Dietary supplementation with Pluronic L-81 modifies hepatic secretion of very low density lipoproteins in the rat

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Abstract Supplementation of high fat/cholesterol-enriched diets with polyoxypropylene-polyoxyethylene copolymers containing 90% hydrophobic constituents has been found to impair enteric secretion of chylomicrons, lower plasma levels of very low density (VLDL) and low density (LDL) lipoprotein cholesterol and prevent diet-induced hypercholesterolemia and atherosclerosis. These agents are known to be absorbed from the gastrointestinal tract and excreted in bile. In order to determine whether dietary supplementation with this group of hydrophobic poloxalenes influences hepatic secretion of triglyceride-rich lipoproteins, groups of rats were maintained for 21–34 days on either *1*) standard chow, *2*) semisynthetic diet containing 10.0% safflower oil/1.0% cholesterol, or *3*) each of the above diets supplemented with the hydrophobic poloxalene Pluronic L-81. At the end of the feeding period, newly secreted hepatic VLDL were isolated from 2-hr recirculating liver perfusates, quantitated, and characterized. Compared to perfusions in chow-fed rats, perfusion experiments in rats fed the high fat/cholesterol-enriched semisynthetic diet revealed *1*) a 3.1-fold increased net hepatic VLDL secretion rate; *2*) enrichment of secretory VLDL in cholesteryl esters and in C18:2 core lipid fatty acids; and *3*) a shift in the size distribution of secretory VLDL towards larger particles. When the 0.5% Pluronic L-81 was included in the high fat/cholesterol-enriched semisynthetic diet, the net hepatic VLDL secretion rate fell significantly and the physicochemical properties of secretory VLDL in these rats were found to resemble those of chow-fed animals. Supplementation of the chow diet with L-81 resulted in a significant fall in the net hepatic VLDL secretion rate from that observed in rats fed chow alone. Compared to rats fed chow alone, perfusate VLDL from rats fed each of the other experimental diets contained markedly lower amounts of both apoB molecular weight variants, as analyzed by gradient gel electrophoresis and densitometric gel scanning. Since previous studies have demonstrated that *1*) VLDL are the major cholesterol transport lipoproteins following fat/cholesterol feeding; *2*) a precursor-product relationship exists between fat/cholesterol-induced hepatic VLDL and plasma VLDL; *3*) such particles are capable of delivering cholesterol to the arterial wall; and *4*) dietary supplementation with hydrophobic poloxalenes prevents both the increase in plasma VLDL-cholesterol and diet-induced atherosclerosis, it is possible that dietary supplementation with hydrophobic poloxalenes may influence the atherogenic process through direct and/or indirect effects on hepatic VLDL transport.—**Manowitz, N. R., P. Tso, D. S. Drake, S. Frase, and S. M. Sabesin.** Dietary supplementation with Pluronic L-81 modifies hepatic secretion of very low density lipoproteins in the rat. *J. Lipid Res.* 1986. **27:** 196–207.

Supplementary key words hydrophobic poloxalenes • diet-induced hypercholesterolemia • atherosclerosis • nascent hepatic lipoproteins • apolipoprotein B variants

Studies in several animal species have demonstrated that administration of cholesterol/fat-enriched diets supplemented with hydrophobic poloxalenes at subtoxic levels prevents diet-induced hypercholesterolemia and atherosclerosis, and lowers plasma levels of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol (1–4). Pluronic L-81, a member of this class of agents, is a nonionic poloxalene copolymer surfactant whose basic structure contains a hydrophobic polyoxypropylene base positioned between hydrophilic polyoxyethylene end groups. The final molecule (M_r 2800) is synthesized to yield 90% of its weight as hydrophobic residues and 10% as hydrophilic.

Tso and co-workers (5–7), using the lymph duct fistula rat model, have shown that intraduodenal infusion of L-81 in combination with dietary lipid results in markedly depressed intestinal transport of absorbed lipid into

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SGPT, serum glutamic-pyruvic transaminase; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; $apoB_H$ and $apoB_L$, high and low molecular weight apoB variants; TG, triglyceride; L-81, Pluronic L-81; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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lymph, associated with enterocyte accumulation of absorbed re-esterified lipid. Electron microscopic studies in rats following infusion of a lipid emulsion containing L-81 at a dosage of 0.5 or 1.0 mg/hr of surfactant revealed the presence only of 200–800-Å sized particles in lymph, the absence of chylomicrons (1000–4000 Å particles), and the accumulation of large lipid droplets within enterocyte smooth endoplasmic reticulum (6), suggesting an effect of L-81 on assembly and/or secretion of triglyceride-rich lipoproteins.

Rodgers et al. (8) have recently demonstrated in the rat that following intraduodenal infusion of a radiolabeled analogue of L-81 (poloxalene 2930), approximately 20% of an infused dose was recovered in bile, indicating that hydrophobic poloxalenes can be absorbed and metabolized by the liver. Since the liver utilizes fatty acids of dietary triglyceride origin as one substrate for synthesis of triglyceride-rich lipoproteins (9, 10), it is conceivable that hydrophobic poloxalenes may impair hepatic secretion of these particles by reducing the intestinal flux of dietary fatty acids to the liver, by direct effect on hepatic VLDL assembly, or by a combination of these effects. The objective of the current study was to address the primary question of whether chronic dietary supplementation with hydrophobic poloxalenes does, in fact, modify hepatic VLDL secretion. Using the isolated perfused rat liver, we investigated the effect of L-81 supplementation of either a basal chow diet or a fat/cholesterol-enriched semisynthetic diet on secretion rates and physicochemical properties of hepatic VLDL. Investigation of the effect of dietary L-81 on hepatic VLDL secretion in fat/cholesterol-fed rats was of particular interest because a number of studies have demonstrated VLDL to be the major cholesterol transport particles under various conditions of dietary fat and cholesterol supplementation (1, 11, 12).

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were received at least 10 days prior to experimentation, and were maintained on standard laboratory chow (Purina Rodent Chow 5001, Ralston Purina, St. Louis, MO) and water ad lib during this period. At the beginning of the experimental feeding period, their weights ranged from 212 to 288 g. For the dietary studies, rats were divided into four groups and maintained on one of the following diets, which were administered in powdered form for periods ranging from 21 to 34 days: *1*) standard chow (containing ~ 4.5% fat as stated by manufacturer and 0.007–0.008% cholesterol (w/w) as analyzed by gas–liquid chromatography; *2*) high fat/cholesterol: the fat-free semisynthetic diet of Wooley and Sebrell (13) was modified according to Tso, Balint, and Rodgers (7) and contained 10.0% safflower oil and 1.0% cholesterol (w/w, formulated by ICN Nutritional Biochemicals, Cleveland, OH); *3*) chow + L-81: standard chow containing 0.5% Pluronic L-81 (w/w) (L-81 was a gift from BASF Wyandotte, Wyandotte, MI); *4*) high fat/cholesterol + L-81: diet No. 2 containing 0.5% L-81 (w/w) (7). The approximate percentages of total calories derived from carbohydrate, protein, and fat in the standard chow diets were 60.0, 28.0, and 12.0%, respectively, and were 52.1, 23.2, and 24.7%, respectively, for the high fat/cholesterol-supplemented defined semisynthetic diets. L-81 was incorporated into the diets as an ether spray, after which the solvent was evaporated in a fume hood overnight. Upon termination of the feeding period, rats were used either for liver perfusion or killed for blood collection between 9 AM and 11 AM without prior fasting. Rats were not fasted because of the rapid reversibility of the effects of L-81, as previously shown in intestinal lipid transport studies (6).

