Lipoprotein kinetic studies have demonstrated that a large proportion of S, 60-400 very low density lipoprotein (VLDL) is cleared directly from the circulation in Type IV hypertriglyceridemic subjects, at an unknown tissue site. The present studies were designed to investigate the role of hepatocytes in this process and to define the conditions, whereby Type IV S, 60-400 VLDL would induce lipid accumulation in HepG2 cells. Type IV VLDL (S, 60-400) failed to augment the total cholesterol, esterified cholesterol, or triglyceride content of HepG2 cells following 24-h incubations. Coincubation of bovine milk lipoprotein lipase (LPL) and Type IV VLDL with HepG2 cells induced a 3-fold increment in cellular esterified cholesterol mass (p < 0.005) and a 7-fold increase in cellular triglyceride mass (p < 0.005), compared to VLDL alone. The increased cellular lipid mass was associated with increased cholesterol esterification into cellular cholesterol esters and triglycerides. Exogenous LPL hydrolyzed 76% of the VLDL triglyceride over 24 h. LPL action on Type IV VLDL was sufficient to promote cellular uptake of these lipoproteins, while elevated media-free fatty acid levels were not. Although HepG2 cells secrete apolipoprotein (apo) E, we assessed the role of VLDL-associated apoE in the lipid accumulation induced by VLDL plus LPL. ApoE-rich and apoE-poor Type IV VLDL subfractions induced similar increments in cellular esterified cholesterol in the presence of LPL, despite a 4-fold difference in apoE content. S, 60-400 VLDL, from subjects homozygous for the defective apoE2 plus LPL, behaved identically to Type IV VLDL plus LPL. Type IV VLDL plus LPL, preincubated with anti-apoE (1D7) and apoB (5E11) monoclonal antibodies, known to block the binding of apoE and -B, respectively, to the LDL receptor failed to block lipid accumulation. In contrast, apoE-poor Type IV VLDL, apoE2 VLDL, and VLDL plus 1D7 were taken up poorly by J774 cells, cells that secrete LPL, but not apoE. These studies suggest that lipolytic remodeling of large Type IV VLDL by LPL is a prerequisite for their uptake by HepG2 cells and that HepG2 cell-secreted apoE rather than VLDL-associated apoE is the ligand involved in uptake.

Very low density lipoproteins (VLDL) transport hepatically synthesized triglycerides to sites of utilization in peripheral tissues. In normolipidemic human subjects, VLDL catabolism involves progressive lipolytic conversion of these triglyceride-rich particles into cholesterol ester-rich low density lipoproteins (LDL). Elevations of plasma VLDL due to excessive production and/or impaired catabolism are characteristic of Type IV hypertriglyceridemia, and have been associated with an increased risk of coronary artery disease (1–3). In contrast to normolipidemic VLDL, Type IV VLDL displays substantial metabolic heterogeneity, with the abnormal aspects of VLDL metabolism being primarily confined to the larger S, 60–400 particles (4–7). In particular, substantial amounts of VLDL in these subjects are directly removed from the plasma by a catabolic shunt that bypasses the usual conversion to LDL (6, 7). While the exact tissue site of this direct VLDL catabolism and the contribution of this catabolic route to the development of coronary artery disease remain unclear, various studies have indicated that hepatocytes (8, 9), nonparenchymal liver cells (10), fibroblasts (11), and macrophages (12, 13) may be involved. Evidence strongly suggests that human VLDL can be directly catabolized by macrophages in culture, however, much less is known about the potential for direct VLDL catabolism by hepatocytes in vitro.

The human hepatoblastoma-derived cell line, HepG2, has become an accepted model for studying lipoprotein metabolism in human hepatocytes. The various properties of HepG2 cells in this regard have been recently reviewed by Javitt (14). Previous studies concerning the interaction of human VLDL with this cell line have focused on the binding, internalization, and degradation of 125I-labeled VLDL apolipoproteins and the ability of VLDL to stimulate the incorporation of [1-14C]oleic acid into cellular cholesterol esters (8, 9, 15). While there has been agreement between reports demonstrating the binding of 125I-VLDL to HepG2 cells (8, 9), there have been conflicting observations regarding the subsequent uptake of these particles. Dashti and Wolfbauer (8) found that iodinated normolipidemic VLDL was internalized and degraded by HepG2 cells, while Craig et al. (16) reported that iodinated normolipidemic VLDL was not taken up or modified in any way by these cells. Furthermore, Ranganathan and Kottke (15) observed that normolipidemic human VLDL stimulated cholesterol esterification in HepG2 cells, while Dashti and Wolfbauer (8) reported that it did not.

The interpretation of studies using 125I-VLDL to investigate the uptake of intact VLDL particles by cells in culture may...

* This work was supported in part by Medical Research Council of Canada Grant MA8014 (to M. W. H.). The costs of publication of this article were defrayed in part by the payment of page charges. The interpretation of studies using 125I-VLDL to investigate the uptake of intact VLDL particles by cells in culture may...
be complicated by the dissociable nature of VLDL-associated apoE and apoC. We reasoned that measuring increases in the mass of cellular esterified cholesterol and triglyceride, as well as increases in oleate incorporation into esterified cholesterol and triglyceride, would better reflect the uptake of VLDL particles by HepG2 cells. Craig and Cooper (17) reported that rat chylomicron remnants and rat β-VLDL induced lipid accumulation in these cells, however, the ability of hypertriglyceridemic, human, Sf 60–400 VLDL to augment HepG2 cell-esterified cholesterol and triglyceride mass has not been described. The primary objective of the studies presented in this report was to define the conditions under which large Type IV VLDL would induce accumulation of esterified cholesterol and triglyceride mass in HepG2 cells. In addition, we have also studied the role of apoE associated with VLDL, as isolated from plasma, in the uptake of hypertriglyceridemic VLDL by these cells.

