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Research Article

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Isolation and Characterization of Hepatic Golgi Lipoproteins from Hypercholesterolemic Rats

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ABSTRACT The feeding of cholesterol-rich diets alters the serum lipoproteins of a number of mammalian species. These lipoproteins are characterized by the presence of several classes of particles enriched in cholesteryl esters and apolipoprotein E (apo E). It was the aim of this study to determine whether one or more of these particles arises by *de novo* hepatic synthesis by characterizing nascent lipoproteins isolated from the hepatic Golgi apparatus of hypercholesterolemic rats. Characterization of these lipoproteins afforded the opportunity to assess morphologic, biochemical, and biophysical properties of newly synthesized lipoproteins before enzymatic alterations and apoprotein transfer known to occur after secretion into the plasma compartment. Golgi very low density lipoproteins (VLDL, $d < 1.006$ g/ml) from hypercholesterolemic rats contained nearly four times the total cholesterol mass found in control Golgi VLDL. They exhibited electrophoretic mobility intermediate between beta and pre-beta and were devoid of apo C. A second population of hepatic Golgi lipoproteins was isolated from hypercholesterolemic rats at 1.006–1.040 g/ml *d*. These low density lipoproteins were smaller than VLDL, displayed beta electrophoretic mobility, were enriched in cholesteryl esters, and contained apo E as well as apo B. The fatty acid composition of the core lipids of the nascent lipoproteins was found to reflect that of dietary triglyceride. The liver of the hypercholesterolemic rat thus plays an active role in dietary-induced hypercholesterolemia by synthesizing a modified VLDL and a low density lipoprotein resembling serum low density lipoprotein.

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INTRODUCTION

Hypercholesterolemia, induced by a cholesterol-enriched diet, is associated with distinctive modifications in the lipoproteins of both atherosclerosis-susceptible and resistant species (1). In experimental animals, such diets are often supplemented with saturated fat, conjugated bile salts, and antithyroid agents. One or more of the following changes have been observed in cholesterol-fed animals and humans: appearance of a beta-migrating very low density lipoprotein (B-VLDL);[1] increase in the intermediate (IDL) and low density (LDL) lipoproteins; decrease in normal alpha$_1$-migrating high density lipoproteins (HDL); and prominence of an alpha$_2$-migrating lipoprotein, HDL$_c$. B-VLDL are cholesterol-enriched lipoproteins isolated at $d < 1.006$ g/ml that display beta electrophoretic mobility, and are similar to the B-VLDL present in the serum of patients with Type III hyperlipoproteinemia (2). HDL$_c$ are similar to LDL with respect to size and lipid composition, but are different from LDL in that HDL$_c$ contain apolipoproteins (apo) E and A$_1$ as major apoproteins and do not contain apo B, the major apoprotein of LDL (1, 3). A common feature shared by cholesterol-induced B-VLDL, IDL, LDL, and HDL$_c$ is enrichment in cholesteryl ester and apo E, and as the plasma cholesterol levels increase, these four classes of lipoprotein particles assume the major share of cholesterol and apo E transport from HDL.

Both biosynthetic and catabolic mechanisms have been suggested for the origin of cholesterol and apo E-enriched lipoproteins. Roheim and co-workers (4) demonstrated that the perfused livers of cholesterol-fed rats secreted 50% more lipoprotein cholesterol

[1] *Abbreviations used in this paper*: B-VLDL, beta-migrating very low density lipoproteins; HC, hypercholesterolemic; HDL, high density lipoproteins; HMW, high molecular weight; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; LPT, lard, propylthiouracil, taurocholate; SDS, sodium dodecyl sulfate.

than control livers, and 87% of this cholesterol was carried in the $d < 1.019$-g/ml lipoprotein fraction. Cholesterol feeding has also been shown to increase apolipoprotein synthesis rates as measured by labeled amino acid incorporation into VLDL synthesized either by rabbit liver slices (5) or by the intact rat (6). In vivo turnover studies suggest that cholesterol feeding results in an increased synthesis of apo E (7), which may be associated with the synthesis of one or more of the cholesterol-enriched lipoprotein classes.

On the other hand, the studies of Redgrave et al. (8) and Ross and Zilversmit (9) suggest that in the cholesterol-fed rabbit cholesterol-enriched lower density lipoproteins arise as a consequence of catabolism within the plasma compartment. These investigators have presented evidence that cholesterol-enriched chylomicron remnants accumulate in the plasma of these animals. These remnants could contribute significantly to the increased concentrations of lower density lipoproteins because of impairment or saturation of the hepatic clearance mechanism for remnants.

We investigated hepatic lipoprotein synthesis in the hypercholesterolemic (HC) rat by characterizing the lipoproteins released from the isolated hepatic Golgi apparatus of these rats. This method of studying lipoprotein synthesis was chosen because it afforded the opportunity to assess the morphologic, biochemical, and biophysical characteristics of nascent particles before enzymatic modifications and apoprotein transfer known to occur as a postsecretory event. The results to be presented demonstrate that the HC rat liver synthesizes a cholesterol-enriched VLDL and a beta-migrating cholesteryl ester-rich lipoprotein resembling serum LDL.

METHODS

Animals and diet. Male Sprague-Dawley albino rats (Holtzman Co., Madison, Wisc.) were used for all experiments. Animals to be fed the cholesterol diet weighed 80–100 g upon initiation of feeding. They were fed a diet of laboratory chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill., 4.1% fat) supplemented with 5.0% lard, 0.1% 6-*N*-propyl-2-thiouracil (Sigma Chemical Co., St. Louis, Mo.), 0.3% sodium taurocholate and 1% cholesterol (ICN Nutritional Biochemicals, Cleveland, Ohio), (cholesterol diet 10.1% fat) for 21 d (3). At the end of this period they weighed 146.2±2.2 g (n = 118). As controls, some animals were fed the above diet without cholesterol (lard, propylthiouracil, taurocholate diet, [LPT-diet]) for 21 d. The weight gain for these animals was similar to those on the cholesterol diet with average weights at the end of the 21-d period being 152.8±1.9 g (n = 29). Animals fed the cholesterol and LPT diets gained from 38 to 40% of the weight gained by chow-fed rats over the same 21-d period. Weight-matched groups of rats (150–160 g), maintained on standard laboratory chow, served as chow-fed controls. All animals were housed under constant temperature and humidity conditions, and were permitted access to food and water ad lib. Procedures for isolation of the hepatic Golgi apparatus were begun at 11:00 a.m., after a 6-h fast.

