreased and, under these conditions, there is little secretion of lipoprotein (40). Administration of lysophosphatidylcholine to these animals results in a restoration of polysome profiles, lipoprotein synthesis, and chylomicron release (40). Related to this, evidence from our earlier studies (10) indicates that intestinal phospholipid synthesis may also have a hormonal component, and is affected by puromycin. In females, the level of [1-$^{14}$C]oleic acid incorporation into intestinal phospholipid was almost three times that seen in males. Administration of puromycin to females resulted in a marked decrease in labeled phospholipid to a level comparable to that in control males; in contrast, the inhibitor had no effect on intestinal phospholipid labeling in males. These data mimic the comparative effects of puromycin on VLDL protein synthesis in female and male rats as shown in the present studies. The relationships of these separate observations have yet to be elucidated.

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