Hepatic perfusate very low density lipoproteins obtained from fat-fed nonhuman primates stimulate cholesterol esterification in macrophages

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Abstract The livers of both baboons and rhesus monkeys fed a high fat, high cholesterol diet secreted very low density lipoproteins (VLDL) that were enriched in cholesteryl ester and apoE as compared to VLDL secreted by the livers of chow-fed animals. Stimulation of macrophage cholesterol esterification by the experimental VLDL was compared to that produced by the standard β-VLDL obtained from the plasma of a rhesus monkey fed 25% coconut oil plus 2% cholesterol. This standard β-VLDL stimulated 7- to 10-fold more esterification than did the bovine albumin control. Hepatic VLDL from fat-fed animals stimulated esterification in J774 macrophages 50 to 150% as well as did the standard β-VLDL, even though hepatic VLDL did not display beta electrophoretic mobility on agarose gel electrophoresis. Plasma VLDL from lard-fed baboons did not exhibit beta electrophoretic mobility but did stimulate esterification in macrophages. Baboons were divided into high and low responders based on the change in plasma cholesterol levels in response to a high fat, high cholesterol diet. Both plasma and hepatic VLDL from high responders stimulated cholesterol esterification, whereas hepatic VLDL obtained from low responders or chow-fed baboons did not stimulate cholesterol esterification in macrophages. There was a strong positive correlation (r = 0.866) between the number of apoE molecules per VLDL particle in VLDL obtained from chow-fed, lard-fed, or coconut oil-fed primates and the rate of cholesterol esterification in macrophages. Our results show that hepatic perfusate VLDL obtained from fat- and cholesterol-fed primates have compositional and functional properties usually ascribed to circulating β-VLDL, without displaying beta mobility, and indicate that the liver may be an important source of atherogenic lipoproteins. —**Soltys, P. A., H. Gump, L. Hennessy, T. Mazzone, K. D. Carey, H. C. McGill, Jr., G. S. Getz, and S. R. Bates.** Hepatic perfusate very low density lipoproteins obtained from fat-fed nonhuman primates stimulate cholesterol esterification in macrophages. *J. Lipid Res.* 1988. **29:** 191–201.

Supplementary key words β-VLDL • hepatic VLDL • baboon • rhesus monkey

The role of macrophages in the development of atherosclerosis has been the subject of numerous studies and reviews (1–7). Macrophages can accumulate large amounts of cholesteryl ester and may comprise a large proportion of the foam cells found in atherosclerotic plaques. Cholesteryl ester synthesis is stimulated in mouse peritoneal macrophages by beta very low density lipoproteins (β-VLDL) isolated from animals fed cholesterol-rich diets (5, 6). This finding suggests that β-VLDL may have an important role in the accelerated atherosclerosis which has been observed in these animals. Recent studies have shown that β-VLDL also stimulates cholesterol esterification in J774 and P388D$_1$ macrophage-like cells (8–10). β-VLDL also interacts with a macrophage cell surface receptor, for which β-VLDL apoprotein E appears to be the ligand (8, 11).

The origin of β-VLDL has been the subject of study for the last decade or more. Early studies pointed to the intestine as the source of β-VLDL. Studies in rabbits (12) and rhesus monkeys (13), using either labeled retinyl acetate or retinol as markers for lipoproteins of intestinal origin, concluded that the cholesteryl ester-rich, slow beta lipoproteins in the plasma of these animals were of intestinal origin. Subsequent studies, however, have shown that in dogs (14) and rabbits (15) some of the cholesteryl ester-rich fraction of plasma VLDL is probably of hepatic origin. Recently Kroon, Thompson, and Chao (16) showed that perfused livers from cholesterol-fed rabbits secrete a VLDL particle whose composition is similar to that of plasma β-VLDL. Cholesterol feeding in the rat and African green monkey have also been shown to induce the hepatogenous production of a cholesteryl ester-enriched VLDL (17–20).

Abbreviations: β-VLDL, beta very low density lipoproteins; TC, total cholesterol; TG, triglyceride; PBS, phosphate-buffered saline; LDL, low density lipoproteins; RIA, radioimmunoassay.

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In this study we have compared the composition of plasma VLDL and nascent hepatogenous VLDL derived from nonhuman primates fed a high fat, cholesterol-rich diet, and have also explored the capacity of these VLDL to promote cholesterol esterification in J774 macrophages. In contrast to normolipidemic VLDL, β-VLDL stimulates cholesterol esterification in freshly isolated mouse macrophages (5, 6) and in several macrophage cell lines (8–10). This characteristic allows one to explore the interactions of nascent hepatic VLDL with macrophages. The similarity between the J774 macrophages and mouse peritoneal macrophages in regard to high affinity uptake and metabolism of β-VLDL has been reported in detail elsewhere (8, 21, 22).

MATERIALS AND METHODS

Animals and diet

Rhesus monkeys (*Macaca mulatta*) and baboons (*Papio cynocephalus*) served as plasma and hepatic VLDL donors. The animals included in this study were fed standard Purina monkey chow or various high fat, high cholesterol diets in order to determine the effect of diet on hepatic lipoprotein production. The stimulation of cholesterol esterification in macrophages by the experimental VLDL samples was compared to that produced by a standard β-VLDL obtained from the plasma of a donor rhesus monkey fed a semisynthetic diet containing 2% cholesterol and 25% coconut oil (23) for the duration of the experiment (mean total serum cholesterol level (TC) = 932 mg/dl, mean serum triglyceride level (TG) = 108 mg/dl). Male rhesus monkeys were fed a semisynthetic atherogenic diet containing either 30% peanut oil and 1% cholesterol (23) (TC = 560 mg/dl, TG = 4 mg/dl), or 25% lard and 1% cholesterol (by weight) (24) (TC = 744 mg/dl, TG = 21 mg/dl) for 12–20 weeks or maintained on standard monkey chow (TC = 111 mg/dl, TG = 36 mg/dl). Female baboons were fed a diet based on chow supplemented with 30% peanut oil and 1% cholesterol for 9 weeks (TC = 182 mg/dl, TG = 4 mg/dl) or standard Purina monkey chow (TC = 118 mg/dl, TG = 36 mg/dl). A group of male baboons was fed the semisynthetic diet containing 25% lard and 1% cholesterol (24) for 8 months or standard Purina monkey chow (TC = 105 mg/dl, TG = 23 mg/dl). These male baboons were divided into two sets: *1*) high responders, those who responded to an 8-week challenge diet with total cholesterol levels equal to or greater than 200 mg/dl and LDL cholesterol levels equal to or greater than 80 mg/dl (TC = 225 mg/dl, TG = 26 mg/dl); and *2*) low responders, those who responded to the challenge diet with total cholesterol levels of less than 200 mg/dl and LDL cholesterol levels of less than 80 mg/dl (TC = 146, TG = 24). More detailed plasma lipid compositional data for the diet groups examined in this study is published elsewhere (25).

Liver perfusion

Livers were removed from fasted donor animals and perfused as described previously (26). The portal vein was cannulated and a perfusion mixture based on Krebs-Ringer bicarbonate buffer (pH 7.4) containing 20% washed human erythrocytes, 0.1% glucose, 0.06% of amino acid mixture, 0.05% hepatin, 0.005% gentamycin, and a continuously infused, physiological level of insulin was recirculated through the liver. The flow rate was maintained at a constant rate of 1 ml/g of liver/min and the perfusate was oxygenated as previously described (26). Liver function was monitored by measuring the incorporation of [^{3}H]leucine into secreted protein at regular intervals and by measuring the release of glutamic pyruvate transaminase and blood urea nitrogen into the perfusate (26). The perfusate collected during the first hr of perfusion was considered plasma washout and was discarded. The perfusate collected during the following 90 min was used as the source of the nascent hepatic VLDL in this study.