Isolation of lipoproteins from serum and hepatic Golgi apparatus. Under light ether anesthesia, rats were exsanguinated from the distal abdominal aorta, and the blood was permitted to coagulate at 4°C. Serum was separated by centrifugation at 2,000 rpm for 30 min at 4°C. Serum lipids were determined on pooled or individual serum samples. Lipoproteins were isolated from pooled serum.

Immediately after exsanguination, livers were excised, and the Golgi apparatus was isolated from liver homogenates according to a modification[2] of the procedures of Morré et al. (10) and Mahley et al. (11). The livers were minced finely with scalpel blades and placed in a homogenization buffer (1.5 ml/g wet wt liver) consisting of 0.1 M phosphate buffer (pH 7.3), 0.25 M sucrose, 0.1% dextran (Sigma Chemical Co.), and 0.01 M $MgCl_2$. The minced livers were homogenized for 15–18 s with a Polytron homogenizer at setting 0.5 (model PT-10, Brinkmann Instruments, Inc., Westbury, N. Y.). The homogenate was centrifuged in an SW 27 (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.) rotor at 4°C at 2,500 rpm for 10 min and 9,500 rpm for 30 min using a Beckman L3-50 ultracentrifuge. The upper one-third of each pellet was resuspended in 5.0 ml of the homogenization buffer, layered on sucrose pads consisting of 23 ml of 1.25 M sucrose, and centrifuged in an SW 27 rotor in the following manner: 5,000 rpm for 10 min, 10,000 rpm for 10 min, and 25,000 rpm for 45 min. At the end of the centrifugation, the band (Golgi-rich fraction) at the interface was recovered, diluted with cold saline, and pelleted in the SW 27 rotor at 20,000 rpm for 30 min.

When isolating Golgi-rich fractions from rats fed the cholesterol diet, an additional wash of the final pellet at 1.59×10^6 g-min was necessary to remove contaminating particulate lipid. This particulate lipid was assumed to arise from cytoplasmic lipid droplets that were observed in the livers of these rats by electron microscopy. The Golgi-rich fractions were studied by negative staining electron microscopy using 2% phosphotungstic acid.

To release nascent lipoproteins, the Golgi-rich pellet was suspended in 0.04 M sodium barbital buffer, pH 8.5, and passed twice through a French pressure cell (American Instrument Co., Travenol Laboratories, Inc., Silver Spring, Md.), according to Ehrenreich et al. (12). The density of the suspension was then adjusted to 1.006 g/ml with 0.52 M NaCl, and the lipoproteins were isolated by preparative ultracentrifugation. VLDL from chow-fed and LPT-fed controls were isolated from the Golgi apparatus using a Beckman type 40 rotor in an L3-50 ultracentrifuge at 35,000 rpm for 20 h at 4°C. The VLDL were removed by tube slicing in a volume of ~2 ml, resuspended in 0.15 M NaCl-0.01% EDTA, pH 7.2, and washed once by recentrifugation under identical conditions. The infranates did not contain sufficient quantities of lipoproteins to permit characterization. VLDL were isolated from the Golgi apparatus of rats fed the cholesterol diet using a Beckman 60 Ti rotor spun at 48,000 rpm for 16 h. The 60 Ti rotor was necessary because of the larger volumes of Golgi membrane suspensions from these rat livers. The VLDL fraction, removed by tube slicing, contained small amounts of particulate lipid, which was separated from the VLDL as described below. The density of the infranate was raised to d = 1.040 g/ml and centrifuged at 48,000 rpm for 20 h in the 60 Ti rotor. The lipoproteins floating at this density were designated HC Golgi LDL. They were removed by tube slicing and washed in the 40 rotor at 35,000 rpm for 20 h.

The particulate lipid was separated from the VLDL by centrifuging the $d < 1.006$-g/ml fraction for 3×10^6 g-min in an

[2] R. L. Hamilton, personal communication.

SW 39 rotor, and removed by tube slicing in a volume of 0.5–1.0 ml. The VLDL were refloated in the 40 rotor at 35,000 rpm for 20 h. Negative stains of this washed fraction, HC Golgi VLDL, demonstrated complete absence of globular fat and the presence of spherical lipoproteins no larger than 1,000 Å Diam.

Serum lipoproteins were isolated by the methods of Havel et al. (13) using the 40 rotor. After isolation of serum VLDL, fractions were sequentially floated at salt *d* 1.006–1.040, 1.040–1.080, and 1.080–1.210 g/ml, raising densities with solid NaCl (1.006–1.080 g/ml) or solid KBr (1.080–1.210 g/ml). The 1.006–1.080-g/ml fractions were isolated and washed once at 35,000 rpm for 20 h, while the 1.080–1.210-g/ml *d* fraction was isolated at 35,000 rpm for 48 h, and washed for 24 h at the same speed. All fractions of Golgi or serum lipoproteins were dialyzed at 4°C for 72 h against three changes of 500 vol of 0.01% EDTA, pH 7.2. Aliquots were retained for electron microscopy, lipoprotein electrophoresis, and protein assay, and the remainder was lyophilized.

Morphologic studies. Small portions of the Golgi-rich fraction pellet and lipoproteins were negatively stained on carbon stabilized Formvar-coated nickel grids using 2% aqueous phosphotungstic acid, pH 6.5. Grids were viewed in a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.), and random fields were selected and photographed. Lipoprotein diameters were determined by measuring all spherical lipoproteins within a given field.