Liver perfusion and VLDL isolation

Rats were anesthetized with sodium pentobarbital, 60 mg/kg i.p. Livers were perfused by the in situ technique (14) in recirculating fashion, using a temperature-controlled 37°C cabinet. Immediately prior to portal vein cannulation, a 2-ml blood sample was drawn from the abdominal aorta into dipotassium EDTA solution (1.5 mg/ml blood) for analysis of plasma lipid and transaminase concentrations. The basic perfusate consisted of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 0.3% glucose and 3.0% bovine albumin (Fraction V, Sigma Chemical Co., St. Louis, MO), and was combined with sufficient thrice-washed outdated human erythrocytes to give a hematocrit of 23–24%. The perfusate was equilibrated with 95% O_2/5% CO_2 by passage through a silastic tubing "lung" (15). The starting reservoir perfusate volume was 125 ml. Forty ml was initially flushed through the liver to discard, after which the remaining 85 ml was recirculated for 2 hr. Flow rate was maintained at 13 ml/min over the course of the perfusions. Liver viability was monitored by analysis of pre- and post-liver perfusate samples for pO_2 and pH every 30 min, using an IL Micro 13 blood gas analyzer (Instrument Laboratories, Lexington, MA). Perfusate pH was maintained between 7.32 and 7.46 by small additions of 8.4% $NaHCO_3$. Hepatic O_2 uptake data after 2 hr of perfusion were (μmol/g liver per min, mean ± SEM n=5 for each group): chow, 1.77 ± 0.03; chow + L-81, 1.67 ± 0.04; high fat/cholesterol, 1.50 ± 0.04; high fat/cholesterol + L-81, 1.64 ± 0.06. The slightly lower O_2 uptake in the high fat/cholesterol-fed group may have been due to hepatomegaly secondary to fatty infiltration (livers in the high fat/cholesterol-fed

group averaged 15.4 ± 0.3 g, which was ~ 20% greater than livers from the other groups). Viability was also reflected by maintenance of flow rate without perfusate leakage, and by the absence of visible hepatic edema, mottling, or cyanosis. The perfusion was terminated by flushing the perfusion circuit with 25 ml of ice-chilled 0.9% NaCl, after which livers were weighed. Erythrocytes were removed by low speed centrifugation at 4°C and the perfusate plasma was combined with 1/100 volume of d 1.006 g/ml NaCl, containing 0.01% chloramphenicol, 2.0% NaN_3, 0.005% gentamicin sulfate, and 1.0% disodium EDTA. Ultracentrifugal isolation of VLDL was begun within 24 hr of each perfusion. Eight ml of perfusate per tube was overlaid with 4 ml of d 1.006 g/ml NaCl solution containing 0.01% EDTA, 0.02% NaN_3, 0.0001% chloramphenicol, and 0.00005% gentamicin and centrifuged in an SW 41 Ti rotor for 24 hr at 35,000 rpm in a Beckman L8 70 ultracentrifuge (Beckman Instruments, Spinco Div., Palo Alto, CA). VLDL were separated by tube slicing and washed once by centrifugation under the above conditions. Plasma VLDL for apoprotein identification were similarly isolated from fed rats exsanguinated under diethyl ether anesthesia. VLDL were dialyzed at 4°C for 48 hr against two changes of at least 100 volumes of 0.01% EDTA, 0.05% NaN_3, 0.0001% chloramphenicol, and 0.00005% gentamicin, pH 7.4. Aliquots were retained for protein assay and negative stain electron microscopy, and the remainder was lyophilized.

Lipid extraction and quantitative analyses

VLDL were delipidated and the lipid classes were separated and isolated by thin-layer chromatography (TLC) and solvent elution procedures of Swift et al. (16). VLDL cholesterol was assayed by the method of Rudel and Morris (17), VLDL triglycerides by a modification of the method of Stern and Shapiro (18), and VLDL lipid phosphorus according to Parker and Peterson (19), using a correction factor of 25× to convert to phospholipid. VLDL protein was estimated in aqueous samples by the Coomassie microprotein technique (20), using a bovine albumin standard.

Plasma for lipid and transaminase assay was obtained from non-fasted rats exsanguinated under ether, and was analyzed for triglycerides, cholesterol, and phospholipid according to previously described methods (21). Glutamic-pyruvic transaminase (GPT) was assayed by the method of Wroblewski and LaDue (22), using the Sigma UV spectrophotometric assay (Sigma Chemical Co., St. Louis, MO, kit No. 55UV). Cholesterol content of standard chow was measured by GLC following extraction (23); 5α cholestane was used as an internal standard.

Qualitative analyses of fatty acids

For analysis of VLDL fatty acid composition, final chloroform extracts of eluted TLC fractions used for quantitative lipid analyses were dried under N_2, methylated according to Balint et al. (24), and analyzed on a Hewlett-Packard 5840A gas chromatograph, using 6′ × 1/8″ glass columns packed with 10% SP2330 on 100/120 mesh Chromosorb W (Supelco Inc., Bellefonte, PA). Dietary triglyceride fatty acids were analyzed by GLC following extraction (23), TLC isolation, and methylation. Peaks were identified by use of standard mixtures of fatty acid methyl esters (Nu-Chek Prep, Elysian, MN).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Apoproteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by a modification of the method of Laemmli (25), using 3.5–20.0% acrylamide gradient slab gels. Bands were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in methanol–acetic acid–water 5:1:4 and destained in the solutions described by Weber and Osborn (26). Apoproteins were identified on the basis of molecular weights relative to known protein standards, and by comparison with published SDS gel electrophoretograms of rat apoproteins (21, 27, 28). When plasma apoVLDL was not present on the same gel for identification of perfusate VLDL apoB variants (see results, Fig. 4C), the variants were identified on the basis of their expected mobility relative to molecular weight standards, as determined from gels run in identical fashion in which plasma apoVLDL (containing high and low molecular weight apoB variants) and standards were run simultaneously (see results, Fig. 4A and 4B). Stained gels were densitometrically scanned using an LKB 2202 Ultroscan Laser Densitometer interfaced to a Model 2220 recording integrator (LKB Instruments, Bromma, Sweden). In order to estimate the triglyceride (TG)/apoB ratio, the "mass" output of apoB was calculated by multiplying the average percent dye uptake for total apoB by the average total protein output. Although this does not yield a true estimate of apoB mass (owing to differences among apoprotein chromogenicities), it is useful for comparing relative outputs under identical experimental conditions.