VLDL isolation

VLDL was isolated from fasted plasma and perfusate by ultracentrifugation. All ultracentrifugation was performed in Beckman centrifuges and rotors (Beckman Instruments, Inc., Palo Alto, CA). The VLDL was isolated in a 60 Ti rotor at 55,000 rpm for 16 hr at 10°C, and then washed in a 30.2 rotor at 29,000 rpm for 16 hr at 10°C. All ultracentrifugation solutions contained 0.05% ethylenediamine tetraacetic acid and 1 mM phenylmethylsulfonylfluoride.

Analysis of lipoproteins and plasma

Plasma from the experimental animals was obtained after a 16-hr fast just before the liver was removed. Plasma from the coconut oil diet donor monkey was obtained after a 16-hr fast. Plasma triglycerides and cholesterol were determined on Zeolite-treated isopropanol extracts run on the Technicon Auto Analyzer II™ (27). Phospholipid was estimated by liberating the inorganic phosphorus from purified lipid extracts by sulfuric acid digestion and then measuring the liberated phosphorus using the Bartlett inorganic phosphorus assay (28). VLDL protein and cellular protein were determined according to the method of Lowry et al. (29). VLDL cholesterol and cholesteryl ester were determined by gas–liquid chromatography as described previously by Bates et al. (4). VLDL triglyceride was quantitated by the method of Marsh and Weinstein (30) as modified by Kritchevsky et al. (31) and is described by Bates et al. (4). In some cases VLDL cholesterol, cholesteryl ester, and triglyceride were quantitated by

thin-layer chromatography and densitometric scanning (32). The data obtained from these methods yielded comparable values. ApoA-I, apoB, and apoE were determined by radioimmunoassay (RIA) (26, 33) in the lard-fed baboon VLDL. The VLDL from the lard-fed rhesus monkeys was subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and scanned densitometrically to quantitate apoA-I, $apoB_{100}$, $apoB_{48}$, and apoE as has been described elsewhere (26). In selected samples, scanning and RIA yielded similar values for the apoproteins of interest. Agarose electrophoresis was performed using "The Corning Electrophoresis System to Analyze Body Fluids" (Corning Medical, Palo Alto, CA).

Macrophages

The macrophage-like cell line J774A.1 (J774) (34) was obtained from the American Type Tissue Culture Collection, Bethesda, MD, and was maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Grand Island Biological Co., Grand Island, NY) plus 10% heat-inactivated fetal calf serum (K. C. Laboratory Supply Co., Indianapolis, IN) at 37°C in a 5% CO_2 atmosphere. Prior to experiments, the J774 cells were seeded to achieve a final cellular protein content of 0.2 to 0.5 mg/well in multiwell tissue culture plates (Costar, 12 wells/plate) after 2 days of incubation.

At the start of an experiment, the macrophages were washed twice with phosphate-buffered saline (PBS), pH 7.2, prior to addition of the experimental media. Cells were incubated with experimental media containing DMEM plus the isolated lipoprotein of interest for 2 hr before 0.04 μCi of [1-^{14}C]oleate–bovine serum albumin (BSA) was added to each well during the last 5 hr of incubation. The oleate:BSA molar ratio was 6.8:1 (1.7 mg of BSA/ml) in all experiments except those comparing the plasma and hepatic perfusate VLDL from lard-fed rhesus monkeys to the standard β-VLDL from the coconut oil donor when the oleate:BSA molar ratio was 0.8:1 (1.7 mg of BSA/ml). The [^{14}C]oleate-BSA complex was made according to the method of St. Clair (35) using [1-^{14}C]oleic acid (60 mCi/mol) from Amersham (Des Plaines, IL) and fatty acid-free BSA, fraction V powder, from Miles Laboratories (Kankakee, IL). The macrophage incubations were terminated in the following manner. The medium was removed and the cells were washed twice with PBS. The cells were then dissolved in 0.1 N NaOH for 20 min. A sample was removed for lipid extraction and neutralized immediately with concentrated acetic acid; a second sample was saved for a protein determination.

Assay of [^{14}C]cholesteryl oleate

[^{14}C]Oleate incorporation into cholesteryl ester was quantified by fractionation of the lipid extracts on plastic silica gel G thin-layer chromatography plates (J. T. Baker, Phillipsberg, NJ) developed with petroleum ether–ethyl ether–acetic acid 75:25:1. The separated lipids were cut out from the plate and placed in Econofluor scintillant (New England Nuclear, Boston, MA) for counting. An internal standard, [1,2-^{3}H]cholesterol, was added during the extraction and the results were corrected for recovery (mean recovery = 80%).

RESULTS

Effect of the peanut oil diet on plasma and perfusate VLDL composition

The composition of plasma VLDL and nascent hepatic VLDL (isolated from perfusate) secreted from the livers of rhesus monkeys and baboons fed normal chow or the peanut oil diet is shown in **Table 1.** Both the plasma and perfusate VLDL from chow-fed rhesus monkeys contained primarily triglyceride with little cholesteryl ester. The chow-fed female baboons, on the other hand, had more cholesteryl ester in both plasma and perfusate VLDL than did the rhesus monkeys. The plasma VLDL from both monkeys and baboons fed the peanut oil diet was rich in cholesteryl ester and low in triglyceride, reflecting the patterns seen in plasma (25). Only the rhesus monkey plasma VLDL was beta-migrating upon agarose gel electrophoresis; perfusate VLDL never exhibited beta electrophoretic mobility. The perfusate VLDL in both species of primates fed peanut oil were also highly enriched in cholesteryl ester although they contained more triglyceride and less cholesteryl ester than did the corresponding plasma VLDL. In the female baboons the cholesteryl ester content of both plasma and perfusate VLDL increased with the peanut oil diet although not quite as markedly as was seen in the rhesus monkey.

Effect of lard diet on plasma and hepatic VLDL composition

A more detailed analysis of the apoproteins of both plasma and hepatic VLDL was performed in animals consuming another atherogenic diet: 25% lard, 1% cholesterol. The rhesus monkeys consuming this diet have serum cholesterol values in excess of 700 mg/dl (25). **Table 2** shows that the plasma VLDL from these animals was even more cholesteryl ester-rich than was the plasma VLDL from the coconut oil-fed rhesus monkey which was used as our standard for β-VLDL production. The plasma VLDL of rhesus monkeys consuming peanut oil (Table 1) and lard (Table 2) were similar in lipid and especially cholesteryl ester content and showed beta electrophoretic mobility. With lard feeding, the perfusate VLDL contained much more triglyceride than did the plasma VLDL of these same animals. The lard plus cholesterol diet increased serum cholesterol less in baboons than in

TABLE 1. Composition of plasma and hepatic VLDL from monkeys and baboons fed a peanut oil plus cholesterol diet or a chow diet