EXPERIMENTAL PROCEDURES

Subjects—The lipid and lipoprotein profiles of the patients used in this study are shown in Table I. The Type IV hypertriglyceridemic subjects were classified according to the criteria of Schaefer and Levy (18) following visits to the Outpatient Endocrinology Clinic at University Hospital, London, Ontario, Canada. These subjects all presented with primary hypertriglyceridemia and none displayed fasting chylomicronemia or had a metabolic disorder such as diabetes, hypothyroidism, obesity, or renal dysfunction. In addition, none of the subjects used in these experiments were being treated with lipid lowering agents or any drugs known to exacerbate hypertriglyceridemia. The apoE homozygotes were identified during routine screening of plasma samples for apoE phenotype. Four of the apoE4 homozygotes were being treated by diet and were not overtly hyperlipidemic.

Lipoprotein Isolation—Approximately 50 ml of blood was collected from the fasted donors and placed in tubes containing Na2EDTA at a final concentration of 0.15%. Plasma was obtained by centrifugation at 1000 x g for 25 min at 4 °C. Approximately 25 ml of plasma was immediately layered under Buffer A (1.006 g/ml density solution containing 0.195 M NaCl, 1 mM Tris, pH 7.4, 1 mM EDTA, 10 μM thrombin (200 units/ml), heat inactivated at 56 °C for 30 min, filtered using sterile 0.22-μm filters, and stored at −20 °C.

All lipoprotein samples were analysed for protein content by a modification of the Lowry method (19) and for total cholesterol and triglyceride using diagnostic kits from Boehringer Mannheim Canada, Laval, Quebec (CHOD-PAP and triglycerides without free glycerol, respectively). All lipoprotein samples were stored at 4 °C and used for tissue culture experiments within 1 week of isolation. ApoE phenotypes were determined on all VLDL samples used in these studies by analytical isoelectric focusing gel electrophoresis as described previously (4).

Type IV, Sf 60–400 VLDL, was subfractionated into apoE-poor and apoE-rich subfractions using heparin-Sepharose chromatography as described previously (5). The VLDL subfractions from duplicate columns were pooled together and reisolated by ultracentrifugation at 40,000 rpm, at 12 °C in Buffer A in a Beckman 50.3 Ti rotor. Protein, cholesterol, and triglyceride contents and the ratios of apoE to apoC of the reisolated VLDL subfractions were determined as described previously (4, 5). Free fatty acid concentrations were measured as described by Novak (20). For some samples, apolipoproteins B, E, CII, and CIII were measured by modifications to a standard enzyme-linked immunosorbent assay technique involving a modified coating buffer and by aligning individual steps to allow each assay to be completed in a single day (21, 22).

HepG2 Cells—HepG2 cells were obtained from the American Type Culture Collection (HB-8065) (Rockville, MD) and grown on 100-mm culture dishes (Falcon, Canlab Scientific, Mississauga, Ontario) in a humidified atmosphere of 95% air, 5% CO2 at 37 °C. The cells were maintained in 10 ml of modified Eagle's media (MEM) (MEM) with Earle's salts containing: 0.20% sodium bicarbonate, 1 mM sodium pyruvate, 10% fetal bovine serum (Flow Laboratories Inc., Mississauga, Ontario), 10 IU/ml of penicillin, 10 μg/ml streptomycin, and 0.25 μg/ml Fungizone (Flow Laboratories Inc., Mississauga, Ontario). The MEM was supplemented with 0.3 mg/ml 1-glutamine every 14 days and the cells received fresh media every 3 days. The cells were split (1:3) using trypsin-EDTA (Flow) on a 7-day cycle up to a maximum of 10 passages. For all of the experiments in the present report, HepG2 cells were plated in 6-well (35-mm) culture plates (Linbro, Flow Laboratories Inc.) in 2 ml of MEM containing 10% fetal bovine serum and grown for 2–3 days. Once the monolayers had become confluent the media was replaced with MEM containing 5% LPDS. The appropriate concentrations of lipoproteins were added to duplicate dishes of cells and were incubated for 5 (oldest incorporation studies) or 24 h (mass determinations) unless otherwise indicated. The cells were not preincubated in MEM containing LPDS prior to the experiments as this had no influence on the results obtained (data not shown). The experiment assessing the effect of medium-free fatty acids on the uptake of large Type IV VLDL was conducted by adding 650 μg of oleic acid (Sigma), complexed to fatty acid-free bovine serum albumin (BSA, Sigma) in a molar ratio of 12:1, to lipoprotein-deficient media in the presence or absence of VLDL. At the conclusion of one experiment, the cells were washed, stained with oil red O (12), and examined by light microscopy using transmitted light in order to demonstrate the presence of neutral lipid droplets.

J774 Cells—J774A.1 cells, a murine macrophage-like cell line, were obtained from the American Type Culture Collection (TIB 67) and grown on 100-mm culture dishes (Falcon) in a humidified atmosphere of 95% air, 5% CO2 at 37 °C. The cells were maintained in 10 ml of Dulbecco's modified Eagle's medium (low glucose) (Gibco, Burlington, Ontario) containing 0.37% sodium bicarbonate, 0.1 mg/ml Galamin (Schering, Pointe Claire, Quebec), 0.25 μg/ml Fungizone, and 10% fetal bovine serum. The cells were maintained on a 7-day cycle.