Morphologic studies

Lipoproteins were studied by negative stain electron microscopy according to Cartwright et al. (21). The diameters of 200 free-standing particles in each group were measured with an ocular micrometer and calculated as the mean of two estimates perpendicular to each other.

For histologic studies, livers of rats exsanguinated for plasma lipid analyses were either perfusion-fixed via the portal vein with 0.5% glutaraldehyde in 0.2% M cacodylate buffer at a flow rate of 13 ml/min or were immersion-fixed in the same solution. Tissue was post-fixed in 2.0% OsO_4 in cacodylate buffer, dehydrated through graded alcohols, and embedded in Spurr's epoxy resin. Thick sections (1.5 μm) were photographed after staining with toluidine blue.

Statistics

Unless otherwise indicated, data are reported as mean ± SEM. Significance of group differences was analyzed by two-tailed Student's *t*-test for independent means.

RESULTS

Effect of diet on growth rates, plasma lipids, and transaminase concentration

The effect of the various dietary regimens on growth rates, plasma lipids, and SGPT concentrations is shown in **Table 1.** Since no trends were observed in plasma lipid or transaminase levels among rats sampled after 21 or 22 days of feeding and those sampled at later terminal feeding points (days 28–34), data from all rats fed for the time interval specified in each group were averaged. In addition, since there were no significant differences in plasma lipid or SGPT concentrations between rats used for liver perfusion and those used for exsanguination in each feeding group, the data in Table 1 represent values obtained from rats used for both purposes. As seen in Table 1, animals consuming the high fat/cholesterol-enriched semi-synthetic diet gained weight at a significantly greater rate than those consuming the other diets. Neither L-81 nor lipid supplementation of the various diets produced hepatocellular injury, as reflected by lack of elevation of plasma SGPT in comparison to chow-fed animals. Divergent results were obtained for the effect of the surfactant on plasma triglycerides; L-81 exerted a hypotriglyceridemic effect when compared to chow diet alone (group B vs A) but did not lower plasma triglycerides from levels induced by the high fat/cholesterol-enriched diet (group D vs C). The same divergence was found with respect to the effect of L-81 on plasma phospholipid levels.

Effect of diet on hepatic morphology

The light microscopic appearance of livers from rats in the four groups is shown in **Fig. 1.** When compared to livers from chow-fed rats (Fig. 1A) the abundant osmiophilic intracytoplasmic droplets from the enlarged livers of rats fed the high fat/cholesterol-enriched diet are apparent (Fig. 1C). Compared to high fat/cholesterol-fed rats, intracytoplasmic droplets were much less abundant in hepatocytes from rats fed the high fat/cholesterol + L-81-supplemented diet. The morphologic changes observed in livers of rats fed the high fat/cholesterol-enriched diet were consistent with biochemical evidence of fatty liver previously demonstrated to be produced by administration of this diet (4, 29). Likewise, the paucity of cytoplasmic droplets in hepatocytes of rats fed the high fat/cholesterol + L-81-supplemented diet in the present study is consistent with the finding of Bochenek and Rodgers (4) that dietary L-81 reduces the hepatic lipid content from levels produced by the high fat/cholesterol diet alone.

TABLE 1. Effect of diet upon growth rates, plasma lipids, and transaminase concentrations[a]

Group[b]	Days Fed	Weight Gain[c]	Total Cholesterol[d]	Triglycerides[e]	Phospholipids[f]	GPT (Wroblewski-LaDue units/ml)
		g/day	*mg/dl*	*mg/dl*	*mg/dl*	
(A) Chow	21–34 (34)	3.2 ± 0.2 (34)	60.5 ± 1.4 (29)	51.2 ± 2.5 (29)	184.2 ± 1.9 (29)	35.4 ± 3.1 (10)
(B) Chow + L-81	21–32 (22)	2.9 ± 0.2 (22)	46.8 ± 2.0 (22)	36.6 ± 2.5 (22)	171.9 ± 4.3 (22)	36.5 ± 1.4 (12)
(C) High fat/ cholesterol	21–28 (21)	4.4 ± 0.1 (21)	95.1 ± 4.3 (17)	68.1 ± 4.1 (17)	211.1 ± 4.0 (17)	25.3 ± 1.6 (9)
(D) High fat/ cholesterol + L-81	22–30 (20)	2.8 ± 0.2 (20)	78.1 ± 2.1 (20)	79.3 ± 3.9 (20)	220.1 ± 4.2 (20)	30.9 ± 3.7 (8)

[a]Values are means ± SEM; numbers in parentheses denote number of rats in each group. Only those statistical differences relevant to the text are cited in the legend.
[b]Data pooled from rats used for perfusion and for exsanguination.
[c]Represents total weight gain/days fed. Since growth rates were not monitored frequently, linearity of growth rate should not be inferred. Weight gain for (C) was significantly greater versus (A), (B), and (D).
[d]Significant differences ($P < 0.001$): (A) versus (B); (C) versus (D).
[e](A) significantly greater than (B) at $P < 0.001$; $P > 0.05$ for (C) versus (D).
[f](A) significantly greater than (B) at $P < 0.02$; $P > 0.05$ for (C) versus (D).