VLDL Source	n	Lipid[a] Triglyceride	Free Cholesterol	Cholesteryl Ester	Protein[a]	Beta Mobility	Plasma Cholesterol[b]
			%		%		mg/dl
Chow diet							
Rhesus plasma VLDL	3	81.7 ± 4.4[c]	7.9 ± 0.1	10.4 ± 1.6[c]	16.3 ± 0.8	No	111
Rhesus hepatic VLDL	5	79.8 ± 3.9[d]	12.6 ± 1.4	7.7 ± 3.1[d]	12.8 ± 2.2	No	
Baboon plasma VLDL	4	48.9 ± 7.2[e]	11.4 ± 1.0	39.8 ± 6.7[g]	42.1 ± 7.8	No	118
Baboon hepatic VLDL	4	61.4 ± 3.8[f]	12.7 ± 1.8	25.9 ± 2.7[h]	36.6 ± 4.5	No	
Peanut oil diet							
Rhesus plasma VLDL	5	3.8 ± 1.8[c]	12.2 ± 2.7	84.2 ± 4.5[c]	17.8 ± 4.5	Yes	560
Rhesus hepatic VLDL	6	24.2 ± 4.3[d]	12.5 ± 1.1	63.2 ± 5.2[d]	13.3 ± 2.3	No	
Baboon plasma VLDL	4	11.9 ± 4.8[e]	14.7 ± 1.4	73.2 ± 5.6[g]	38.7 ± 14.8	No	182
Baboon hepatic VLDL	4	30.2 ± 6.3[f]	13.3 ± 2.8	56.6 ± 4.7[h]	17.8 ± 3.8	No	

The chow diet consisted of standard Purina monkey chow fed to male rhesus monkeys and female baboons. The peanut oil diet consisted of a semisynthetic diet containing 30% (by weight) peanut oil and 1% (by weight) cholesterol fed to male rhesus monkeys for 12–20 weeks or a standard chow diet supplemented with 30% peanut oil and 1% cholesterol fed to female baboons for 9 weeks. Results are means ± SD.

[a] Lipid is reported as percentage of the sum of triglyceride plus free cholesterol plus cholesteryl ester. Protein is reported as percentage of lipid plus protein.

[b] Mean plasma cholesterol values for these animals have been published previously (25) and are listed here for clarity.

[c–h] Comparisons reveal the following significant differences between the effect of the chow diet and the peanut oil diet in each of the lipid components of the VLDL: [c], triglyceride and cholesteryl ester in rhesus monkey plasma VLDL ($P < 0.001$); [d], triglyceride and cholesteryl ester in rhesus monkey hepatic VLDL ($P < 0.001$); [e], triglyceride in baboon plasma VLDL ($P < 0.005$); [f], triglyceride in baboon hepatic VLDL ($P < 0.005$); [g], cholesteryl ester in baboon plasma VLDL ($P < 0.001$); and [h], cholesteryl ester in baboon hepatic VLDL ($P < 0.001$).

TABLE 2. Composition of plasma and hepatic VLDL from rhesus monkeys and baboons fed a lard plus cholesterol diet or a chow diet

VLDL Source	Lipid[a] Triglyceride	Free Cholesterol	Cholesteryl Ester	Protein[a]	Beta Mobility	PL:P Ratio[b]	Plasma Cholesterol[b]
		%		%			mg/dl
Chow diet[c]							
Baboon plasma	65.8 ± 6.5	8.8 ± 4.3	25.4 ± 2.6	16.8 ± 5.9	No	1.6	105
Baboon liver	73.2 ± 6.8	11.8 ± 0.5	14.8 ± 7.0	33.7 ± 9.7	No	1.1	
Lard diet							
Rhesus monkey plasma	8.5 ± 1.6[d]	11.1 ± 1.8	79.5 ± 3.8[d]	8.2 ± 2.5	Yes	2.3	744
Rhesus monkey liver	61.9 ± 4.8[e]	10.4 ± 0.8	27.6 ± 4.1[f]	13.6 ± 1.5	No	2.5	
High responder baboon plasma	61.4 ± 13.0	7.6 ± 1.2	31.0 ± 12.0	10.0 ± 1.3	No	1.7	225
High responder baboon liver	77.8 ± 17.3	7.4 ± 6.0	14.8 ± 1.2	30.0 ± 9.3	No	1.4	
Low responder baboon plasma	54.2 ± 17.5	14.6 ± 9.6	31.2 ± 12.8	15.7 ± 2.3	No		146
Low responder baboon liver	63.6 ± 9.0	13.3 ± 1.2	23.1 ± 8.8	32.6 ± 11.4	No	1.6	
Coconut oil diet							
Rhesus monkey plasma	33.3 ± 18.4	12.5 ± 1.6	54.2 ± 15.0	11.4 ± 3.9	Yes	3.2	932

The results are means ± SD for three animals in each group except for the coconut diet plasma VLDL in which the VLDL obtained from the single donor monkey was measured on three separate occasions. The chow diet consisted of standard Purina monkey chow fed to male baboons. The lard diet consisted of semisynthetic diet containing 25% (by weight) lard and 1% (by weight) cholesterol which was fed to male rhesus monkeys for 12–20 weeks and to high and low responder male baboons for 8 months. The coconut oil diet consisted of a semisynthetic diet containing 25% coconut oil and 2% cholesterol fed to a single male rhesus monkey over a period of 2 years.

[a] Lipid composition is reported as the percentage of the sum of triglyceride plus free cholesterol plus cholesteryl ester. Protein is reported as the percentage of lipid plus protein.

[b] PL:P is phospholipid to protein ratio for those samples that were analyzed for phospholipid. The phospholipid content of the particles can be estimated from the phospholipid to protein ratio which was calculated when sufficient sample was available for the phospholipid assay. Mean plasma cholesterol values for these animals have been published previously (25) and are listed here for clarity.

[c] The values for the chow-fed rhesus monkey plasma and hepatic VLDL are in Table 1. The phospholipid:protein ratio for chow-fed rhesus plasma VLDL is 1.6 and for perfusate is 2.9. The chow diet baboon VLDL in this table were obtained from a group of chow-fed male baboons in contrast to those reported in Table 1 which are for chow-fed female baboons.

[d] These VLDL lipids are significantly different from chow-fed rhesus monkey plasma VLDL (Table 1) ($P < 0.001$).

[e] Triglycerides are significantly different from chow-fed rhesus monkey hepatic VLDL (Table 1) ($P < 0.05$).

[f] Cholesteryl esters are significantly different from chow-fed rhesus monkey hepatic VLDL (Table 1) ($P < 0.02$).

rhesus monkeys reaching a maximum value of only 270 mg/dl. The observed increase varied considerably among individual baboons (25) and the animals in our study were divided into high responder and low responder groups on the basis of this response (see Materials and Methods). The lipid composition of both plasma and nascent hepatic VLDL from these animals is shown in Table 2. Baboon plasma VLDL from lard-fed animals did not exhibit beta electrophoretic mobility. The plasma VLDL of the lard-consuming baboons was not nearly as enriched in cholesteryl ester as was the VLDL of rhesus monkeys fed the lard diet or of rhesus monkeys and baboons eating the peanut oil diet. In contrast to the rhesus monkeys fed the lard diet or standard Purina chow, there were no significant differences in the lipid composition of either perfusate or plasma VLDL from the lard-fed animals as compared to the chow-fed animals or between the high responder and low responder baboon groups (Table 2). Comparison of the plasma and perfusate VLDL of lard-fed male baboons revealed the same relative enrichment of perfusate VLDL with triglyceride as was observed in the other groups in these studies. Phospholipids were analyzed when sufficient sample was available and the data are reported in Table 2 as the ratio of phospholipid to protein (PL/P).