TABLE I

| Lipoprotein phenotype | Plasma | VLDL | LDL | HDL |
|-----------------------|--------|------|-----|------|
|                       | C      | TG   | C   | C    |
| Type IV (n = 10)*     | 6.0 ± 0.4 | 7.5 ± 1.5 | 2.0 ± 0.5 | 5.7 ± 1.3 | 2.6 ± 0.3 | 0.8 ± 0.1 |
| ApoE homozygote un-  | 11.2 ± 1.0 | 7.1 ± 2.0 | 5.6 ± 1.2 | 4.7 ± 1.1 | 4.5 ± 0.6 | 1.1 ± 0.2 |
| treated (n = 4)       | 5.4 ± 0.5 | 2.0 ± 0.4 | 0.9 ± 0.2 | 1.5 ± 0.3 | 3.3 ± 0.1 | 1.2 ± 0.2 |
| ApoE homozygote      | 5.4 ± 0.5 | 2.0 ± 0.4 | 0.9 ± 0.2 | 1.5 ± 0.3 | 3.3 ± 0.1 | 1.2 ± 0.2 |
| treated (n = 4)       | 5.4 ± 0.5 | 2.0 ± 0.4 | 0.9 ± 0.2 | 1.5 ± 0.3 | 3.3 ± 0.1 | 1.2 ± 0.2 |

* Type IV subjects were all homozygous for apo E2.
being split 1:3 on days 1 and 5 and having fresh media added on day 3. J774 cells were plated in 6-well (35-mm) culture plates in 2.0 ml of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and grown for 1–2 days. When the monolayers had become 70–90% confluent, the media was changed with Dulbecco's modified Eagle's medium containing 5% LPDS. The appropriate dilutions of lipoproteins were added to duplicate dishes of cells and were incubated for 24 h. The cells were not preincubated in Dulbecco's modified Eagle's medium containing LPDS prior to the experiments as we had previously shown that such treatment has no influence on the results described previously (31).

Monoclonal Antibodies—Affinity purified human apoE and apoB monoclonal antibodies and Fab fragments were prepared as described previously (24). The anti-apoE antibodies used in this study were: (i) 1D7, an antibody that specifically inhibits binding by apoE to the apoE receptor, and (ii) 3C5, an antibody that reacts with an epitope that is not involved with receptor recognition (25). The anti-apoB antibodies used in this study were: (i) 5E11, an antibody that specifically inhibits binding by apoB to the apoB/E or LDL receptor, and (ii) 1D1, an antibody that reacts with an epitope that is not involved with receptor recognition (26, 27). These antibodies were generously provided by Drs. Ross Milne and Yves Marcel, Clinical Research Institute of Montreal. The Fab fragments were incubated with VLDL preparations (2.5 μg of Fab fragment/μg of VLDL protein) for 0.5 h at 37 °C before addition to cells.

Determination of Cellular Lipid Content—Five experiments in which total cholesterol content was measured using a total cholesterol assay (i.e. free cholesterol plus esterified cholesterol) were conducted. In each experiment, the cells were grown to confluence, washed once with Dulbecco's modified Eagle's medium containing 5% LPDS and grown for 1–2 days. When the monolayers had become 70–90% confluent, the media was changed with Dulbecco's modified Eagle's medium containing 5% LPDS. The appropriate lipoproteins were added to duplicate dishes of confluent monolayers and incubated for 0.5, 1, 6, or 24 h. The conditioned media was collected in sterile tubes and stored at −80 °C until used in the assay. The cells were washed 3 times with Buffer B without BSA and the cell protein was determined. The activity of hepatic triglyceride lipase (HTGL) in the conditioned media was determined using a stable [1-14C]glycerol trioleate-gum arabic emulsion as a substrate, according to the method of Blaese et al. (31). Duplicate 250-μl aliquots of media were added to incubation buffer (1 M NaCl, 0.4 M Tris, 0.05 mM fatty acid-free BSA, pH 8.0), and 100 μl of the substrate. Samples were incubated for 1 h at 37 °C and the release of [1-14C]oleic acid was determined as described previously (31). HTGL activity was expressed in units/mg of cell protein, where 1 unit of activity was defined as 1 nmol of free fatty acid released per ml of media per h.

Oleate Incorporation into Cellular Cholesterol Ester and Triglyceride—The incorporation of [1-14C]oleic acid into cellular cholesterol esters and triglycerides was determined by a modification of the method described previously (31). The appropriate lipoproteins were added to duplicate dishes of confluent monolayers and incubated for 5 h in MEM containing 5% LPDS. Each dish received 0.04 μCi of [1-14C] oleic acid (Amersham) complexed with fatty acid-free BSA. The molar ratio of oleic acid to BSA was 5:1. The cells were washed 3 times with Buffer B without BSA, the lipids extracted in situ as described above, and the cell protein was determined. The lipids were separated by TLC as described above, using [3H]cholesterol olate and [1-3H]trioleate as internal standards to assess recovery. The cholesterol ester and triglyceride bands were scraped from the TLC plates and counted in Aquasol-2 (Du Pont Canada, Mississauga, Ontario) using a Beckman LS 3801 counter. The specific activity of the added [1-14C]oleate-BSA was corrected for dilution by the amount of free fatty acid released from lipoproteins incubated in the absence of cells.