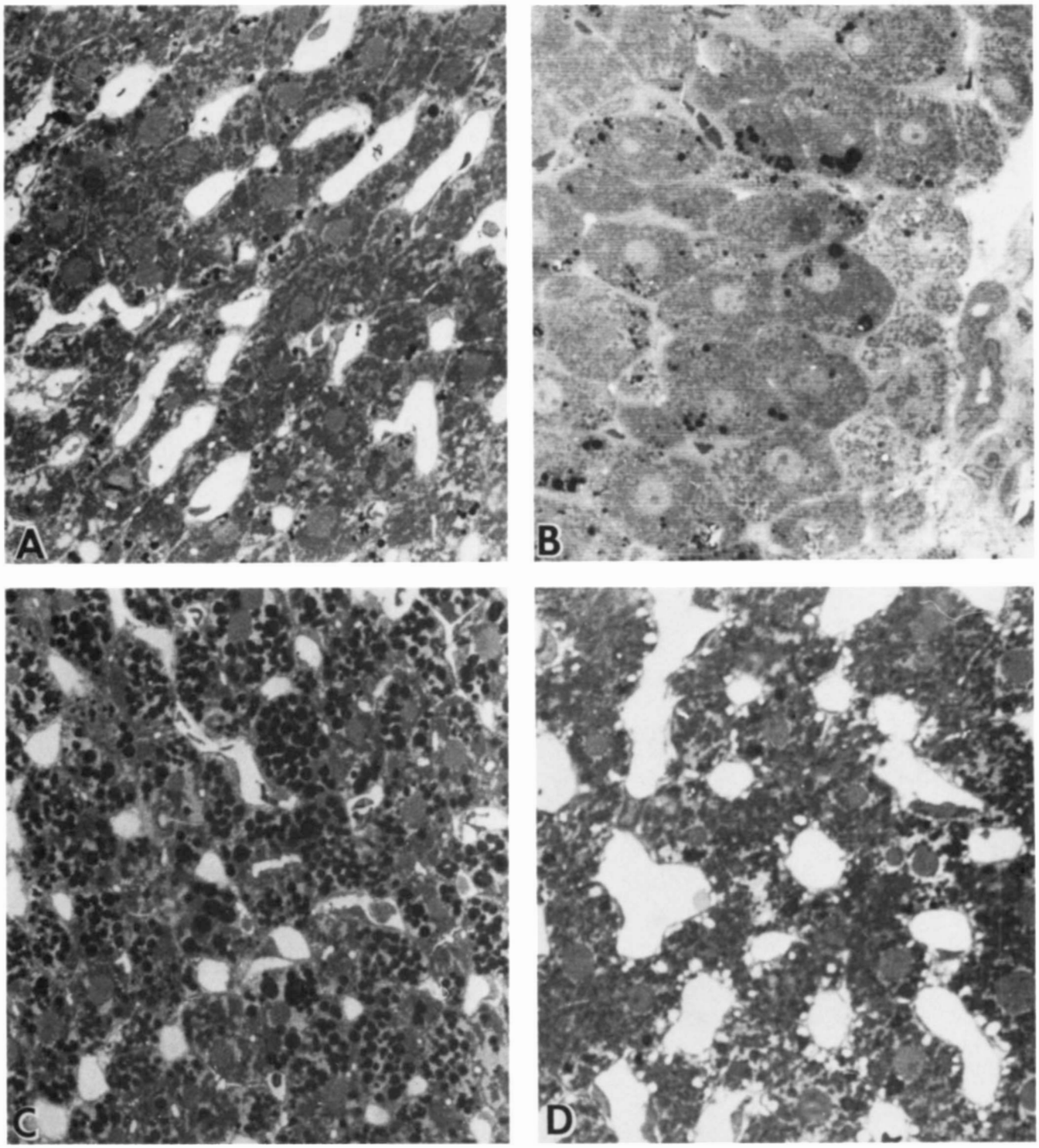

Fig. 1. Effect of diet on histologic appearance of liver. Thick sections (1.5 μm) were prepared according to Methods and stained with toluidine blue. Magnification × enlargement = 1600. A, Chow; B, chow + L-81; C, high fat/cholesterol; D, high fat/cholesterol + L-81.

Effect of diet on morphology and particle size distribution of newly secreted hepatic VLDL

The morphologic characteristics of hepatic perfusate VLDL were evaluated by negative stain electron microscopy (**Fig. 2**). In all groups, VLDL appeared as essentially spherical particles. In chow-fed and chow + L-81-fed rats (Fig. 2A and 2B), perfusate VLDL ranged from approximately 250 to 1000 Å in diameter, while administration of the high fat/cholesterol-enriched semisynthetic