The apoprotein composition of perfusate and plasma VLDL was analyzed by scanning VLDL protein samples separated by SDS-PAGE techniques (rhesus monkeys) or by radioimmunoassay (baboons). The results are shown in **Table 3.** Plasma VLDL from both lard-fed and coconut oil-fed rhesus monkeys had considerably more apoprotein B_{48}, as identified by its electrophoretic mobility, than did the hepatic VLDL. The perfusate VLDL of baboons, as analyzed by SDS-PAGE (data not shown), contained little $apoB_{48}$ and the small amount observed did not appear to be newly synthesized as assessed by its labeling with [^{3}H]leucine. The plasma and perfusate VLDL from the lard-fed rhesus monkeys contained two to four times as much apoE as did plasma VLDL from the coconut oil-fed donor animal. There was also a significant increment ($P < 0.05$) in the percentage of apoE in the plasma VLDL of the high responder baboons as compared to the plasma VLDL of the low responder or the chow-fed baboons. The relative apoprotein E enrichment is reflected in the calculated molecular composition of the VLDL particles (**Table 4**). Assuming a single $apoB_{100}$ molecule per VLDL particle, the apparent apoE/apoB molar ratio ranges from 12.4 for the plasma VLDL from lard-fed monkeys to 2.3 for the plasma VLDL from the coconut oil-fed donor monkey. Among the baboons, too, the apoE/apoB molar ratio extended from 4.3 for plasma VLDL of high responders to 1.0 for plasma VLDL of chow-fed baboons.

Stimulation of cholesterol esterification in J774 macrophages by hepatic and plasma VLDL

The first experiments were designed to ask whether nascent hepatic VLDL from either monkeys or baboons

TABLE 3. Effect of diet on apoprotein composition of plasma and hepatic VLDL

VLDL Source	ApoB	$ApoB_{48}$	$ApoB_{100}$	ApoE	ApoA-I	n
			% of total protein ± SD			
Assay by SDS-PAGE						
Rhesus monkey plasma VLDL						
Lard diet		4 ± 2	52 ± 5	44 ± 8	<0.5	3
Coconut oil diet		7 ± 0	78 ± 7	12 ± 4	4 ± 3	1[a]
Rhesus monkey hepatic VLDL						
Lard diet		1 ± 1	74 ± 9	23 ± 9	1 ± 1	3
Assay by radioimmunoassay						
Baboon plasma VLDL						
Lard diet; high responder	77 ± 6			22 ± 5[b]	2 ± 2	3
Lard diet; low responder	89 ± 3			7 ± 2[b]	4 ± 5	3
Chow diet	93 ± 3			6 ± 3[b]	1 ± 0	3
Baboon hepatic VLDL						
Lard diet; high responder	80 ± 1			10 ± 4	6 ± 7	3
Lard diet; low responder	78 ± 9			4 ± 1	17 ± 13	3
Chow diet	91 ± 1			4 ± 1	5 ± 1	3

The apoprotein compositions were obtained for the rhesus monkey samples from gel scanning of SDS-PAGE electrophorograms; the amount of low molecular weight apoproteins was less than 5%. The apoprotein composition of plasma VLDL from chow-fed rhesus monkeys has been reported previously (26) and the gel scan data for those samples indicated that the VLDL contained 59% apoB, 35% small molecular weight proteins (apoC and/or apoA-II), and the remaining 6% was apoE and apoA-I. The apoprotein compositions of the baboon VLDL samples were obtained by radioimmunoassay. SDS-PAGE of baboon plasma and hepatic VLDL revealed very minor amounts of $apoB_{48}$.

[a] Three separate preparations of VLDL from a single monkey fed the coconut oil plus cholesterol diet were analyzed.

[b] Lard-fed, high responder plasma VLDL was significantly different from either lard-fed, low responder plasma VLDL or chow-fed baboon plasma VLDL ($P < 0.05$).

TABLE 4. Molar composition of baboon and rhesus monkey VLDL

VLDL Source	ApoB	ApoE	Cholesteryl Ester	Free Cholesterol	Triglyceride
			mol		
Rhesus monkey VLDL					
Lard diet, plasma	1.0	12.4	13069	3158	1055
Lard diet, hepatic	1.0	4.6	1817	1185	3076
Coconut oil diet, plasma	1.0	2.3	4158	1660	1928
Baboon VLDL					
High responder, plasma	1.0	4.3	2783	1243	4133
High responder, hepatic	1.0	1.9	336	271	1319
Low responder, plasma	1.0	1.2	1404	1176	1847
Low responder, hepatic	1.0	0.8	460	450	966
Chow diet, plasma	1.0	1.0	1007	628	2008
Chow diet, hepatic	1.0	0.7	246	340	903

These values were calculated assuming molecular weights of $apoB_{100}$ = 513,000 (36); apoE = 34,200 (37); cholesteryl ester = 668; free cholesterol = 386; and triglyceride = 885. Assuming that each VLDL particle contains only one apoB molecule, the molar composition indicates the number of each of the component molecules per VLDL particle.

fed the peanut oil diet had the ability to enhance the synthesis of cholesteryl esters in macrophages. Plasma VLDL of monkeys fed coconut oil has ben identified as having a large β-VLDL component and has been shown to stimulate cholesterol esterification in J774 macrophages (4, 8). β-VLDL isolated from the plasma of a single coconut oil-fed donor rhesus monkey was used as the positive control or standard throughout these experiments. As shown in **Fig. 1,** the standard β-VLDL stimulated cholesteryl ester synthesis by J-774 macrophages 7- to 10-fold over that produced by albumin alone. When adjusted to the same total cholesterol concentration, plasma VLDL from the peanut oil-fed monkeys produced a response similar to that seen with the standard β-VLDL. The cholesteryl ester-rich VLDL isolated from hepatic perfusate of peanut oil-fed monkeys also promoted cholesterol esterification in J774 macrophages. In accord with previous reports, VLDL isolated from the plasma or liver perfusate of chow-fed monkeys did not affect cellular cholesteryl ester synthesis (4, 5). At this low concentration (40 μg of cholesterol/ml) and short time period (7 hr), LDL, isolated from the plasma of the coconut oil-fed donor monkey, stimulated cholesterol esterification in the J774 cells only slightly. This is consistent with the report of Tabas, Weiland, and Tall (38) who showed that even 300 μg of human LDL protein (greater than 600 μg of LDL cholesterol) did not cause large accumulations of cholesteryl ester after only 7 hr of incubation. For most experiments, 40 μg/ml of VLDL cholesterol was close to a saturating concentration in promoting cholesterol esterification in macrophages. To conserve the limited amount of perfusate VLDL, all subsequent experiments were performed at this VLDL cholesterol concentration (40 μg/ml). **Fig. 2** illustrates that both plasma and hepatic VLDL from rhesus monkeys and baboons fed the cholesterol and peanut oil diet were able to stimulate macrophage cholesterol esterification to a similar extent. In both species the liver produced a VLDL particle capable of stimulating cholesterol esterification.

Hepatic and plasma VLDL from lard-fed rhesus monkeys were also examined for their ability to promote cholesterol esterification in J774 macrophages. As illustrated in **Fig. 3,** plasma VLDL from the lard-fed monkeys stimulated cholesterol esterification to a much greater extent than did the standard β-VLDL. Hepatic VLDL from the lard-fed monkeys, although not as cholesteryl ester-rich as the standard β-VLDL (Table 2), also stimulated more cholesterol esterification than did the standard β-VLDL.

Effect of high and low responder phenotypes on VLDL stimulation of cholesteryl esterification in macrophages

Plasma VLDL from high responder baboons stimulated cholesterol esterification in macrophages to the same extent as the standard β-VLDL from the coconut oil-fed rhesus monkey (**Fig. 4**) when adjusted to the same total cholesterol concentration (40 μg total cholesterol/ml). In contrast, plasma VLDL from low responder baboons showed only one-third as much activity as the standard β-VLDL. High responder plasma VLDL was significantly more active than either low responder plasma VLDL ($P < 0.05$) or control plasma VLDL ($P < 0.025$). Nascent hepatic VLDL from the high responder baboons fed lard was 40% as effective as the standard β-VLDL in promoting cholesterol esterification in macrophages. This was a significant increase over the esterification caused by nascent hepatic VLDL from chow-fed animals ($P < 0.05$).