Agarose gel electrophoresis. Electrophoretic mobility of lipoproteins was determined by a modification of the method of Noble (14), using 0.8% agarose (Bio-Rad Laboratories, Richmond, Calif.) in 50 mM barbital buffer, pH 8.6, containing 0.2% bovine serum albumin (Sigma Chemical Co.).

Analytical methods. Total serum cholesterol was determined on ethanol-ethyl acetate extracts as described by Babson et al. (15). Serum triglycerides and phospholipids were quantitated by gas-liquid chromatography after separation of lipid classes by thin-layer chromatography and methylation of the fatty acids of the lipid esters (16). Samples were run on a Varian 2100 gas chromatograph (Varian Associates, Inc., Instrument Group, Palo Alto, Calif.) equipped with 6 ft × ¼ in. glass columns, packed with SP-2340 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefonte, Pa.) using N_2 carrier gas at a flow rate of 60 ml/min. Temperature was programmed from 175–225°C at 6°/min, and detection was by flame ionization. Quantitation was performed by reference to internal standards of trieicosenoin (C20:1) (Nu-Chek Prep, Elysian, Minn.) and ditetradecanoyl-L-alpha lecithin (C14:0) (Supelco, Inc.). Peak areas were computed on a Varian Integrator, model 485, and summed peak areas related to that of the standard. Peaks were identified by comparison with standard mixtures of fatty acid methyl esters (Nu-Chek Prep).

Serum lipoprotein protein was estimated by the method of Lowry et al. (17) using bovine serum albumin as standard and Triton X-100 to extinguish turbidity. To conserve material, Golgi lipoprotein protein was estimated by the Coomassie microprotein assay (18), using the bovine serum albumin standard.

Lyophilized lipoproteins were delipidated on a multipurpose rotator, using the solvent system of Bersot et al. (19). The total lipid extract was separated into individual lipid classes on silica gel 60 (EM Laboratories, Elmsford, N. Y.) thin-layer plates developed in petroleum ether, diethyl ether, acetic acid, 80:20:1. For quantitation of lipids, the individual lipid classes were visualized by iodine vapors, identified by reference to standards (nonpolar lipid mix A, Supelco, Inc.), scraped from the plates, and eluted from the gel (20). Cholesterol (free and esterified) was analyzed by the method of Babson et al. (15), triglyceride according to Van Handel and Zilversmit (21), and phospholipid according to Bartlett (22). For fatty acid analysis of individual lipid esters, the lipid spots were visualized by rhodamine 6G, and scraped from the plates. Fatty acid methyl esters were prepared and analyzed by gas-liquid chromatography as described above.

To determine fatty acid composition of dietary triglycerides, portions of the control or experimental diet were extracted according to Folch et al. (23). The triglycerides were isolated by thin-layer chromatography and the fatty acids analyzed by gas-liquid chromatography as described above.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. The spectrum of apoproteins associated with HC and chow-fed Golgi and serum lipoproteins was analyzed by a modification of the SDS gel electrophoretic method of Weber and Osborn (24). 30 μg of delipidated protein were completely solubilized at 37°C in 0.4% SDS, 0.05 M Tris, 0.38 M glycine buffer, pH 8.2, and applied to 11% polyacrylamide gels prepared in the same buffer containing 0.1% SDS. Proteins were migrated at 2 mA/gel, stained for 1.5 h with 0.1% (wt/vol) Coomassie Brilliant Blue, and destained.

The apoprotein bands were identified on the basis of molecular weights, as determined by reference to purified protein standards, and by comparison to published SDS gel electrophoretograms of rat apolipoproteins (1).

To quantitate cholesterol-induced alterations in Golgi VLDL apoprotein distribution, rats were anesthetized with sodium pentobarbital and injected intravenously in the jugular bulb with 75 μCi of DL-[4,5-^{3}H]leucine (50 Ci/mmol, New England Nuclear, Boston, Mass.). 15 min after injection the rats were killed and the Golgi apparatus and associated lipoproteins were isolated. This time period was chosen because Nestruck and Rubinstein (25) have shown that the specific activity of the Golgi VLDL protein is maximal 15 min after injection of [^{3}H]leucine. The radioactive Golgi apo VLDL was separated on SDS gels as described above. Stained protein bands and unstained regions were sliced from each gel and prepared for liquid scintillation counting (26). Gel slice samples were counted in a Beckman LS 233 liquid scintillation counter. Because quenching was the same in all samples, no quench correction was necessary. In all cases >82% of the radioactivity applied to the gels was recovered.

Statistical analyses. Significance of the data was evaluated by grouped Student's *t* test. Values are expressed as mean ±SEM.

RESULTS

Serum lipid and lipoprotein concentrations. Table I presents the serum lipid concentrations from rats fed cholesterol, LPT, and chow diets. Total serum cholesterol of rats fed the cholesterol diet for 21 d averaged 432 mg/dl. These rats will be referred to as HC rats. Rats fed the LPT diet had serum cholesterol concentrations of 94 mg/dl compared with 80 mg/dl for chow-fed control rats. An increasing prominence of the lower density lipoproteins paralleled the degree of hypercholesterolemia in HC rats. In chow-fed control animals VLDL ($d < 1.006$ g/ml) contained 25% of the total serum cholesterol and 10% of the lipoprotein protein. In a group of HC rats that had an average serum cholesterol of 692 mg/dl, 71% of the

TABLE I

Serum Lipid Concentrations

Group	Cholesterol	Triglycerides	Phospholipids
		mg/dl	
HC	432.3±64.4 (36)	21.9±8.7*	160.0±26.8‡
LPT-fed control	94.0±5.9 (6)	39.5±4.8 (6)	112.7±10.7 (6)
Chow-fed control	79.8±1.7 (12)	39.0±0.6§	88.6±6.2§

Numbers in parentheses refer to number of rats individually analyzed.
* Mean±SEM of three pools of rats. Five to six rats per pool.
‡ Mean±SEM of four pools of rats. Five to ten rats per pool.
§ Mean±SEM of three pools of rats. Three to five rats per pool.

cholesterol and 79% of the lipoprotein protein was present in the $d < 1.006$-g/ml fraction.