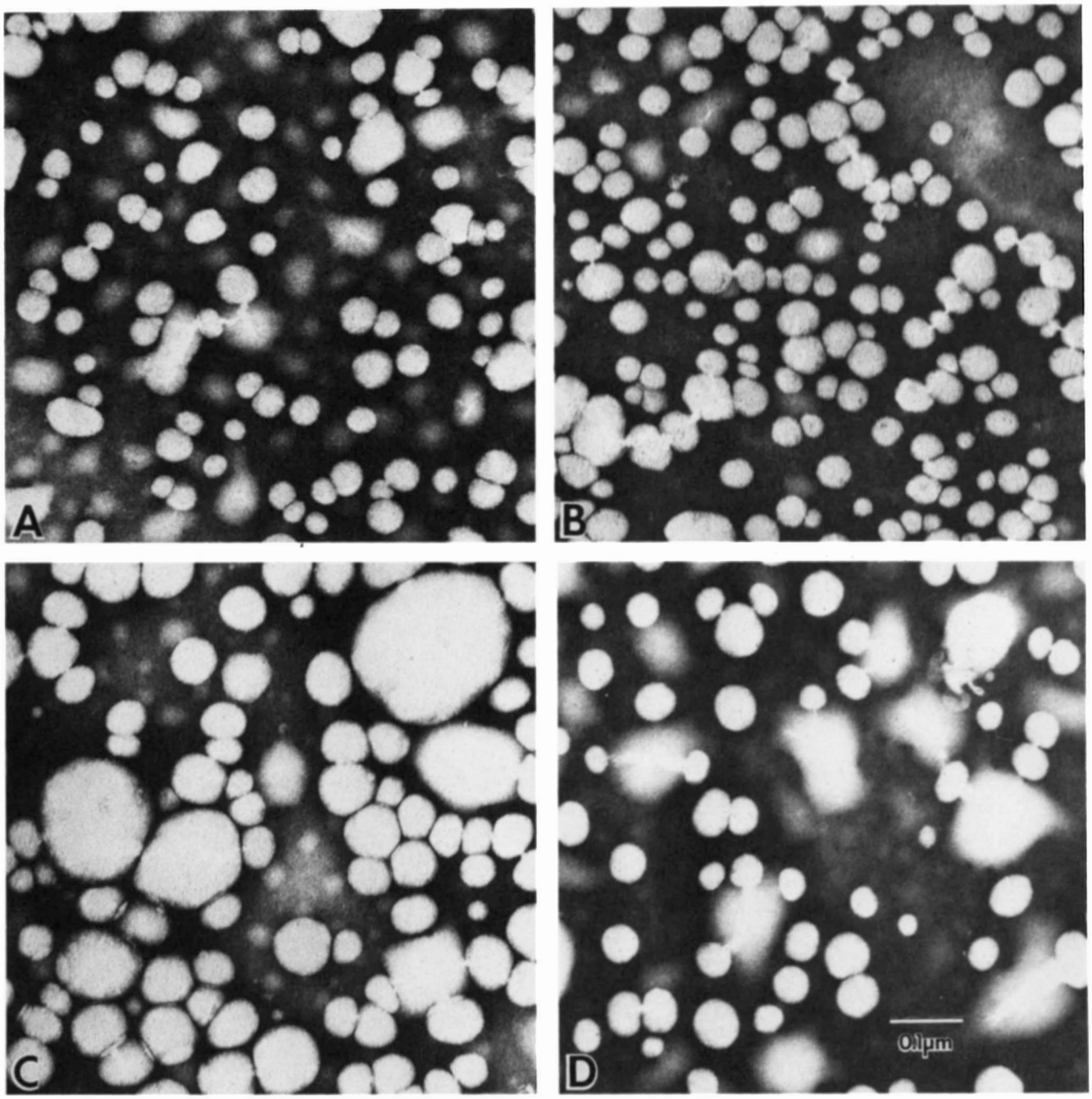

Fig. 2. Negative stain electron photomicrographs of hepatic perfusate VLDL. Particles were stained with 2.0% Na phosphotungstate and sized with an ocular micrometer. Magnification × enlargement = 120,000. A, Chow; B, chow + L-81; C, high fat/cholesterol; D, high fat/cholesterol + L-81.

diet resulted in secretion of a spectrum of particles containing VLDL of considerably larger size (Fig. 2C). Inclusion of L-81 in the high fat/cholesterol-enriched diet resulted in secretion of VLDL having a size distribution similar to that of perfusate VLDL from chow-fed rats.

The differences in particle size distribution are reflected in the histogram in **Fig. 3.** Perfusate VLDL from the high fat/cholesterol-fed group demonstrated a clear shift in distribution toward larger particles (Fig. 3C). The average size VLDL in this group was nearly 1.5-fold larger in diameter than perfusate VLDL from chow-fed rats (Fig. 3A legend).

Effect of diet on net secretory rates of hepatic VLDL

Table 2 presents the net secretion rates of the lipid and protein constituents of hepatic VLDL. The data are expressed on the basis of output per organ rather than per

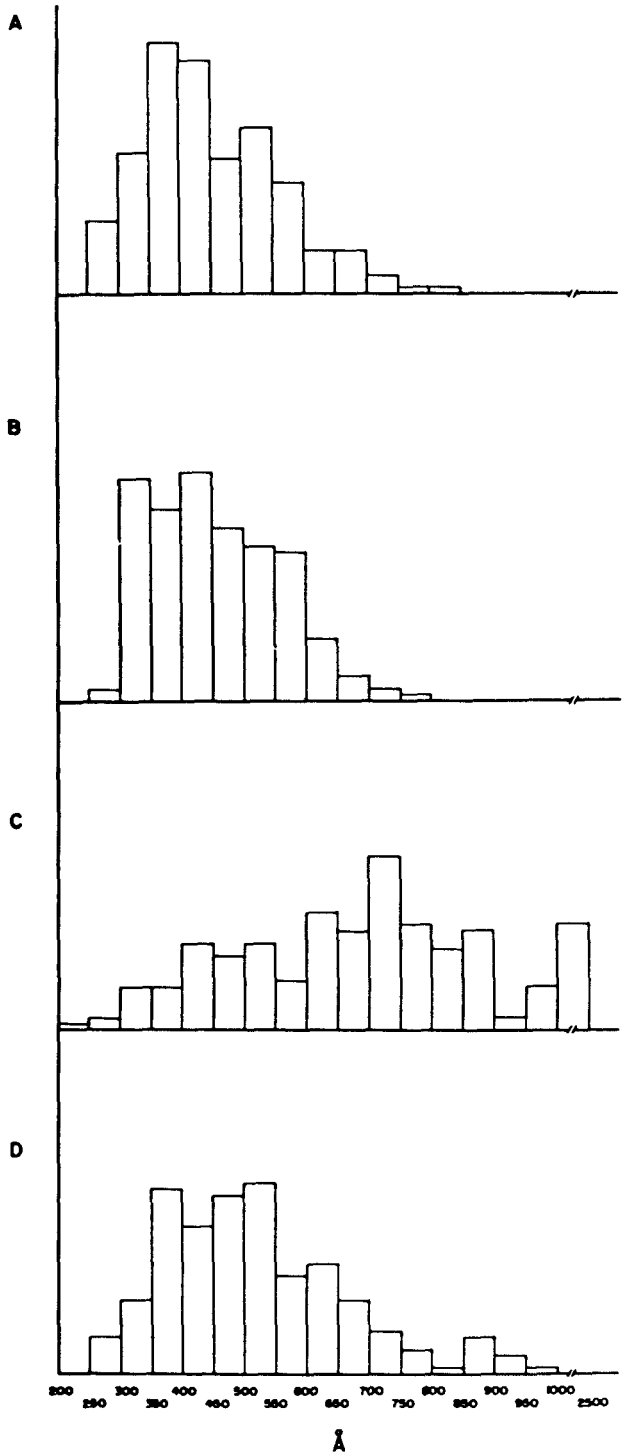

Fig. 3. Histogram showing VLDL size distribution in liver perfusates. Two-hundred particles were sized for each panel. Bar height is proportional to number of particles in each interval. A, Chow; B, chow + L-81; C, high fat/cholesterol; D, high fat/cholesterol + L-81. Mean diameters (± SE): A, 449.3 ± 7.8 Å; B, 453.7 ± 7.3 Å; C, 662.3 ± 28.2 Å; D, 497.0 ± 17.5 Å.

unit mass because of the increased liver mass secondary to fatty infiltration in the high fat/cholesterol-fed group.

The net secretory rate of VLDL triglyceride in chow-fed rats averaged approximately 3100 μg/liver per 2 hr. In contrast, when L-81 was included in the chow diet, the net secretory rate of VLDL triglyceride as well as that of total VLDL was reduced by approximately 75%. The highest VLDL secretory rates were observed in rats fed the high fat/cholesterol-enriched semisynthetic diet. In contrast to chow-fed rats, net VLDL triglyceride secretion in these rats averaged nearly 8300 μg/liver per 2 hr, a 2.7-fold increase over chow-fed rats. The difference in VLDL esterified cholesterol secretory rate was even more pronounced, e.g., livers of rats fed the high fat/cholesterol diet were found to secrete over seven times as much VLDL cholesterol as livers of chow-fed rats (1715 vs 233 μg/liver per 2 hr). The average total VLDL secretory rate in the high fat/cholesterol-fed rats was approximately 3.1-fold higher than in chow-fed rats. Inclusion of L-81 in the high fat/cholesterol-enriched diet significantly reduced the net secretory rates of each of the VLDL moieties.