HepG2 Cell Hepatic Triglyceride Lipase Assay—Confluent monolayers were incubated with MEM containing 5% LPDS in the presence or absence of 10 IU/ml of heparin (Hepalet, Organon Canada Ltd.) for 1, 6, or 24 h. The conditioned media was collected in sterile tubes and stored at −80 °C until used in the assay. The cells were washed 3 times with Buffer B without BSA and the cell protein was determined. The activity of hepatic triglyceride lipase (HTGL) in the conditioned media was determined using a stable [1-14C]glycerol trioleate-gum arabic emulsion as a substrate, according to the method of Blaese et al. (31). Duplicate 250-μl aliquots of media were added to incubation buffer (1 M NaCl, 0.4 M Tris, 0.05 mM fatty acid-free BSA, pH 8.0), and 100 μl of the substrate. Samples were incubated for 1 h at 37 °C and the release of [1-14C]oleic acid was determined as described previously (31). HTGL activity was expressed in units/mg of cell protein, where 1 unit of activity was defined as 1 nmol of free fatty acid released per ml of media per h.

Bovine Milk Lipoprotein Lipase—Bovine skim milk milk P L was partially purified by a modification of the method of Sorocco and Jackson (32) as described previously (33). All experiments involving the addition of bovine milk milk P L were conducted by adding 0.25 units of activity (1 unit is defined as 1 nmol of free fatty acid released per ml of enzyme solution per h) per ml of media containing 5% LPDS and human LDL. Control incubations containing media conditioned by 5774 cells were identically treated to test the absence of VLDL activity. The amount of milk milk P L added to the media of HepG2 cells was identical to the amount of P L activity we measured in conditioned conditioned by J774 cells. To assess the effect of the concentration of milk milk P L in the media, one specific experiment was conducted using 0.5- or 2-fold the amount of milk milk P L activity described above.

The extent of VLDL lipolysis by milk milk P L was estimated by incubating Type IV VLDL at 37 °C for 5 or 24 h, in 35-mm culture dishes containing MEM with 5% LPDS, and milk milk P L in the absence of HepG2 cells. The lipids in aliquots of VLDL not treated with milk milk P L were isolated by thin layer chromatography. The VLDL were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (6). Samples were separated on gels containing 4 and 12% acrylamide.

Results

The lipid, protein, and apoprotein composition of the various VLDL samples used in these studies is summarized in Table II. The effect of Type IV VLDL on the lipid content of HepG2 cells is shown in Fig. 1A. Type IV VLDL failed to induce significant increments in the esterified cholesterol content of these cells following 24-h incubations.

A. J. Evans, M. W. Huff, C. G. Sawyez, B. M. Wolfe, P. W. Connelly, and G. F. Maguire, unpublished observations.
Uptake of Large VLDL by HepG2 Cells

**TABLE II**

| Characteristics of S<sub>60-400</sub>-VLDL |
|------------------------------------------|
| Values expressed as means ± S.E.; C, cholesterol; TG, triglyceride; FFA, free fatty acids. Ratios are weight ratios. | |

| Type IV | C/TG | C/protein | TG/protein | apoE/apoC<sup>+</sup> | apoE/apoB<sup>+</sup> | FFA/protein |
|---------|------|-----------|------------|----------------------|----------------------|-------------|
| Type IV substractions<sup>a</sup> | EP | 0.17 ± 0.03 | 1.54 ± 0.27 | 9.67 ± 1.40 | 0.25 ± 0.05<sup>b</sup> | 0.30 ± 0.06<sup>b</sup> |
| Type IV lipolysed in vitro<sup>b</sup> | Control | 0.10 ± 0.001 | 1.72 ± 0.27 | 14.73 ± 1.89 | 0.25 ± 0.05 | 0.30 ± 0.06 | 0.01 ± 0.001 |
| Type III apoE<sub>2</sub> homozygotes | Lipolyzed | 0.27 ± 0.04<sup>d</sup> | 1.02 ± 0.10<sup>d</sup> | 4.12 ± 1.03<sup>d</sup> | 0.38 ± 0.04 | 0.15 ± 0.03 | 0.96 ± 0.03<sup>d</sup> |
| Untreated | 0.21 ± 0.02 | 1.86 ± 0.10 | 8.51 ± 1.22 | 0.41 ± 0.03 | 2.39 ± 1.20 |

<sup>a</sup> Values were determined by enzyme-linked immunosorbent assay, with the exception of the Type IV EP and ER subfractions which were determined by densitometric scanning of analytical isoelectric focusing gels.

<sup>b</sup> Values are mean ± S.E. from 5 patients.

<sup>c</sup> VLDL S<sub>60-400</sub> was subfractionated by heparin-Sepharose chromatography as described under "Experimental Procedures"; EP, apoE-poor fraction; ER, apoE-rich fraction.

<sup>d</sup> VLDL S<sub>60-400</sub> from four Type IV subjects was incubated with bovine milk lipoprotein lipase (5 mg of VLDL triglyceride/unit of enzyme) for 3 h and the lipolyzed particles were reisolated as described under "Experimental Procedures."

<sup>p < 0.02</sup>, compared to EP.