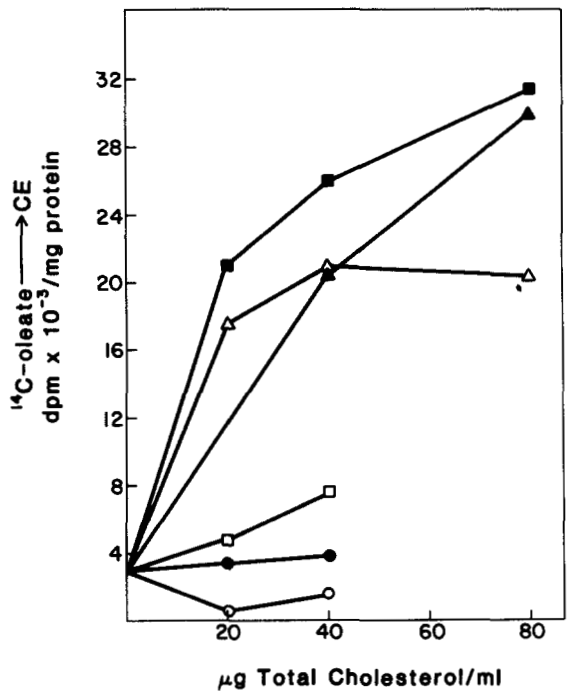

Fig. 1. Cholesterol esterification in J774 macrophages. J774 macrophages were grown for 7 hr in the presence of various concentrations of plasma and hepatic VLDL isolated from rhesus monkeys fed a peanut oil plus cholesterol diet, a standard Purina monkey chow diet, or a coconut oil plus cholesterol diet. [^{14}C]Oleate complexed to bovine serum albumin (molar ratio 6.8:1) was added during the last 5 hr of incubation and then the cells were harvested and the incorporation of labeled oleate into cellular cholesterol was assayed. The data were gathered from five separate experiments. The data are duplicate determinations except for the normal plasma VLDL for which a single determination was made at each point. The normal plasma VLDL data are included to reinforce the previous observation (4, 5) that these samples do not promote cholesterol esterification. Values between experiments were normalized to cholesterol esterification induced by the plasma VLDL of the coconut oil-fed rhesus monkey which served as the positive control in all of the experiments. The albumin control always stimulated the incorporation of 3×10^{-3} dpm (or less) into cholesteryl ester (CE) and is represented here as 3×10^{-3} dpm/mg cell protein for all of the incubations. Legend: (■) plasma VLDL from coconut oil-fed donor; (▲) plasma VLDL from peanut oil-fed monkey; (△) hepatic VLDL from peanut oil-fed monkey; (□) plasma LDL from coconut oil-fed donor; (●) plasma VLDL from chow-fed monkey; and (○) hepatic VLDL from chow-fed monkey.

DISCUSSION

We examined the effects of two high fat, high cholesterol diets on both nascent hepatogenous VLDL and plasma VLDL in rhesus monkeys and baboons in order to ascertain whether the properties of the nascent hepatic VLDL, harvested from the recirculated perfusate of livers, resemble those of circulating plasma VLDL. Specifically, we wanted to determine whether nascent hepatic VLDL is capable of stimulating cholesterol esterification in macrophages, potentially leading to the production of foam cells. Although there were large quantitative differences in the plasma cholesterol response of rhesus monkeys and baboons fed the same atherogenic diets, the plasma and hepatic VLDL from both species were usually enriched in cholesteryl ester and apoE and promoted cholesterol esterification in macrophages. In the case of lard-fed baboons, there was minimal cholesteryl ester enrichment in the plasma and hepatic VLDL; nevertheless, macrophage cholesterol esterification was promoted by the plasma and hepatic VLDL of the high responder baboons and the plasma VLDL of the low responder baboons. The capacity of hepatogenous VLDL to promote cholesterol esterification in macrophages has not previously been reported.

The plasma VLDL of hyperlipemic rhesus monkeys differed from that of baboons in its relative electrophoretic mobility in agarose gels. Hyperlipemic rhesus monkey plasma VLDL exhibited beta electrophoretic mobility while hyperlipemic baboon plasma VLDL did not. Nevertheless, VLDL from both species were similarly active in promoting cholesterol esterification in macrophages. While it has been previously shown that VLDL isolated from hyperlipemic dogs has a small pre-β-VLDL component (less than 10% of the total VLDL fraction) which stimulates esterification in macrophages almost as well as the β-VLDL component (5), our study describes stimulation of esterification in macrophages by VLDL isolated from plasma which contains no β-VLDL component. This hyperlipemic baboon VLDL may reflect the

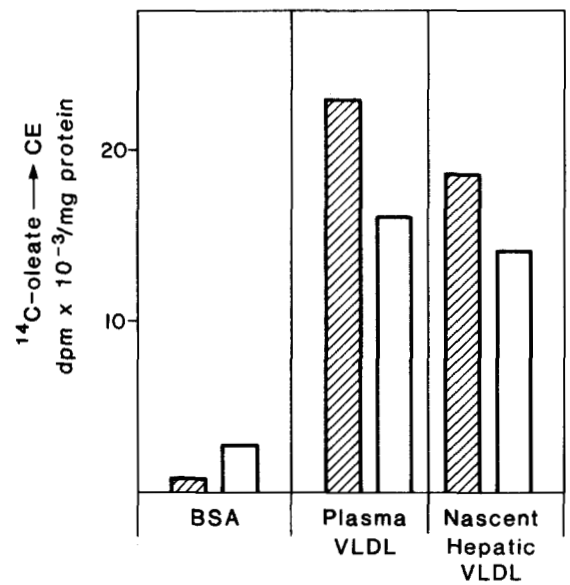

Fig. 2. Macrophage cholesterol esterification promoted by VLDL of two species of primates. The stimulation of cholesterol esterification in J774 macrophages by plasma and nascent hepatic (perfusate) VLDL (40 μg of VLDL cholesterol/ml) from baboons (striped bars) and rhesus monkeys (open bars) fed a peanut oil plus cholesterol diet is compared to the stimulation produced by the bovine serum albumin (BSA) (2 mg/ml) control. The values are the averages of duplicate determinations from one experiment with each species; CE, cholesteryl ester.

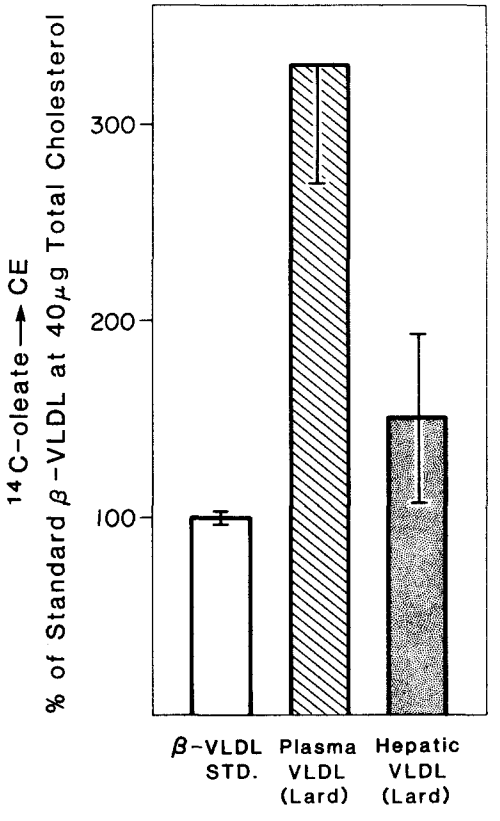

Fig. 3. Stimulation of cholesteryl ester synthesis in macrophages by plasma VLDL or nascent hepatic VLDL isolated from rhesus monkeys fed a 30% lard plus 1% cholesterol diet. The data represent the mean ± SEM of duplicate experiments performed with three separate animal preparations. The VLDL were added to give a final concentration of 40 μg/ml of VLDL cholesterol. The cholesterol esterification induced by the standard β-VLDL from the coconut oil-fed donor (40 μg of VLDL cholesterol) was used to normalize the data among the three experiments; CE, cholesteryl ester.

condition found in humans who consume high fat, high cholesterol diets but who do not have β-VLDL in their plasma.