Effect of diet on lipid and protein composition of newly secreted hepatic VLDL

The lipid and protein composition of hepatic perfusate VLDL is shown in **Table 3.** While VLDL in all groups were found to be essentially triglyceride-rich particles, the weight percent of triglycerides was significantly greater (66.0%) in VLDL from chow-fed rats than in VLDL from high fat/cholesterol-fed rats (55.1%). This was accompanied by a significant (2.2-fold) increase in weight percent of VLDL cholesteryl esters in high fat/cholesterol-fed rats versus chow-fed animals. Inclusion of L-81 in the high fat/cholesterol diet significantly reduced the weight percent of VLDL cholesteryl esters to a value that was not significantly different from that of chow-fed rats.

Table 4 presents the fatty acid composition of the core lipid moieties of hepatic secretory VLDL. In chow-fed rats, C18:2 fatty acids represented 15.1 and 16.7% of VLDL triglyceride and cholesteryl ester fatty acids, respectively. When rats were fed the high fat/cholesterol-supplemented diet (enriched in C18:2 triglyceride fatty acids), the percentage of C18:2 triglyceride fatty acids was found to be approximately 2.4-fold greater than in VLDL from chow-fed rats. Likewise, the percentage of C18:2 cholesteryl ester fatty acids was approximately 1.5-fold greater in VLDL from high fat/cholesterol-fed rats than in VLDL from chow-fed animals. Inclusion of L-81 in the high fat/cholesterol-enriched diet significantly reduced the percentages of C18:2 triglyceride and cholesteryl ester fatty acids to values that were not significantly different from those of chow-fed animals.

The distribution of the major core lipid fatty acids in perfusate VLDL from chow + L-81-fed rats was similar to that of chow-fed animals. The percentage of C18:2 phospholipid fatty acids was not significantly different among the four groups.

TABLE 2. Effect of diet on net secretion of hepatic VLDL[a]

Group[b]	Triglycerides	Phospholipids	Unesterified Cholesterol	Esterified Cholesterol	Protein
			μg per liver per 2 hr		
(A) Chow[c,d,e]	3103 ± 580	446 ± 86	214 ± 21	233 ± 31	490 ± 58
(B) Chow + L-81	737 ± 209	73 ± 17	87 ± 23	159 ± 29	191 ± 47
(C) High fat/ cholesterol[f]	8266 ± 809	1407 ± 206	649 ± 80	1715 ± 233	1902 ± 264
(D) High fat/ cholesterol + L-81	4485 ± 1309	911 ± 206	318 ± 51	438 ± 105	851 ± 168

[a]Livers were perfused with erythrocyte-containing buffer for 2 hr in recirculating fashion as described in Methods. VLDL were isolated from perfusates by ultracentrifugation at d 1.006 g/ml. Values are means ± SEM. Only those statistical differences relevant to the text are cited in the legend.
[b]N = 5 for each group.
[c](A) significantly greater than (B) for all paramenters at $P < 0.01$, except for esterified cholesterol ($P < 0.001$).
[d](A) significantly less than (C) for all parameters at $P < 0.001$, except for phospholipids ($P < 0.01$).
[e](A) not significantly different from (D) ($P > 0.05$) for all parameters.
[f](C) significantly greater than (D) for all parameters at $P < 0.01$, except for triglycerides ($P < 0.05$).

Effect of diet on apolipoprotein composition of newly secreted hepatic VLDL

The apolipoprotein composition of hepatic perfusate VLDL was analyzed by gradient gel SDS-PAGE and densitometric gel scanning, and is presented in **Fig. 4** and **Table 5.** The principal apoproteins of VLDL from chow-fed rats (Fig. 4A) were the two apoB molecular weight variants ($apoB_H$ or high molecular weight apoB and $apoB_L$ or low molecular weight apoB), apoE, and the low molecular apoC + A-II bands. As seen in Table 5, VLDL apoB from chow-fed rats comprised approximately 25% of Coomassie blue-stainable material. By SDS-PAGE, a band migrating between $apoB_H$ and $apoB_L$ was consistently observed in perfusate VLDL from chow-fed rats. Although the identity of this band is not known, it was not considered to be either artifactual, because of its reproducibility, nor a degradation product because of its absence from plasma apoVLDL (lane 2), which was prepared under identical conditions. By SDS-PAGE and gel scanning, the apoprotein composition of perfusate VLDL from rats fed the high fat/cholesterol-enriched semisynthetic diet (Fig. 4B) was found to differ from that of chow-fed rats. Compared to chow-fed rats, VLDL from the high fat/cholesterol-fed group were relatively depleted of both $apoB_H$ and $apoB_L$ and were relatively (though not significantly) enriched in apoE. When Pluronic L-81 was added to either the chow diet or to the high fat/cholesterol-enriched diet, hepatic VLDL from each of these groups (Fig. 4C) were likewise observed to be markedly depleted in content of both apoB variants compared to chow-fed rats.

TABLE 3. Effect of diet on lipid and protein compostion of hepatic VLDL[a]

Group[b]	Triglycerides	Phospholipids	Unesterified Cholesterol	Cholesteryl[c] Esters	Protein
			% Wt		
(A) Chow	66.0 ± 3.0[d]	9.6 ± 1.4	4.8 ± 0.5	8.6 ± 1.4[e,f]	10.9 ± 0.9
(B) Chow + L-81	59.2 ± 4.1	6.1 ± 1.3	7.9 ± 1.1	10.5 ± 2.5	16.4 ± 0.5
(C) High fat/ cholesterol	55.1 ± 1.0	9.2 ± 0.8	4.3 ± 0.2	18.8 ± 0.8	12.6 ± 1.0
(D) High fat/ cholesterol + L-81	59.2 ± 2.9	13.2 ± 1.4	4.8 ± 0.5	10.4 ± 1.0[e]	12.3 ± 1.1

[a]Rat livers were perfused and VLDL were isolated as in Table 2. Values are means ± SEM. Only those statistical differences relevant to the text are cited in the legend.
[b]N = 5 for each group.
[c]Cholesteryl ester, esterified cholesterol × 1.67 (16).
[d](A) significantly greater than (C) at $P < 0.05$.
[e](A) and (D) significantly less than (C) at $P < 0.001$.
[f](A) not significantly different from (D) ($P > 0.05$).