Morphologic studies. With the use of negative contrast electron microscopy the isolated, intact hepatic Golgi apparatus from HC rats exhibited the same architectural features that have been previously described for the Golgi apparatus isolated from control rat livers (10). These features are illustrated in Figs. 1A and 1B. The cisternae, appearing as flattened central plates in Fig. 1A or as fenestrated plates in Fig. 1B, were continuous with an anastomosing tubular network terminating in lipoprotein-filled secretory vesicles. In chow-fed control rats, the intratubular and intravesicular particles measured from 300–1,000 Å. (Fig. 2a). In contrast HC Golgi vesicles, seen in Fig. 1C, D, and E, contained particles with smaller diameters than those seen in chow-fed control vesicles. This shift to smaller diameters can be readily seen in the histograms (Fig. 2a, b, c). In addition, the HC Golgi vesicles appeared to contain an additional population of lipoproteins with diameters as small as 200–300 Å. (Fig. 2c). The size of these particles is characteristic of plasma lipoproteins normally found in the LDL range (1). These small particles were not observed in Golgi vesicles isolated from either chow-fed or LPT-fed control rat livers. It was because of the observation of these small particles within the HC Golgi vesicles that attempts were made to isolate lipoproteins from HC Golgi preparations at densities >1.006 g/ml.

Fig. 2d, e, and f present the particle size distributions of isolated Golgi VLDL from chow-fed, LPT-fed, and HC rats. Golgi VLDL from chow-fed rats (Fig. 2d) averaged 555 Å Diam, whereas Golgi VLDL from LPT-fed and HC rats averaged ~480 Å (Fig. 2e, f). Golgi lipoproteins in the 1.006–1.040-g/ml d range could only be isolated from HC rat livers. These LDL ranged from 200 to 400 Å in size with a mean of 297 Å (Fig. 2g).

Agarose gel electrophoresis. Figs. 3A and 3B present agarose electrophoretograms of control and HC serum, control and HC Golgi VLDL, and HC Golgi LDL. HC Golgi VLDL migrated with a mobility intermediate between beta and pre-beta, whereas chow-fed control Golgi VLDL migrated with characteristic pre-beta mobility. (Fig. 3A). The HC Golgi LDL fraction contained only beta migrating lipoproteins (Fig. 3A). Golgi VLDL from LPT-fed rats exhibited pre-beta mobility (Fig. 3B).

Lipoprotein compositional studies. The chemical compositions of VLDL isolated from hepatic Golgi apparatus of HC, chow-fed, and LPT-fed control rats are presented in Table II. The composition of HC Golgi VLDL was significantly different from both LPT- and chow-fed control Golgi VLDL. Triglyceride comprised 40% of the mass of HC Golgi VLDL compared with 64.5% in chow-fed control and 66.8% in LPT-fed Golgi VLDL. Cholesteryl esters of HC Golgi VLDL were increased nearly threefold compared with LPT-fed Golgi VLDL and fourfold compared with chow-fed Golgi VLDL. HC Golgi VLDL contained significantly less phospholipid and more protein than chow-fed control Golgi VLDL.

Table III presents the compositions of HC Golgi and serum LDL. The composition of these lipoproteins was very similar, with cholesteryl esters comprising ~50% of the mass of each particle.

Fatty acid composition of dietary triglycerides and Golgi lipoprotein cholesteryl esters and triglycerides. Table IV presents the fatty acid composition of the dietary triglycerides, and cholesteryl esters and triglycerides of nascent lipoproteins from chow-fed control and HC rats. In both control and HC rats, 16:0, 18:1, and 18:2 were the major triglyceride and cholesteryl ester fatty acids. However, the percentage of 18:1 was significantly greater in HC Golgi VLDL triglycerides and cholesteryl esters than in the respective lipid classes from chow-fed control rats ($P < 0.05$). The differences in fatty acid compositions of the Golgi lipoprotein triglycerides and cholesteryl esters reflect differences in the fatty acid composition of the dietary triglycerides, with the HC diet containing increased 18:1 compared with the chow diet.

Apolipoprotein composition. The apoprotein composition of serum and Golgi VLDL from HC and