The plasma VLDL of the two primate species fed peanut oil was fairly similar (Table 1) with core lipid compositions in which cholesteryl ester was the major component and triglyceride was a minor component. This VLDL composition is not surprising in view of the very low plasma triglyceride levels reported in peanut oil-fed male rhesus monkeys (25, 39) and female baboons (25). All hepatic VLDL had relatively more triglyceride than the homologous plasma VLDL and this triglyceride enrichment occurred at the expense of cholesteryl ester, the other core lipid. However, the perfusate VLDL from fat-fed primates was still enriched in cholesteryl ester as compared to chow-fed primates in proportion to the enrichment of the corresponding plasma VLDL but at a somewhat lower level. The hepatic secretion of cholesteryl ester-enriched VLDL has been previously reported in rat, rabbit, and African green monkey liver perfusion studies (16–20) and the secretion of such VLDL from the livers of two additional species of cholesterol-fed primates is documented in this report.

The data from the lard-fed baboon study (Fig. 4) is particularly interesting because there were no significant differences in the lipid composition of the VLDL isolated from the chow-fed and the lard-fed high responder and low responder animals even though the animals were divided into high and low responders on the basis of their serum cholesterol response to the challenge diet. The serum cholesterol concentration appears to be genetically mediated in baboons (40, 41). Indeed, several distinct lipoprotein phenotypes have been shown to be heritable (40, 42) and are modulated by diet (43) as well as by breeding. Our data, obtained from phenotypically designated high responder and low responder baboons, suggests that a genetic component may be similarly involved in the response of the liver to dietary stimulus. The VLDL secreted by the liver of high responder baboons, who are only mildly hyperlipemic and who do not have β-VLDL in their plasma, stimulated cholesterol esterification in macrophages, whereas the hepatic VLDL of the low responder baboons did not promote cholesterol esterification. This finding suggests that the liver may play an important role in determining whether atherogenic lipoproteins are produced in response to dietary stimuli.

In considering which determinants of VLDL are most closely related to the stimulation of cholesterol esterification in macrophages, we examined both the apoprotein composition of the VLDL, which affects the amount as well as the efficiency of VLDL uptake by the macrophage cell surface receptor, and the cholesteryl ester content of the VLDL particle. Since hepatogenous VLDL, which contained little or no $apoB_{48}$, was capable of promoting cholesterol esterification in macrophages, our data indicate that apoprotein B_{48} is not a requirement for interaction with macrophages. In addition, the extent of the relative cholesteryl ester enrichment of the VLDL lipids (Tables 1 and 2) was not, by itself, an accurate indicator of the stimulation of esterification in the macrophage assay. For example, when VLDL lipid composition was similar (i.e., comparing plasma VLDL from lard-fed high responders to plasma VLDL from chow-fed baboons; Table 2) only the VLDL from the diet-fed animals promoted macrophage cholesteryl ester synthesis.

Recent investigations have drawn attention to the importance of apoprotein E as the ligand for the interaction of VLDL with the macrophage cell surface receptor (8, 11). When the apoprotein content of the VLDL samples was estimated by radioimmunoassay or gel scanning, it was found that the molar ratio of apoE/apoB differed. If one assumes that each VLDL particle has only one

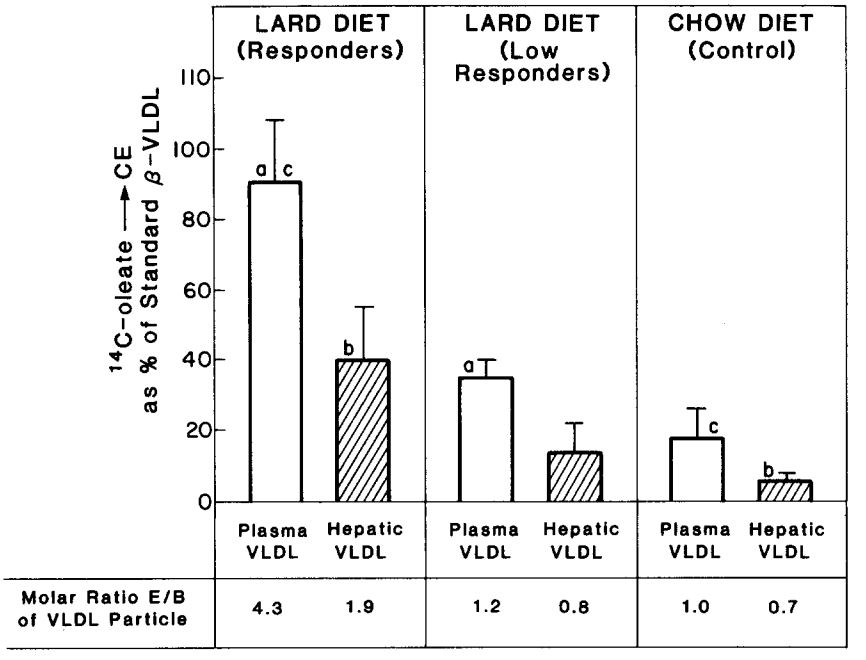

Fig. 4. Stimulation of esterification in macrophages by hepatic and plasma VLDL from high and low responder baboons fed a lard diet. The data in each of the groups, high responder, low responder, and control, compare the stimulation of esterification by VLDL obtained from the liver perfusion and circulating plasma of three baboon experiments. The data represent the averages of duplicate determinations from each of the three animals included in each group and were normalized to the esterification (100%) induced by the standard β-VLDL from the coconut oil-fed donor. The VLDL were added to the macrophages at a concentration of 40 μg of total cholesterol per ml. The following comparisons were significantly different: a) high responder plasma VLDL versus low responder plasma VLDL ($P < 0.05$); b) high responder hepatic VLDL versus chow hepatic VLDL ($P < 0.05$); and c) high responder plasma VLDL versus chow plasma VLDL ($P < 0.025$); CE, cholesteryl ester.

molecule of apoB_{100}, it appears that there must be an average of greater than one apoE molecule per particle (i.e., a molar apoprotein E/B ratio of greater than 1.0) in order to stimulate cholesterol esterification over control levels (Fig. 4). Thus the lard-fed high responder baboon livers secreted an apoE-enriched VLDL that stimulated cholesterol esterification in macrophages even though this particle was not particularly cholesteryl ester-rich as compared to VLDL from chow-fed animals. We examined the relationship between the number of moles of apoE per VLDL particle and cholesterol esterification in macrophages (**Fig. 5**), and found a highly significant correlation ($r = 0.866$; $P < 0.001$; n = 24). This enrichment of apoE in the isolated VLDL may well be only a minimal estimation of the in vivo enrichment, since apoE is known to be displaced from VLDL during centrifugation (44). Since acyl-CoA cholesterol acyltransferase (ACAT) activity is stimulated by increased availability of substrate cholesterol (45), we also calculated the simple correlation between cholesterol esterification and the moles of cholesteryl ester per VLDL particle and found a significant correlation ($r = 0.737$; $P < 0.001$; n = 24). However, these simple correlations assume that the apoE content and the cholesteryl ester content of the VLDL particle are independent variables. Since the apoE content and the cholesteryl ester content of the VLDL particles used in these experiments are probably not independent variables, we also used multiple regression analysis, a more stringent statistical test. This analysis showed that the amount of apoE per VLDL particle is a highly significant predictor of cholesterol esterification ($P < 0.001$; n = 24) whereas the amount of cholesteryl ester per VLDL particle is not ($P > 0.5$). Thus the amount of apoE in the VLDL particle appears to be the important determinant in stimulating cholesterol esterification in macrophages, while the cholesteryl ester may be necessary but not sufficient to cause the stimulation that was observed.