<sup>p < 0.01</sup> compared to control.

relative to cells to which no lipoproteins were added (referred to as control cells). In contrast to Type IV VLDL, the addition of normolipidemic human LDL resulted in a 3-fold increase in both total (not shown) and esterified cholesterol mass (p < 0.01 relative to control cells). Therefore, we proposed that lipolytic modification was required for interaction of Type IV VLDL with HepG2 cells. Fig. 1A demonstrates that the addition of bovine milk LPL along with Type IV VLDL resulted in a 3-fold elevation of esterified cholesterol mass compared to control dishes or cells incubated with VLDL in the absence of enzyme (p < 0.005). Fig. 1B shows that the addition of increasing amounts of VLDL failed to augment the cellular esterified cholesterol content unless bovine milk LPL was added to the media. Addition of more than 50 μg of VLDL cholesterol/ml of media in the presence of milk LPL did not result in significantly greater increments in esterified cholesterol mass.

Type IV VLDL alone failed to increase HepG2 cell triglyceride content as shown in Fig. 2A. However, coincubation of Type IV VLDL and milk LPL with HepG2 cells induced a 5-fold increase in cellular triglyceride content over a 24-h period (p < 0.005 relative to control cells). As was the case for esterified cholesterol mass, the addition of increasing amounts of VLDL had no effect on cellular triglyceride content in the absence of LPL activity (Fig. 2B). In contrast to the pattern of esterified cholesterol mass accumulation (Fig. 1B), the increase in cellular triglyceride content was linear with the amount of VLDL added to the media. Adjusting the amount of milk LPL added to the media to 0.5- or 2-fold the amount used above had no effect on the results obtained (data not shown). Type IV VLDL, coincubated with LPL, stimulated the incorporation of [1-<sup>14</sup>C] lauric acid into cellular cholesterol ester and triglyceride to the same extent as observed with the mass of the two lipids (data not shown). In four experiments following incubation of LPL and Type IV VLDL (50 μg of VLDL cholesterol/ml of media) for 24 h with HepG2 cells, heparin was added to the wash buffer in order to release any lipoproteins bound to the cell surface. The heparin wash had no effect on the results obtained. Cellular cholesterol ester and triglyceride contents without the heparin wash were: 15 ± 4 and 393 ± 94 μg/mg cell protein, respectively, and with the heparin wash: 17 ± 8 and 380 ± 112 μg/mg cell protein, respectively.

Control HepG2 cells and cells incubated in the presence and absence of LPL were stained with oil red O and examined by light microscopy. A large number of stained neutral lipid droplets were observed only in cells incubated with VLDL in the presence of LPL. Droplets were observed only in the cytoplasm and did not appear to be associated with the cell surface (data not shown).

The extent of lipolysis of Type IV VLDL by milk LPL was estimated over a 24-h time course by incubating VLDL with the enzyme in the absence of cells under conditions identical to those of the cell experiments. Twenty-six percent of the initial VLDL triglyceride was hydrolyzed within the first hour of incubation, and the extent of lipolysis steadily increased until a maximum of 76% of the VLDL triglyceride had been hydrolyzed by 12 h. This provided an estimate of VLDL lipolysis mediated only by exogenous milk LPL. It was reasonable to speculate that HTGL activity, known to be secreted by HepG2 cells, might enhance the degree of VLDL lipolysis and the subsequent cellular lipid accumulation. HepG2 cells used in the present studies secreted increasing amounts of HTGL activity: 4.3 ± 0.2, 8.1 ± 1.0, and 38.1 ± 2.9 nmol of free fatty acid released per ml of media/h, at 2, 6, and 24 h of incubation, respectively. Accumulation of HTGL activity in the media appeared linear over 24 h and was enhanced 6-8-fold by the addition of heparin (10 IU/ml of media) at each time point. The HTGL activity, present in the media after 24 h, assayed using a synthetic triglyceride emulsion as substrate, was 16% of the added milk LPL activity, assayed using the same substrate. The contribution to VLDL lipolysis made by this amount of HTGL activity, in addition to that made by milk LPL, was not directly determined.

The patterns of total cholesterol, esterified cholesterol, and triglyceride mass accumulation in HepG2 cells over a 24-h time course, induced by Type IV VLDL in the presence of milk LPL, are shown in Fig. 3. The cellular triglyceride content was clearly enhanced during the first 4 h of incubation and steadily increased with time until reaching a maximum value (6-fold greater than control) at 12 h. In contrast, 4 h of incubation were required to detect increases in cellular total and esterified cholesterol mass. In addition, the cellular total and esterified cholesterol content was still increasing at 24 h. These results demonstrate a lag between accumulation of the triglyceride and cholesterol components by HepG2 cells fol-
particles are summarized in Table 1, where it is evident that treated with LPL in the absence of cells. Compared to untreated VLDL, there appeared to be no major proteolytic cleavage of apoE to apoC ratio increased by 50% and the ratio of apoE to apoB decreased by approximately 50%. Fig. 4 shows determinations for five experiments expressed as means

FIG. 1. Esterified cholesterol content of HepG2 cells incubated with Type IV 5, 60–400 VLDL in the absence or presence of oleic acid or bovine milk lipoprotein lipase. A, 50 μg of cholesterol/ml of media of Type IV 5, 60–400 VLDL were incubated with HepG2 cells in the absence or presence of either 0.25 units of bovine milk LPL/ml of media or 325 μg of oleic acid/ml of media (complexed to BSA in a molar ratio of 12:1) for 24 h. The amount of oleic acid was based on the amount of free fatty acid liberated during lipolysis of Type IV VLDL at a concentration of 50 μg of cholesterol/ml of media by LPL in the presence of cells. Lipolyzed VLDL was prepared by incubating Type IV VLDL with bovine milk LPL (5 mg of VLDL triglyceride/unit of LPL activity) for 3 h. 50 μg of cholesterol/ml of media of reisolated lipolyzed VLDL were then incubated with HepG2 cells for 24 h. 50 μg of normolipidemic LDL were incubated with HepG2 cells for 24 h. B, various concentrations of Type IV VLDL cholesterol were incubated with HepG2 cells in the absence (○) or presence (●) of milk LPL as in A. The cellular esterified cholesterol content was determined as described under "Experimental Procedures." The values are the results of duplicate determinations for five experiments expressed as means ± S.E.; *, p < 0.005 relative to control cells (no lipoproteins) and cells receiving VLDL alone.