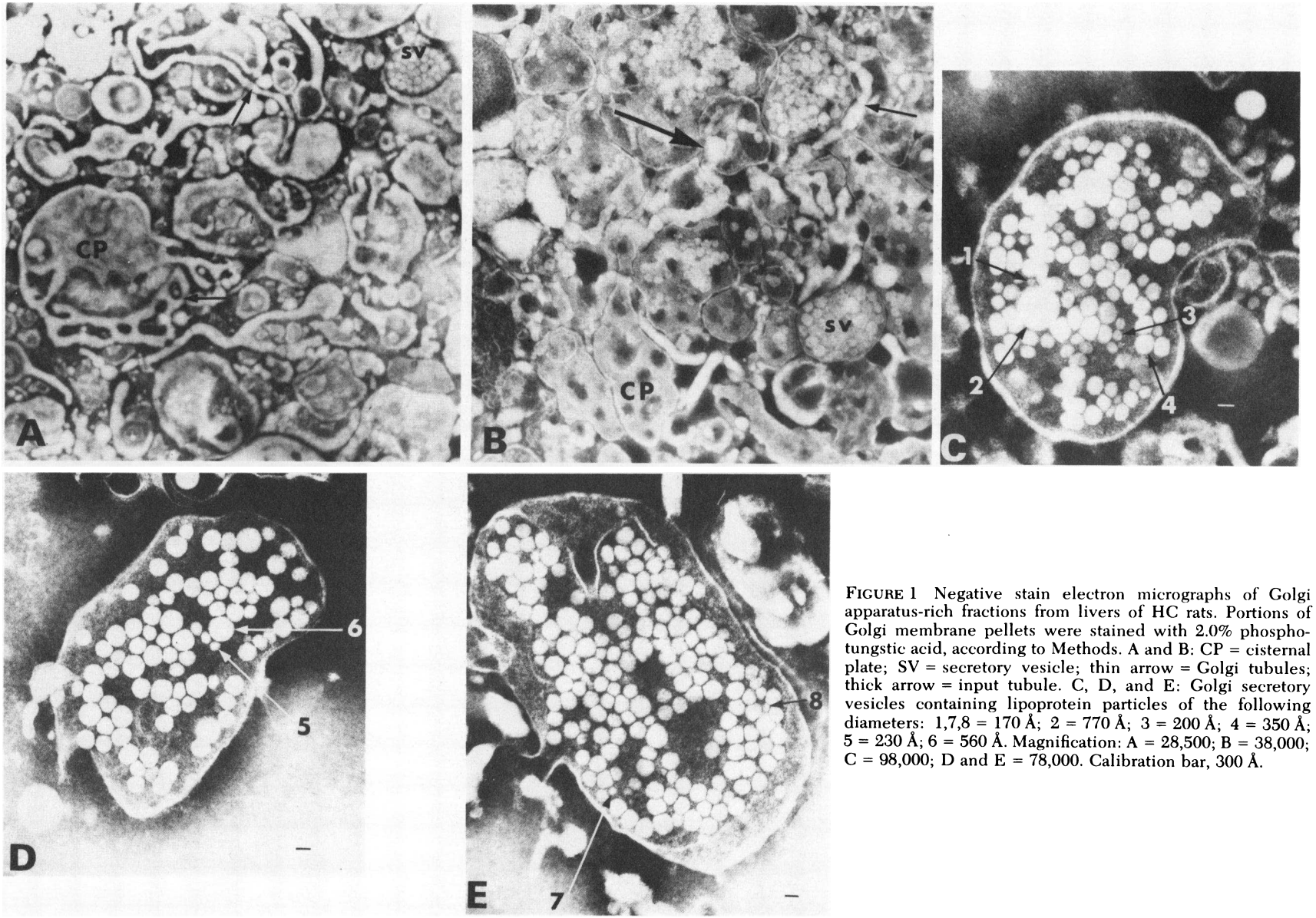

FIGURE 1 Negative stain electron micrographs of Golgi apparatus-rich fractions from livers of HC rats. Portions of Golgi membrane pellets were stained with 2.0% phosphotungstic acid, according to Methods. A and B: CP = cisternal plate; SV = secretory vesicle; thin arrow = Golgi tubules; thick arrow = input tubule. C, D, and E: Golgi secretory vesicles containing lipoprotein particles of the following diameters: 1,7,8 = 170 Å; 2 = 770 Å; 3 = 200 Å; 4 = 350 Å; 5 = 230 Å; 6 = 560 Å. Magnification: A = 28,500; B = 38,000; C = 98,000; D and E = 78,000. Calibration bar, 300 Å.

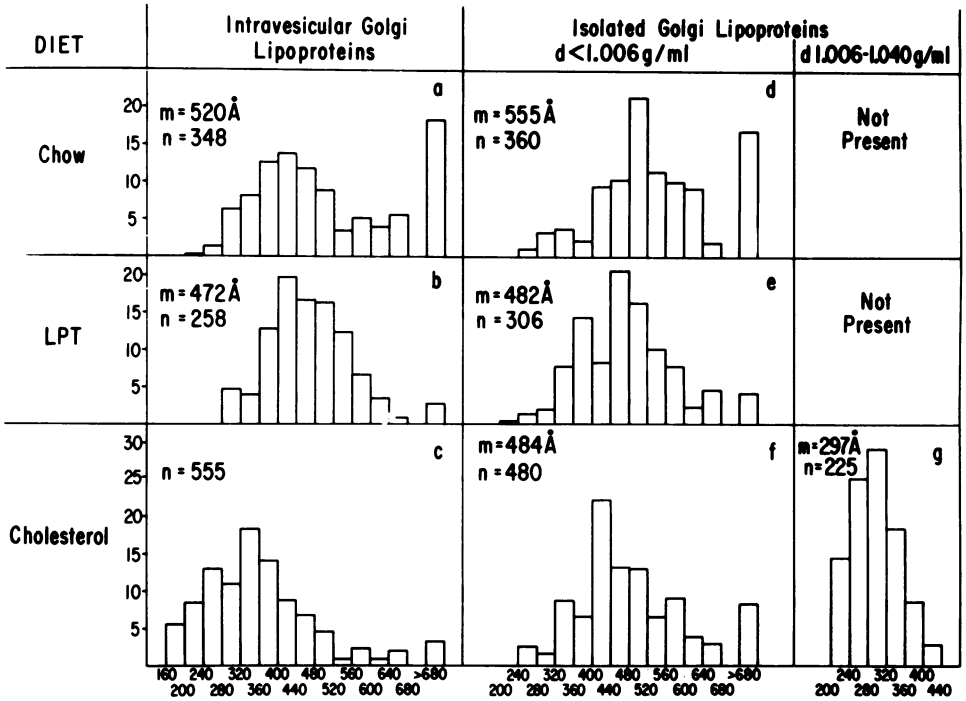

FIGURE 2 Percent distribution of particle diameters of intravesicular hepatic Golgi lipoproteins and isolated Golgi lipoproteins from chow-fed, LPT-fed, and HC rats. a,b,c: intravesicular Golgi lipoproteins from rats fed chow, LPT, and cholesterol diets, respectively. d,e,f: Golgi lipoproteins isolated at $d < 1.006$ g/ml from rats fed chow, LPT, and cholesterol diets, respectively. g, Golgi lipoproteins isolated at 1.006–1.040 g/ml d from HC rats. n = number of particles counted. m, mean Diam.