TABLE 4. Effect of diet on fatty acid composition of the core lipids of hepatic VLDL[a]

Fatty Acid[b]	Chow (A) TG[c] (5)	Chow (A) CE[d] (5)	Chow + L-81 (B) TG (2)	Chow + L-81 (B) CE (3)	High Fat/Cholesterol (C) TG (4)	High Fat/Cholesterol (C) CE (4)	High Fat/Cholesterol + L-81 (D) TG (3)	High Fat/Cholesterol + L-81 (D) CE (3)	Dietary TG Chow (2)	Dietary TG Safflower Oil (2)
					%					
14:0	0.7 ± 0.2	1.1 ± 0.6			0.7 ± 0.2	0.3 ± 0.1	0.7 ± 0.2	0.3 ± 0.1	1.3 ± 0.5	
16:0	31.2 ± 1.3	18.3 ± 1.7	25.5 ± 5.0	19.8 ± 1.9	19.9 ± 1.5	13.5 ± 0.8	23.1 ± 0.8	12.7 ± 0.1	26.4 ± 0.4	6.8 ± 0.1
16:1	5.2 ± 3.4	8.8 ± 1.1	5.3 ± 3.0	7.9 ± 1.2	6.3 ± 0.6	7.2 ± 0.9	7.8 ± 0.7	7.1 ± 0.6		
18:0	8.2 ± 0.6	13.5 ± 0.7	11.8 ± 1.4	15.1 ± 0.8	5.0 ± 1.0	11.6 ± 0.6	9.2 ± 1.2	13.0 ± 0.2	17.4 ± 0.1	2.0 ± 0
18:1	28.5 ± 1.4	14.6 ± 1.7	24.3 ± 8.9	16.8 ± 1.6	18.6 ± 1.0	20.2 ± 1.9	22.0 ± 1.6	19.1 ± 1.0	36.9 ± 0.1	12.7 ± 0.1
18:2	15.1 ± 1.4	16.7 ± 1.5	19.5 ± 0.5	16.0 ± 1.0	36.7 ± 2.0[e]	25.6 ± 0.4[e]	20.9 ± 2.2	17.5 ± 0.4	17.0 ± 0.1	78.5 ± 0.3
18:3	2.1 ± 0.6	9.1 ± 1.1	3.8 ± 3.5	7.5 ± 0.4	2.0 ± 0.6	9.2 ± 0.9	2.6 ± 0.7	9.3 ± 0.4	0.8 ± 0.1	
20:4	1.4 ± 0.2	2.3 ± 1.0	2.9 ± 0.9	0.7 ± 0.7	1.9 ± 1.0	0.4 ± 0.3	2.7 ± 0.7	1.4 ± 0.4		

[a]Values are means ± SEM; numbers in parentheses denote number of individual preparations analyzed. Fatty acids are indicated by ratio of chain length to number of double bonds.
[b]Since fatty acid methyl ester standards > C20:4 were not used, fatty acids > C20:4 were not identified; hence not all columns sum to 100%.
[c]TG, triglycerides.
[d]CE, cholesteryl esters.
[e]Percent C18:2 TG and CE fatty acids were significantly greater than group (A) at $P < 0.001$, and greater than (B) and (D) at $P < 0.01$.

DISCUSSION

Hepatic synthesis and secretion of triglyceride-rich lipoproteins can be modulated by changes in the nutritional, metabolic, or hormonal environment, as well as by exogenous pharmacologic manipulation (30). Since hepatic VLDL synthesis and secretion are influenced in part by changes in free fatty acid flux (10), and since hydrophobic poloxalenes inhibit (intestinal) triglyceride-rich lipoprotein secretion (6), it was hypothesized that the net effect of dietary supplementation with hydrophobic poloxalenes might be to reduce hepatic secretion of triglyceride-rich lipoproteins (VLDL). The results of the current study show that addition of the hydrophobic poloxalene Pluronic L-81 to either a standard chow diet or to a high fat/cholesterol-enriched semisynthetic diet results in significant reduction in the net VLDL secretory rate in rats fed these diets when compared to rats fed the corresponding native diets. The effect of dietary supplementation with L-81 on hepatic VLDL cholesterol secretion was accompanied by hypocholesterolemia.

A number of studies have shown that fat and cholesterol supplementation of various basal diets (including the semisynthetic diet used in the current studies) results in hypercholesterolemia that is associated with an increased proportional transport of plasma cholesterol in the VLDL fraction (1, 11, 12). Furthermore, the increased plasma VLDL cholesterol transport occurring under conditions of diet-induced hypercholesterolemia has been shown to be accompanied by a significant increase in hepatic VLDL cholesterol secretion (31, 32). It has been demonstrated that cholesterol/fat-induced plasma VLDL are capable of delivering cholesterol to the arterial wall (33). Since the prevention of diet-induced hypercholesterolemia and atherosclerosis by dietary hydrophobic poloxalenes is associated with profound lowering of plasma VLDL concentrations (1), it might be reasonably argued that the reduction in hepatic VLDL (cholesterol) secretion produced by dietary supplementation with hydrophobic poloxalenes, as shown in this study, may contribute to the modulation of diet-induced hypercholesterolemia and atherosclerosis.

The apolipoprotein composition of hepatic secretory VLDL was compared under the dietary conditions of the present study. In agreement with previous reports (28, 34–40), livers from chow-fed rats were found to incorporate both $apoB_H$ and $apoB_L$ variants into nascent VLDL, the major secretory product being the low molecular weight form. When rats were fed the high fat/cholesterol-supplemented semisynthetic diet, livers were observed to secrete VLDL that contained greatly reduced amounts of $apoB_H$ and $apoB_L$ compared to chow-fed rats. When L-81 was included in either the high fat/cholesterol-enriched diet or the standard chow diet, the content of $apoB_H$ and $apoB_L$ was also markedly diminished. Further experiments are needed to elucidate the regulation of the incorporation of apoB variants into hepatic VLDL by high fat/cholesterol and L-81. Lastly, the absence of significant differences in secretory rates or physicochemical properties between two populations of VLDL found to differ significantly only in their apoB content (e.g., chow-fed VLDL: TG/apoB ratio approximately 25, versus high fat/cholesterol fed VLDL: TG/apoB ratio approximately 80) suggests that the extent of incorporation of apoB into nascent VLDL may not be critically rate-limiting for the secretion of TG.

The hypocholesterolemic effect produced by inclusion of L-81 in the high fat/cholesterol-enriched diet was consistent with previously reported effects of dietary hydrophobic poloxalenes on plasma cholesterol levels (1–4). The lack of hypotriglyceridemic effect, however, seemed somewhat pardoxical in view of both the marked diminution in hepatic VLDL triglyceride secretion seen in this ex-