The method of coinubating Type IV VLDL and milk LPL with HepG2 cells did not permit us to determine the composition of the lipolytic products ultimately taken up by the cells. Therefore, we incubated Type IV 5, 60–400 VLDL with milk LPL for 3 or 6 h in the absence of cells, and reisolated the lipolyzed particles by ultracentrifugation, and incubated them with HepG2 cells for 24 h. The characteristics of the lipolyzed particles are summarized in Table II, where it is evident that LPL treatment significantly reduced the triglyceride/protein ratio (p < 0.005). The amount of free fatty acid associated with the VLDL particles increased significantly (p < 0.01) such that the amount of free fatty acid represented approximately 15% of the initial VLDL triglyceride and 25% of the total triglyceride hydrolyzed. Relative to untreated VLDL, the apoE to apoC ratio increased by 50% and the ratio of apoE to apoB decreased by approximately 50%. Fig. 4 shows electrophotograms of apo-VLDL from VLDL samples treated with LPL in the absence of cells. Compared to untreated VLDL, there appeared to be no major proteolytic degradation of apoB, E, or C. However, in the treated sample, bands corresponding to apoB-74 and apoB-26 were observed. These bands were separated by densitometric scanning. Type IV VLDL treated with LPL for 3 h increased cellular esterified cholesterol (3-fold) and triglyceride mass (4-fold), whereas untreated VLDL did not (Figs. 1A and 2A). In addition, increasing the incubation time of VLDL with milk LPL from 3 to 6 h had no further effect on the extent of lipid accumulation induced by the lipolyzed VLDL particles (data not shown). The increment in cellular triglyceride was approximately half of that observed with untreated VLDL plus LPL (Fig. 2A), whereas the increment in cellular cholesterol ester content was similar (Fig. 1A). This difference reflects the 2-fold increase in the cholesterol to triglyceride ratio in the treated VLDL particles (Table II).

It was possible that the fatty acid liberated during VLDL lipolysis may have played some role in facilitating the uptake of the cholesterol-carrying remnants by these cells. Thus, Type IV 5, 60–400 VLDL was incubated with HepG2 cells in the presence or absence of oleic acid complexed to BSA. The concentration of oleic acid was based on an estimate of the amount of free fatty acid released during lipolysis of Type IV VLDL by LPL in the absence of cells as described above. Fig. 1A shows that the addition of oleic acid to Type IV VLDL failed to induce accumulation of esterified cholesterol in HepG2 cells when compared to VLDL in the presence of exogenous LPL. Oleic acid alone did stimulate a 7-fold increase in cellular triglyceride content (Fig. 2A), which was similar to that induced by VLDL in the presence of LPL. Coincubation of oleic acid and VLDL, however, did not result
10748

esterified cholesterol were determined at the indicated time points as described under triglyceride accumulation in HepG2 cells induced by Type IV determinations for three experiments expressed as means Sf 60-400 HepG2 cells over a 24-h time course. The total cholesterol saturated by bovine milk lipoprotein lipase on apolipoprotein collected and the lipolyzed VLDL reisolated by ultracentrifugation for 3 h at 37°C. The same VLDL-1D7 complex was incubated with J774 cells, cholesterol ester accumulation was inhibited by approximately 75%, compared to VLDL alone or VLDL plus LPL (data not shown). Incubation of VLDL with the anti-apoB antibody, 5E11, also had no effect on the increase in HepG2 cell cholesterol ester. However, preincubation of LDL with 5E11 inhibited cholesterol ester accumulation in HepG2 cells by approximately 80% (data not shown). None of the antibodies had any effect on the ability of VLDL plus LPL to increase cellular triglyceride (data not shown).

Third, the ability of S, 60–400 VLDL from Type III subjects containing only apoE2 to induce cellular lipid accumulation following coincubation with milk LPL was determined. Experiments with apoE2 homozygotes were carried out with VLDL from untreated Type III patients as well as from treated Type III patients whose plasma lipids approached the normal range (Table I). Since Innerarity et al. (34) have indicated that treatment of a Type III patient to lower plasma lipid concentrations resulted in enhanced binding of VLDL to cellular B/E receptors, the data for treated and untreated subjects are presented separately. VLDL from these subjects plus LPL was incubated with HepG2 cells and the same VLDL preparations (without LPL) were incubated with J774 mac-

FIG. 3. Time course of total and esterified cholesterol and triglyceride accumulation in HepG2 cells induced by Type IV S, 60–400 VLDL in the presence of exogenous bovine milk lipoprotein lipase. 50 μg of VLDL cholesterol/ml of media and 0.25 units/ml of bovine milk LPL/ml of media were coincubated with HepG2 cells over a 24-h time course. The total cholesterol (A) and esterified cholesterol (●) and triglyceride (■) (B) content of the cells were determined at the indicated time points as described under “Experimental Procedures.” The values are the results of duplicate determinations for three experiments expressed as means ± S.E.