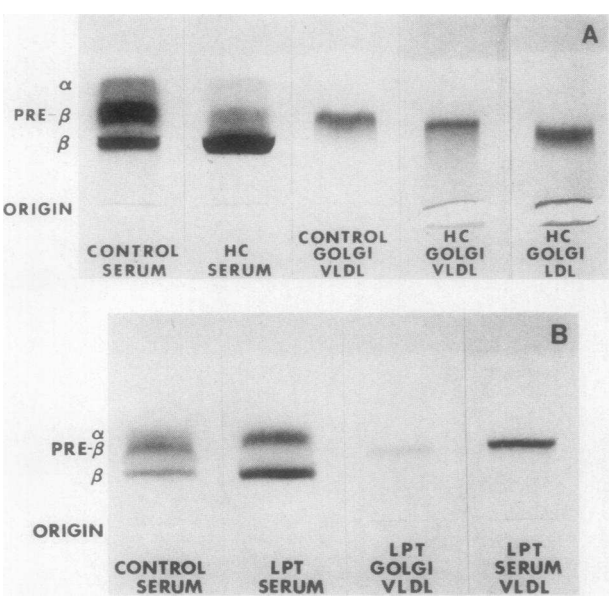

FIGURE 3 Agarose gel electrophoretograms of serum and hepatic Golgi lipoproteins from chow-fed control and HC rats (A) and LPT-fed rats (B). Samples were electrophoresed in 0.8% agarose and stained with oil red O. Gels in A and B were run at different times.

chow-fed control rats was analyzed by SDS polyacrylamide gel electrophoresis. As has been reported (27), HC serum VLDL appeared relatively enriched in apo E and relatively depleted of apo C (Fig. 4B). As shown in Fig. 4A, both control and HC Golgi VLDL contained apo B, apo E, and apo A_1, whereas the apo C group of proteins was present only in control VLDL. On repeated occasions and as shown in the figure we noted a faint split of the apo E band in the HC Golgi VLDL. This split was not observed in control Golgi lipoproteins or in HC or control serum lipoproteins. A similar split in the apo E band has been reported in VLDL secreted by perfused HC rat livers (28) and in human VLDL (29). Cholesterol-induced alterations in hepatic VLDL apoprotein synthesis were studied by comparing the biosynthetic incorporation of intravenously administered [^{3}H]leucine into nascent VLDL apoproteins of HC and chow-fed control rats. The percentage distribution of incorporated radioactivity is shown in Table V. Apo B accounted for 50% of VLDL apoprotein radioactivity in control rats, and 61% in HC rats. The percentage of leucine incorporated into apo E of Golgi VLDL was the same in the two groups. Less than 5.0% of the radioactivity was incorporated into the C apoproteins of Golgi VLDL

TABLE II
Percent Composition of Hepatic Golgi VLDL

	HC (n = 4)	Chow-fed control (n = 3)	LPT-fed control (n = 2)	P*
		% *wt*		
Triglyceride	40.4±0.7	64.5±2.5	66.8	<0.001
Cholesteryl ester	29.9±1.0	7.7±2.6	10.7	<0.01
Unesterified cholesterol	3.0±0.4	1.4±0.4	3.2	>0.05
Phospholipid	10.5±0.5	18.6±0.9	7.1	<0.01
Protein	16.2±0.9	7.8±0.5	12.3	<0.02

Cholesteryl ester = 1.67 × esterified cholesterol.
Values are mean±SEM.
* P values compare HC with chow-fed controls.

from control rats whereas none was present in the C apoproteins of Golgi VLDL from HC rats. In both control and HC groups, 10% of the radioactivity was incorporated into apoproteins whose molecular weight ranged between 50,000 and 77,000 (high molecular weight [HMW] proteins).

Fig. 5 shows the SDS polyacrylamide gels of HC serum and Golgi LDL. The major apoproteins associated with both LDL fractions were apo B and apo E. Smaller amounts of slower migrating HMW proteins were also seen.

DISCUSSION

Our study has shown that the livers of rats made hypercholesterolemic by feeding diets containing cholesterol, lard, taurocholate, and propylthiouracil synthesize (*a*) a cholesterol-enriched VLDL and (*b*) a cholesteryl ester-rich, beta-migrating LDL containing apo E, as well as apo B, as the major apoproteins. In contrast, the livers of rats fed identical diets without cholesterol (LPT-diet) or fed chow, synthesize a triglyceride-rich VLDL, but do not synthesize the cholesteryl ester-enriched LDL. These findings indicate that abnormal hepatic lipoprotein synthesis contributes to the pathogenesis of dietary-induced hypercholesterolemia.

TABLE III
Composition of LDL from Hepatic Golgi Apparatus and Serum of HC Rats*

	Golgi (n = 4)	Serum (n = 2)
	% *wt*	
Triglyceride	4.3±1.0	1.4
Cholesteryl ester	52.4±1.0	53.3
Unesterified cholesterol	8.6±0.8	12.8
Phospholipid	15.6±2.5	13.3
Protein	19.1±1.2	19.4

Values are mean±SEM.
Cholesteryl ester = 1.67 × esterified cholesterol.
* 1.006–1.040 g/ml *d*.

Golgi VLDL isolated from the liver of the HC rat differ from control Golgi VLDL in their morphologic, electrophoretic, and biochemical characteristics. Chow-fed control Golgi VLDL averaged 555 Å Diam and displayed pre-beta mobility on agarose. As has been reported (11) these nascent lipoproteins were triglyceride-rich particles (see Table II). [^{3}H]leucine incorporation into chow-fed control Golgi VLDL demonstrated that apo B and apo E were major apoproteins, comprising 50.7 and 24.2% of the total apoprotein radioactivity, respectively, whereas apo A_1 and apo C were minor apoproteins (Table V). In contrast, HC Golgi VLDL were smaller, slower-migrating particles, enriched fourfold in cholesteryl ester. The decreased particle diameter of HC Golgi VLDL may be a result of the increased degree of saturation of the fatty acids of the lipid esters compared with chow-fed control. Wilcox et al. (30) have shown that VLDL secreted by perfused livers after infusion of saturated fatty acids have slower rate zonal mobility in the ultracentrifuge and are probably smaller particles than VLDL produced after infusion of unsaturated fatty acids. It would not be unexpected then that feeding diets rich in saturated fatty acids might lead to the production of smaller VLDL particles than those synthesized by rats on control diets. In support of this hypothesis, Golgi VLDL from rats fed the saturated fat diet without cholesterol (LPT diet) were identical in size to HC Golgi VLDL. The decreased electrophoretic mobility of HC Golgi VLDL may be due to the absence of apo C as shown by SDS gel electrophoresis and [^{3}H]leucine incorporation studies. HC Golgi VLDL contained relatively more apo B and similar percentages of apo E compared with control (Table V).