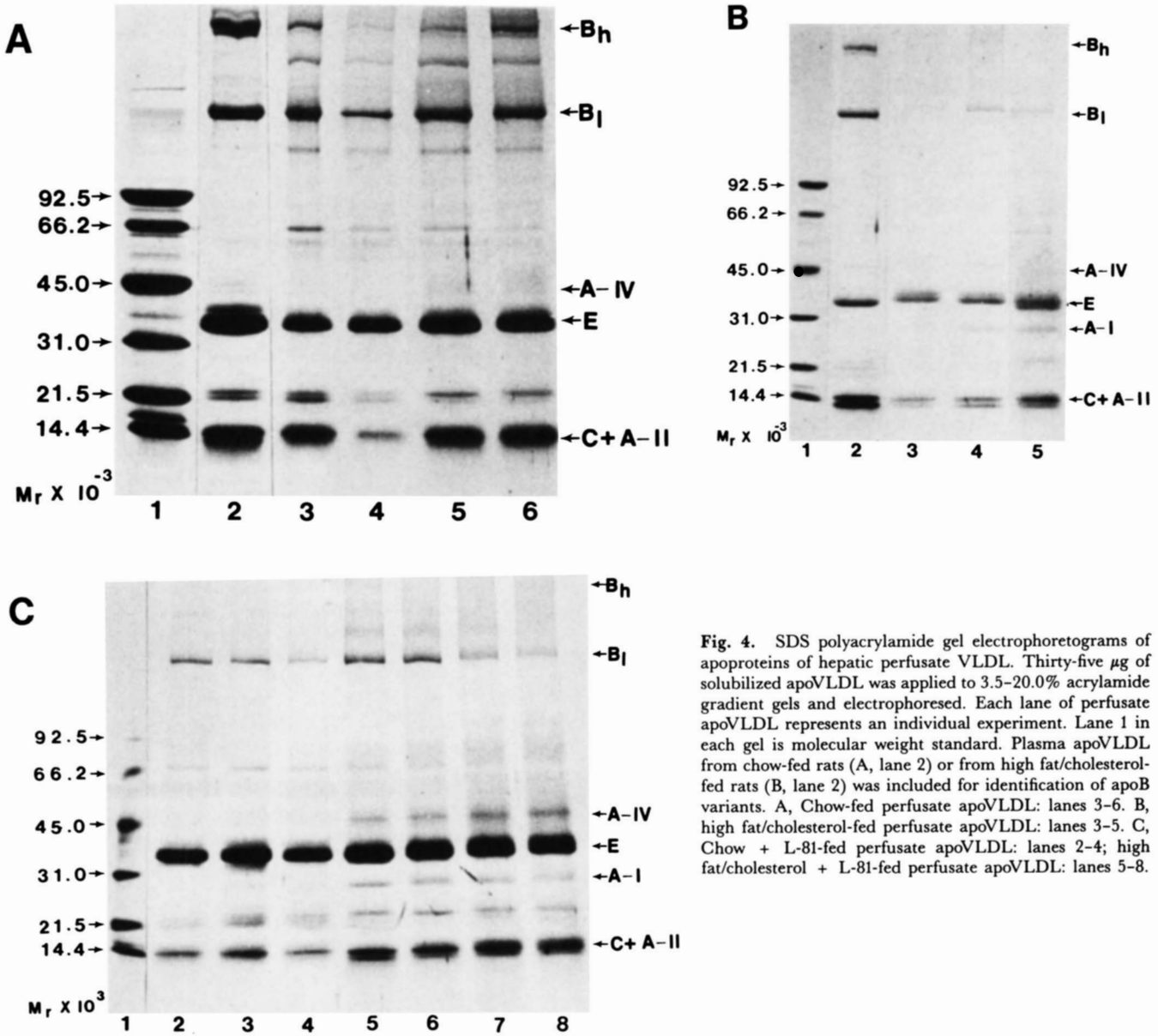

Fig. 4. SDS polyacrylamide gel electrophoretograms of apoproteins of hepatic perfusate VLDL. Thirty-five μg of solubilized apoVLDL was applied to 3.5–20.0% acrylamide gradient gels and electrophoresed. Each lane of perfusate apoVLDL represents an individual experiment. Lane 1 in each gel is molecular weight standard. Plasma apoVLDL from chow-fed rats (A, lane 2) or from high fat/cholesterol-fed rats (B, lane 2) was included for identification of apoB variants. A, Chow-fed perfusate apoVLDL: lanes 3–6. B, high fat/cholesterol-fed perfusate apoVLDL: lanes 3–5. C, Chow + L-81-fed perfusate apoVLDL: lanes 2–4; high fat/cholesterol + L-81-fed perfusate apoVLDL: lanes 5–8.

TABLE 5. Effect of feeding regimen on apolipoprotein compostion of hepatic perfusate VLDL[a]

Apoprotein	Chow (A) (4)	Chow + L-81 (B) (3)	High Fat/Cholesterol (C) (3)	High Fat/Cholesterol + L-81 (D) (4)
			% of total dye uptake	
B_H	3.9 ± 1.6	tr[b]	tr[b]	tr[b]
B_L	21.2 ± 3.1	7.6 ± 2.7	3.6 ± 1.2	7.0 ± 1.0
A-IV	tr[b]		1.7 ± 1.4	4.1 ± 0.3
E	49.2 ± 6.4	77.3 ± 1.9	73.8 ± 9.5	60.5 ± 1.1
A-I			4.2 ± 0.4	3.9 ± 0.2
C + A-II	24.9 ± 1.5	14.5 ± 2.0	16.2 ± 4.9	24.2 ± 2.8

[a]Rat livers were perfused and VLDL were isolated as in Table 2. SDS-PAGE and densitometric gel scanning of delipidated apoVLDL were performed as described in Methods. Values are means ± SEM of total dye uptake.
[b]Dye uptake (≤ 1.0% of total) was detected in these regions of the gels.

periment and the known inhibitory effect of this agent on intestinal chylomicron secretion (7). One possible explanation for the absence of a hypotriglyceridemic effect of L-81 in high fat/cholesterol fed-rats may have been the absorption of sufficient quantities of the hydrophobic detergent during the fat-fed state (during which the rats were exsanguinated for plasma lipid assays) to impair peripheral chylomicron catabolism by way of a "Triton-like" effect. Further studies of the effect of hydrophobic poloxalenes on triglyceride-rich lipoprotein catabolism will be required to answer this question.

The mechanism(s) by which hydrophobic poloxalenes reduce hepatic VLDL secretion remain to be elucidated. Fatty acid compositional data on perfusate VLDL core lipids suggest the possibility that, in the fat-fed state, these agents may reduce availability of dietary fatty acids for hepatic esterified lipid synthesis and secretion. Further studies will be required in order to delineate the various mechanisms by which hydrophobic poloxalenes influence lipoprotein synthesis and catabolism.

This work was supported by grants T32-AM07292, HL 30553, AM 33048, AM 31025, and AM 32288 from the National Institutes of Health, a Grant-in-Aid from the American Heart Association/Tennessee Affiliate, and intramural funds from the University of Tennessee Center for the Health Sciences. Dr. Manowitz was post-doctoral trainee of the National Institutes of Health. We gratefully acknowledge the technical assistance of Mrs. Lois Kuiken and Mrs. Polina Kogan. The editorial assistance of Mrs. Rebecca Potter is gratefully recognized.

Manuscript received 28 November 1983.

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