FIG. 4. Effect of Type IV S, 60–400 VLDL lipolysis mediated by bovine milk lipoprotein lipase on apolipoprotein content. 50 μg of VLDL cholesterol/ml of media were incubated in the absence or presence of 0.25 units of bovine milk LPL/ml of media for 3 h at 37°C in the absence of HepG2 cells. The media was collected and the lipolyzed VLDL reisolated by ultracentrifugation (d < 1.019 g/ml), delipidated, and the apolipoproteins subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on gels containing 4% acrylamide (lanes 1 and 2) or 12% acrylamide (lanes 3 and 4) as described under ”Experimental Procedures.” Lanes 1 and 3, Type IV VLDL incubated in the absence of LPL; lanes 2 and 4, Type IV VLDL incubated with LPL. Apoproteins were identified by comparison to molecular weight standards. Gels were overloaded in order to see any minor bands. The apoB-74 and apoB-26 bands observed in lane 2 represent proteolytic breakdown products of apoB-100 (28).

in a further increment in cellular triglyceride mass over and above that induced by oleic acid alone.

We investigated the role of apoE associated with VLDL, as isolated from plasma, in the lipid accumulation observed following coincubation of Type IV VLDL and milk LPL with HepG2 cells using three approaches. First, apoE-poor and apoE-rich VLDL subfractions, separated by heparin-Sepharose chromatography, were incubated with HepG2 cells in the presence of milk LPL. Fig. 5 demonstrates that in the presence of LPL, both subfractions caused similar increments in cellular cholesterol ester content demonstrating that the 4-5-fold increase in apoE content of the apoE-rich subfraction at the outset of the 24-h incubations had no effect on the extent of cellular esterified cholesterol accumulation.

Second, we assessed whether preincubation of VLDL with the monoclonal antibody 1D7, an antibody that blocks the recognition of apoE by the LDL receptor, or antibody 5E11, which blocks the binding of apoB to the LDL receptor, could inhibit lipid accumulation in HepG2 cells in the presence of LPL. Coincubation of LPL and VLDL plus 1D7 did not inhibit the increased cellular cholesterol ester observed in the absence of 1D7 (Fig. 5) or VLDL and LPL plus control anti-apoE antibody, 6C5 (data not shown). In contrast, when the same VLDL-1D7 complex was incubated with J774 cells, cholesterol ester accumulation was inhibited by approximately 75%, compared to VLDL alone or VLDL plus 6C5. Incubation of VLDL with the anti-apoB antibody, 5E11, also had no effect on the increase in HepG2 cell cholesterol ester. However, preincubation of LDL with 5E11 inhibited cholesterol ester accumulation in HepG2 cells by approximately 80% (data not shown). None of the antibodies had any effect on the ability of VLDL plus LPL to increase cellular triglyceride (data not shown).

FIG. 5. Esterified cholesterol content of HepG2 cells incubated with Type IV S, 60–400 apoE-poor and apoE-rich VLDL subfractions and with Type IV VLDL plus anti-apoE monoclonal antibodies, in the absence or presence of bovine milk lipoprotein lipase. 50 μg of Type IV VLDL cholesterol/ml of media and 0.25 units of bovine milk LPL/ml of media were coincubated with HepG2 cells for 24 h and the cellular esterified cholesterol content was determined as described under ”Experimental Procedures.” VLDL was separated into apoE-poor and apoE-rich subfractions by heparin-Sepharose chromatography as described under ”Experimental Procedures.” Fab fragments of monoclonal anti-apoE antibody 1D7 were preincubated with Type IV VLDL (2.5 μg of Fab/μg of VLDL protein for 0.5 h at 37°C prior to incubation with cells). Antibody 1D7 is an anti-apoE monoclonal antibody that blocks the binding of apoE to the LDL receptor. Values are the results of duplicate determinations for four experiments expressed as means ± S.E.; *, p < 0.01 relative to control cells (no lipoproteins) and cells receiving Type IV VLDL alone.
treated) resulted in significant increases in cellular cholesterol ester and triglyceride content. In contrast to HepG2 cells, such particles may not be optimal substrates for the HepG2 cell-secreted HTGL prior to uptake by a receptor-mediated process. This would be analogous to the multistep mechanism proposed by Mahley et al. (42) for the uptake of chylomicron remnants by hepatic parenchymal cells. Although not addressed in the present study, Havel and coworkers (43) have provided evidence that VLDL remnants are taken up by HepG2 cells exclusively by the LDL receptor.

Using three approaches, we obtained evidence that apoE associated with the VLDL particle as isolated from plasma may not be the primary determinant for uptake of lipolyzed VLDL particles by HepG2 cells. First, both apoE-poor and apoE-rich Type IV VLDL subfractions plus LPL induced similar increments in cellular esterified cholesterol. It is possible that the 24-h end point may have masked differences in the kinetics of uptake of the two subfractions. This is unlikely since in J774 macrophages, using the same protocol, the apoE-rich subfraction caused a 1.6-fold greater accumulation of cellular cholesterol ester compared to the E-poor subfraction.2 A more likely explanation, involving HepG2 cell-secreted apoE, is discussed below.

Second, coincubation of LPL and large VLDL from both treated and untreated Type III subjects, homozgyous for apoE2, resulted in similar degrees of cellular lipid accumulation as were seen with Type IV VLDL plus LPL. This was in agreement with previous reports (39, 40), HTGL was secreted into the media by HepG2 cells, yet the addition of Type IV VLDL alone failed to promote increases in cellular lipolyzed cholesterol containing remnant.