This investigation has shown also that the liver of the HC rat assembles a lipoprotein in the 1.006–1.040-g/ml *d* range which we termed HC Golgi LDL. HC Golgi LDL averaged 297 Å Diam and displayed beta

TABLE IV

Fatty Acid Composition of Dietary Triglycerides and Core Lipids of Hepatic Golgi Lipoproteins

Fatty acid	Chow-fed control			HC				
	Dietary TG*	Golgi VLDL TG	Golgi VLDL CE	Dietary TG*	Golgi VLDL TG	Golgi VLDL CE	Golgi LDL TG*	Golgi LDL CE
	%					%		
16:0	21.1	24.4±0.1	15.0±2.0	25.0	21.2±0.7	14.5±1.0	22.2	13.9±0.1
16:1	3.1	2.5±0.3	2.4±0.2	2.6	2.6±0.3	3.8±0.7	1.1	4.6±0.9
18:0	9.4	2.1±0.3	5.3±0.2	12.5	5.3±0.1	4.9±0.5	9.9	5.6±0.1
18:1	31.0	23.0±5.0	34.3±0.3	43.0	42.2±1.2	54.9±1.2	29.3	57.1±1.2
18:2	30.5	31.6±4.7	26.9±6.7	14.3	24.1±1.2	15.0±0.4	18.7	14.5±2.5
18:3	—	1.9±0.2	1.2‡	—	—	1.3‡	5.5	1.6‡
20:4	—	3.5±0.1	3.6±0.5	—	0.8±0.1	—	0.4	—
Other	4.9	11.0	11.3	2.6	3.8	5.6	12.9	2.7

Fatty acids are denoted by ratio of chain length to number of double bonds.
Values are mean±SEM for duplicate experiments except for * which represents single analysis.
‡ Peak present in only one sample.
TG, triglycerides; CE, cholesteryl esters.

mobility on agarose. They had a composition similar to LDL (1.006–1.040 g/ml *d*) isolated from serum of HC rats. The apoprotein composition of HC Golgi and serum LDL was similar, with apo B and apo E the predominant apoproteins. The *de novo* synthesis of LDL may contribute to the increased levels of this lipoprotein found in the HC rat. Noel et al. (28) have reported that the perfused liver of the HC rat secretes

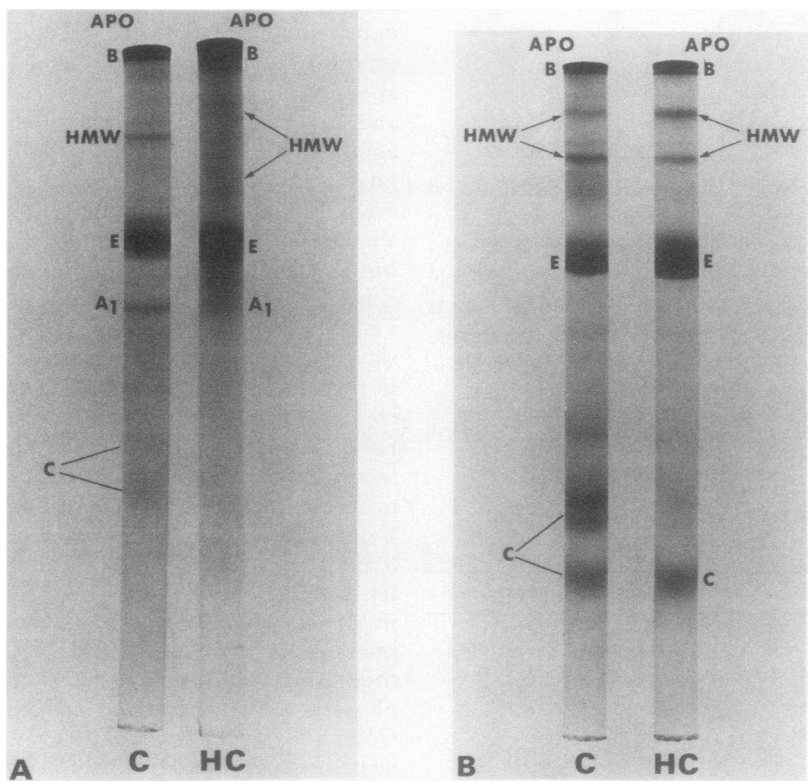

FIGURE 4 SDS polyacrylamide gel electrophoretograms of hepatic Golgi VLDL (A) and serum VLDL (B) from HC and chow-fed control rats. 30 μg of solubilized apoproteins were applied to 11% gels and electrophoresed according to Methods. HMW, high molecular weight proteins (mol wt, 50,000–77,000).