In conclusion, we examined cholesterol esterification rates in J774 macrophages using plasma and hepatic sources of VLDL in order to assess the relative biological reactivity of these particles in an in vitro system. It was not our purpose to describe which of several possible receptors (3, 46, 47) on the macrophage surface is involved in the uptake process, but rather to assess whether VLDL particles produced by the liver are capable of promoting cholesterol esterification which may in turn lead to foam cell formation. We have clearly shown that nascent hepatic VLDL will stimulate cholesterol esterification in macrophages. It remains for future studies to characterize the mechanism by which this occurs. In addi-

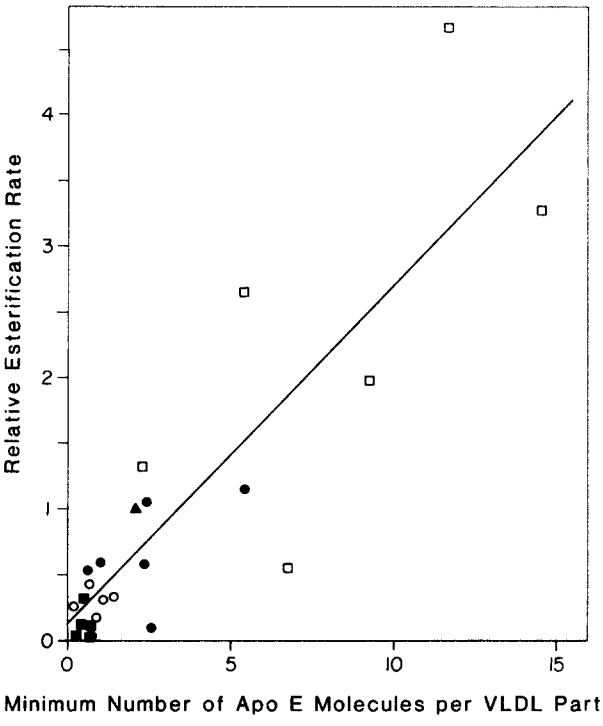

Fig. 5. Correlation between esterification rate and moles of apoE per VLDL particle. The relation between esterification rate and the number of moles of apoE per VLDL particle was plotted for macrophage incubations in which VLDL was added at a concentration of 40 μg of VLDL cholesterol/ml. The relative esterification rate sets the amount of esterification produced by the standard β-VLDL equal to 1. The 24 experimental VLDL samples included plasma and hepatic VLDL from lard-fed rhesus monkeys (□), plasma β-VLDL from the coconut oil-fed donor monkey (▲), plasma and hepatic VLDL from high (●) and low (○) responder lard-fed baboons and plasma and hepatic VLDL from chow-fed male baboons (■). Each macrophage incubation was performed in duplicate. The correlation coefficient is 0.866 ($P < 0.001$; n = 24).

tion, this study has shown that neither beta electrophoretic mobility nor profound cholesteryl ester enrichment of the VLDL is required for the stimulation of cholesterol esterification in macrophages. The amount of apoprotein E seemed to be the most sensitive predictor of the ability of a VLDL particle to stimulate cholesterol esterification in macrophages, regardless of whether the VLDL is obtained from plasma or hepatic perfusate. The basis for the association of apoprotein with particular VLDL particles remains to be determined. The lard and coconut oil diets were associated with different levels of apoprotein E in the VLDL recovered from plasma. One mechanism through which particular fats are atherogenic may be via their propensity to generate VLDL which are enriched in apoE and cholesteryl ester.

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REFERENCES

1. Brown, M. S., and J. L. Goldstein. 1983. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Rev. Biochem.* **52:** 223–261.
2. Mahley, R. W. 1983. Development of accelerated atherosclerosis: concepts derived from cell biology and animal model studies. *Arch. Pathol. Lab. Med.* **107:** 393–399.
3. Mahley, R. W., and T. L. Innerarity. 1983. Lipoprotein receptors and cholesterol homeostasis. *Biochim. Biophys. Acta.* **737:** 197–222.
4. Bates, S. R., P. L. Murphy, Z. Feng, T. Kanazawa, and G. S. Getz. 1984. Very low density lipoproteins promote triglyceride accumulation in macrophages. *Arteriosclerosis.* **4:** 103–114.
5. Goldstein, J. L., Y. K. Ho, M. S. Brown, T. L. Innerarity, and R. W. Mahley. 1980. Cholesteryl ester accumulation in macrophages resulting from receptor-mediated uptake and degradation of hypercholesterolemic canine β-very low density lipoproteins. *J. Biol. Chem.* **255:** 1839–1848.
6. Mahley, R. W., T. L. Innerarity, M. S. Brown, Y. K. Ho, and J. L. Goldstein. 1980. Cholesteryl ester synthesis in macrophages: stimulation by β-very low density lipoproteins from cholesterol-fed animals of several species. *J. Lipid Res.* **21:** 970–980.
7. Gianturco, S. H., W. A. Bradley, A. M. Gotto, J. D. Morrisett, and D. L. Peavy. 1982. Hypertriglyceridemic very low density lipoproteins induce triglyceride synthesis and accumulation in mouse peritoneal macrophages. *J. Clin. Invest.* **70:** 168–178.
8. Bates, S. R., B. A. Coughlin, T. Mazzone, J. Borensztajn, and G. S. Getz. 1987. Apoprotein E mediates the interaction of β-VLDL with macrophages. *J. Lipid Res.* **28:** 787–797.
9. Via, D. P., A. L. Plant, I. F. Craig, A. M. Gotto, Jr., and L. C. Smith. 1985. Metabolism of normal and modified low-density lipoproteins by macrophage cell lines of murine and human origin. *Biochim. Biophys. Acta.* **833:** 417–428.
10. Gianturco, S. H., S. A. Brown, D. P. Via, and W. A. Bradley. 1986. The β-VLDL receptor pathway of murine P388D_1 macrophages. *J. Lipid Res.* **27:** 412–420.
11. Innerarity, T. L., K. S. Arnold, K. H. Weisgraber, and R. W. Mahley. 1986. Apolipoprotein E is the determinant that mediates the receptor uptake of β-very low density lipoproteins by mouse macrophages. *Arteriosclerosis.* **6:** 114–122.
12. Ross, A. C., and D. B. Zilversmit. 1977. Chylomicron remnant cholesteryl esters as the major constituent of very low density lipoproteins in plasma of cholesterol-fed rabbits. *J. Lipid Res.* **18:** 169–181.
13. Lusk, L., J. Chung, and A. M. Scanu. 1982. Properties and metabolic fate of two very low density lipoprotein subfractions from rhesus monkey serum. *Biochim. Biophys. Acta.* **710:** 134–142.
14. Fainaru, M., R. W. Mahley, R. L. Hamilton, and T. L. Innerarity. 1982. Structural and metabolic heterogeneity of

β-very low density lipoproteins from cholesterol-fed dogs and from humans with Type III hyperlipoproteinemia. *J. Lipid Res.* **23:** 702–714.
15. Thompson, K. H., L. B. Hughes, and D. B. Zilversmit. 1983. Lack of secretion of retinyl ester by livers of normal and cholesterol-fed rabbits. *J. Nutr.* **113:** 1995–2001.
16. Kroon, P. A., G. M. Thompson, and Y-S. Chao. 1985. β-Very low density lipoproteins in cholesterol-fed rabbits are of hepatic origin. *Atherosclerosis.* **56:** 323–329.
17. Noel, S-P., L. Wong, P. J. Dolphin, L. Dory, and D. Rubinstein. 1979. Secretion of cholesterol-rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64:** 674–683.
18. Kris-Etherton, P. M., and A. D. Cooper. 1980. Studies on the etiology of the hyperlipemia in rats fed an atherogenic diet. *J. Lipid Res.* **21:** 435–442.
19. Swift, L. L., N. R. Manowitz, G. D. Dunn, and V. S. LeQuire. 1980. Isolation and characterization of hepatic Golgi lipoproteins from hypercholesterolemic rats. *J. Clin. Invest.* **66:** 415–425.
20. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1985. Effects of the degree of saturation of dietary fat on the hepatic production of lipoproteins in the African green monkey. *J. Lipid Res.* **26:** 403–417.
21. Lindqvist, P., A-M. Ostlund-Lindqvist, J. L. Witztum, D. Steinberg, and J. A. Little. 1983. The role of lipoprotein lipase in the metabolism of triglyceride-rich lipoproteins by macrophages. *J. Biol. Chem.* **258:** 9086–9092.
22. Ellsworth, J. L., A. D. Cooper, and F. B. Kraemer. 1986. Evidence that chylomicron remnants and β-VLDL are transported by the same receptor pathway in J774 murine macrophage-derived cells. *J. Lipid Res.* **27:** 1062–1072.
23. Vesselinovitch, D., R. W. Wissler, T. J. Schaffner, and J. Borensztajn. 1980. The effect of various diets on atherogenesis in rhesus monkeys. *Atherosclerosis.* **35:** 189–207.
24. McGill, H. C., Jr., C. A. McMahan, A. W. Kruske, and G. E. Mott. 1981. Relationship of lipoprotein cholesterol concentrations to experimental atherosclerosis in baboons. *Arteriosclerosis.* **1:** 3–12.
25. Getz, G. S., P. A. Soltys, K. D. Carey, H. C. McGill, Jr., and R. Hay. 1987. Nutrition and biogenesis of plasma lipoproteins in nonhuman primates. *Am. Heart J.* **113:** 440–445.
26. Jones, L. A., T. Teramoto, D. J. Juhn, R. B. Goldberg, A. H. Rubenstein, and G. S. Getz. 1984. Characterization of lipoprotein produced by the perfused rhesus monkey liver. *J. Lipid Res.* **25:** 319–335.
27. Lipid Research Clinics. 1974. Manual of Laboratory Operations. Vol 1. National Heart and Lung Institute, Bethesda, MD.
28. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
29. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
30. Marsh, J. B., and D. B. Weinstein. 1966. Simple charring method for determination of lipids. *J. Lipid Res.* **7:** 574–576.
31. Kritchevsky, D., L. M. Davidson, H. K. Kim, and S. Malhotra. 1973. Quantitation of serum lipids by a simple TLC-charring method. *Clin. Chim. Acta.* **46:** 63–68.
32. Portman, O. W., and M. Alexander. 1972. Changes in arterial subfractions with aging and atherosclerosis. *Biochim. Biophys. Acta.* **260:** 460–474.
33. Karlin, J. B., D. J. Juhn, G. Fless, A. M. Scanu, and A. H. Rubenstein. 1978. Measurement of rhesus monkey (*Macaca mulatta*) apolipoprotein B in serum by radioimmunoassay: comparison of immunoreactivities of rhesus and human low density lipoproteins. *J. Lipid Res.* **19:** 197–206.
34. Ralph, P., J. Prichard, and M. Cohn. 1975. Reticulum cell sarcoma: an effector cell in antibody-dependent cell-mediated immunity. *J. Immunol.* **114:** 898–905.
35. St. Clair, R. W., B. P. Smith, and L. L. Wood. 1977. Stimulation of cholesterol esterification in rhesus monkey smooth muscle cells. *Circ. Res.* **40:** 166–173.
36. Law, S. W., S. M. Grant, K. Higuchi, A. Hospattankar, K. Lackner, N. Lee, and H. B. Brewer, Jr. 1986. Human liver apolipoprotein B-100 cDNA: complete nucleic acid and derived amino acid sequence. *Proc. Natl. Acad. Sci. USA.* **83:** 8142–8146.
37. Mahley, R. W., T. L. Innerarity, S. C. Rall, Jr., and K. H. Weisgraber. 1984. Plasma lipoproteins: apolipoprotein structure and function. *J. Lipid Res.* **25:** 1277–1294.
38. Tabas, I., D. A. Weiland, and A. R. Tall. 1985. Unmodified low density lipoprotein causes cholesteryl ester accumulation in J774 macrophages. *Proc. Natl. Acad. Sci. USA.* **82:** 416–420.
39. Vesselinovitch, D., R. W. Wissler, L. Harris, and L. Lusk. 1980. The relationship between lipoprotein levels and xanthomas during progression and regression of atherosclerosis. *Atherosclerosis.* **36:** 351–364.
40. Flow, B. L., T. C. Cartwright, T. J. Kuehl, G. E. Mott, D. C. Kraemer, A. W. Kruski, J. D. Williams, and H. C. Mcgill. 1981. Genetic effects on serum cholesterol concentrations in baboons. *J. Hered.* **72:** 97–104.
41. Flow, B. L., and G. E. Mott. 1982. Genetic mediation of lipoprotein cholesterol metabolism in the baboon (*Papio cynocephalus*). *Atherosclerosis.* **41:** 403–414.
42. Kushwaha, R. S., G. M. Barnwell, K. D. Carey, and H. C. McGill. 1986. Metabolism of apoprotein B in selectively bred baboons with high and low levels of low density lipoprotein. *J. Lipid Res.* **27:** 497–507.
43. Williams, M. C., R. S. Kushwaha, and H. C. McGill. 1987. Quantitation of baboon lipoproteins by high performance gel exclusion chromatography. *Lipids.* **22:** 366–374.
44. Castro, G. R., and C. J. Fielding. 1984. Evidence for the distribution of apolipoprotein E between lipoprotein classes in human normocholesterolemic plasma and for the origin of unassociated apolipoprotein E (Lp-E). *J. Lipid Res.* **25:** 58–67.
45. Field, F. J., A. D. Cooper, and S. K. Erickson. 1982. Regulation of intestinal acyl coenzyme A cholesterol acyltransferase in vivo and in vitro. *Gastroenterology.* **83:** 873–880.
46. Koo, C., M. E. Wernette-Hammond, and T. L. Innerarity. 1986. Uptake of canine β-very low density lipoproteins by mouse peritoneal macrophages is mediated by a low density lipoprotein receptor. *J. Biol. Chem.* **261:** 11194–11201.
47. Ellsworth, J. L., F. B. Kraemer, and A. D. Cooper. 1987. Transport of β-very low density lipoproteins and chylomicron remnants by macrophages is mediated by the low density lipoprotein receptor pathway. *J. Biol. Chem.* **262:** 2316–2325.