TABLE V
Percent Distribution of ^{3}H-labeled Apolipoproteins of Golgi VLDL

Apoprotein	Chow-fed control	HC
B	50.7±0.3	61.1±0.1
HMW*	10.9±1.1	9.8±1.1
E	24.2±2.3	24.8±0.9
A_1	9.7±0.2	4.3±0.3
C	4.5±1.2	‡

Each rat was administered 75 μCi of [^{3}H]leucine 15 min before death. 12 rats were pooled per experiment. 30 μg of protein, containing 1,366–3,880 cpm were applied to each gel. Gels were run in triplicate for each experiment. Values represent mean±SEM of duplicate experiments.
* HMW proteins, mol wt 50,000–77,000.
‡ Not significantly different from background counts.

a cholesteryl ester-rich, apo C-deficient VLDL, with slower electrophoretic mobility than the triglyceride-rich VLDL produced by perfused normal livers. In addition, they have shown that the perfused HC rat liver secretes an LDL with beta mobility, which is rich in cholesterol and contains apo E as well as apo B. The similarities between the lipoproteins found by Noel et al. (28) in the perfusate from HC rat livers and those found in the isolated Golgi apparatus from HC rat livers in the present study strongly suggests that the nascent Golgi lipoproteins are indeed precursors to their cholesterol-induced serum lipoprotein counterparts.

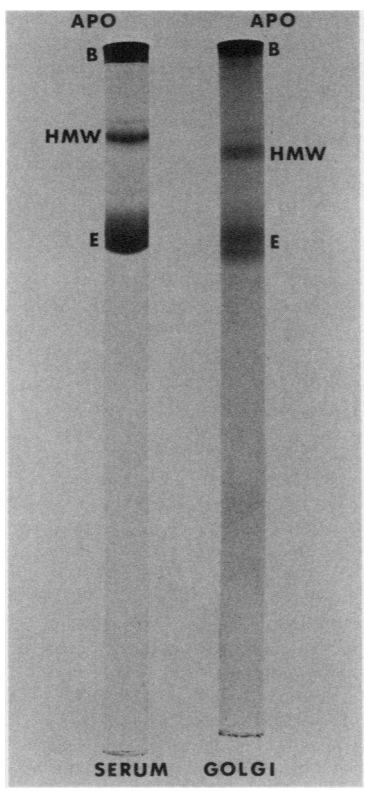

FIGURE 5 SDS polyacrylamide gel electrophoretograms of hepatic Golgi and serum LDL from HC rats. 30 μg of solubilized apoproteins were applied to 11% gels and electrophoresed according to Methods. LDL were isolated between d = 1.006 and 1.040 g/ml.

The triglyceride and cholesteryl ester fatty acid composition of nascent hepatic Golgi lipoproteins was found to reflect the fatty acid composition of the dietary triglycerides, supporting the earlier work of Dunn et al. (31). These workers have shown in the intact rat that the fatty acid composition of plasma triglycerides reflects that of dietary triglycerides. Our finding establishes this relationship for nascent Golgi lipoproteins, which are precursors to the serum lipoprotein pool.

The mechanisms through which lower density lipoproteins become elevated in HC animals have been unclear. It has been suggested that the prominence of B-VLDL, IDL, and LDL reflects a saturation of clearance mechanisms induced by the high cholesterol content of the diet. This hypothesis is supported by the work of Ross and Zilversmit (9), who have found that nearly two-thirds of the esterified cholesterol in the $d < 1.006$-g/ml lipoproteins in cholesterol-fed rabbits was of dietary origin. Furthermore, they found hepatic remnant uptake in these rabbits to be delayed, resulting in a longer circulation time for remnants and subsequent appearance of a substantial fraction of remnants in Svedberg units of flotation (S_f) < 20 ($d > 1.006$ g/ml). Redgrave et al. (8) have shown also that the clearance of chylomicron remnants in the cholesterol-fed rabbit is impaired. Therefore, the accumulation of chylomicron remnants in the plasma of cholesterol-fed rabbits could contribute significantly to the increased concentration of lower density lipoproteins found in these animals.

On the other hand Kris-Etherton and Cooper (32) and Cooper and Yu (33) have demonstrated that there is no defect in removal of chylomicron remnants either in intact HC rats or in the perfused livers from these animals. The ability of the HC rat to clear chylomicron remnants as effectively as normal rats suggests that the cholesterol-induced serum lipoproteins arise by synthetic mechanisms. Several studies support this hypothesis. Kris-Etherton and Cooper (32) reported an increased hepatic secretion of cholesterol-rich VLDL by the perfused liver from HC rats. Noel et al. (28) have reported also that the liver of the HC rat secretes abnormal cholesterol carrying lipoproteins found in the VLDL and LDL density ranges. Our finding of cholesterol-enriched VLDL and LDL in the Golgi apparatus of HC rat livers is further evidence that abnormal hepatic lipoprotein synthesis contributes to dietary-induced hypercholesterolemia.

The *de novo* synthesis of LDL by the HC rat liver is significant in light of the concept that LDL arise solely from VLDL catabolism. Several recent studies in humans and animals have suggested the presence of a *de novo* synthetic pathway for LDL. Soutar et al. (34) found that LDL apo B flux in patients with homozygous familial hypercholesterolemia was 50–100% greater than their VLDL apo B flux, implying a direct secretion of LDL (or IDL) into the plasma compartment. Berman et al. (35) furnished kinetic evidence for VLDL-independent apo B secretion in patients with Type III hyperlipoproteinemia. Illingworth (36) calculated that in the squirrel monkey as much as 14% of the LDL fraction (1.019–1.063 g/ml *d*) might be secreted directly. Fidge and Poulis (37) in studies with rats reported that 85–94% of the LDL with S_f 0–5 (1.040–1.063 g/ml *d*) is secreted directly into the plasma compartment. Our findings complement these studies and provide direct evidence for a *de novo* hepatic synthesis of LDL in an animal model of hypercholesterolemia.

Our study has shown that the HC rat liver synthesizes a cholesterol-enriched VLDL and a low density lipoprotein similar to cholesterol-induced serum LDL. Our findings, coupled with the findings of Noel et al. (28) indicate that nascent HC Golgi lipoproteins, VLDL and LDL, are indeed precursors to their cholesterol-induced serum lipoprotein counterparts. The finding of a *de novo* synthetic pathway for LDL indicates that the liver has the potential for synthesizing this lipoprotein. This pathway may contribute little to the total serum LDL pool in normal states. However, it may assume greater significance in diet-induced as well as in genetic hyperlipidemias.

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