In conclusion, we feel that HepG2 cells would be an appropriate model for all lipoprotein lipase and Type IV VLDL, correlated with the observation of cytoplasmic oil red O-positive lipid droplets, as well as enhanced radiolabeled oleate incorporation into both lipid classes. The appearance of cellular triglyceride was more rapid than the appearance of cellular total and esterified cholesterol. Collectively, these results indicate that the increased cellular mass of triglyceride and cholesterol ester resulted from hydrolysis, uptake, and intracellular re-esterification.

The present investigations were carried out to define the conditions under which HepG2 cells would catabolize S, 60-400 VLDL from Type IV subjects and subjects homozgyous for apoE2, in the absence and presence of bovine milk lipoprotein lipase. 50 μg of VLDL cholesterol/ml of media were incubated with HepG2 cells for 24 h in the absence or presence of 0.25 units of bovine milk LPL/ml of media and with J774 cells in the absence of LPL. The esterified cholesterol (A) and triglyceride (B) contents were determined as described under "Experimental Procedures." Control, incubations in the absence of lipoproteins; Type III-U, untreated Type III (E2 homozgyotes) hyperlipemic subjects; Type III-T, treated Type III (E2 homozgyotes) subjects whose plasma lipid values were normal (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I). Following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates (listed in Table I) following dietary treatment. Values are duplicates.
spite of the known impaired ability of apoE to act as a ligand for the LDL receptor (44), and our observations that apoE-VLDL failed to promote cholesterol accumulation in J774 macrophages (this report and Ref. 23), cells which express the LDL receptor (45).

Third, the anti-apoE monoclonal antibody 1D7 failed to block the cholesterol ester accumulation induced by Type IV VLDL plus LPL, even though the same VLDL-1D7 complex reduced cholesterol ester accumulation in J774 cells by 75%. Anti-apoB monoclonal antibody 5E11 also failed to block cholesterol ester accumulation induced by VLDL plus LPL, a result consistent with previous findings in fibroblasts (46, 47) that apoB is not a primary determinant for VLDL uptake.

It is known that HepG2 cells secrete apoE (48, 49), which is stimulated by free fatty acid (18, 50). It is therefore possible that secreted apoE enhanced the apoE content of the lipolyzed VLDL leading to uptake. This would be analogous to the findings of Eisenberg et al. (51) that supplementation of normal VLDL with exogenous apoE enhanced uptake by fibroblasts and HepG2 cells (52). However, if HepG2 cell-secreted apoE stimulated VLDL uptake in our studies, it was unable to do so in the absence of LPL. LPL treatment of VLDL reduces the triglyceride to cholesterol ratio, decreases apoC content, increases the apoE/apoC ratio, and does not appear to cause any significant proteolytic degradation of apoE or B. This remodeling may allow for the association with HepG2 cell-secreted apoE. This conclusion is supported by studies in perfused rat livers that apoE mediated uptake of triglyceride-rich lipoproteins is inhibited if these particles contain significant amounts of apoC (58, 53–55).

In the present studies, HepG2 cell-secreted apoE may have prevented us from seeing a difference in the uptake of lipolyzed apoE-poor and apoE-rich heparin-Sepharose subfractions. Similarly, the uptake of lipolyzed apoE-containing VLDL particles, may have been promoted by the apoE secreted by HepG2 cells, since such VLDL are not readily internalized by J774 macrophages, cells which do not secrete apoE. Clearly, in vivo, this would not occur since hepatocytes of E<sub>2</sub> homozygotes would secrete apoE. The addition of anti-apoE antibody 1D7 to Type IV VLDL was able to block uptake by J774 cells, but may not have been present in sufficient quantity to block the effect of HepG2 cell-secreted apoE. Friedman et al. (56) demonstrated that anti-apoE 1D7 blocked the binding of intermediate density lipoproteins to HepG2 cells, however, these studies were carried out over 2 h at 4°C, conditions under which cellular secretion of apoE would be minimal. Our studies raise the possibility that apoE secreted by hepatocytes in vivo may be more important for facilitating the uptake of VLDL remnants than previously appreciated. This concept is supported by the findings of Hamilton et al. (57), that apoE is found in high concentrations in the space of Disse. It has been suggested that chylomicron remnants may be able to bind this apoE and facilitate uptake by cellular receptors (58). The results presented in this paper indicate that a similar mechanism may apply to hypertriglyceridemic VLDL.

Our finding that Type IV VLDL is not taken up by HepG2 cells in the absence of lipolysis is consistent with those of Craig et al. (16) who did not observe any uptake or modification of normolipidemic 125I-VLDL by this cell line. However, others have demonstrated binding (8, 9), internalization, and degradation (8) of normolipidemic 125I-VLDL and the binding of large hypertriglyceridemic 125I-VLDL (9) to these cells. Since increases in acyl-coenzyme A cholesterol acyltransferase activity were not observed and only modest increases in cellular cholesterol ester were observed only at very high concentrations of VLDL added (6-fold greater VLDL concentrations than used in the present studies) (8), this suggests that internalization of 125I-VLDL radioactivity does not necessarily reflect uptake of whole VLDL particles.

Studies by Yen et al. (59) have indicated that enrichment of media with oleic acid enhanced the binding of apoE-containing triglyceride-rich lipoproteins to normal human fibroblasts. However, we found that the free fatty acids liberated during VLDL lipolysis did not mediate the uptake of the VLDL remnant particle. Type IV VLDL lipolyzed and reisolated prior to addition to HepG2 cells resulted in marked increments in cellular esterified cholesterol and triglyceride mass. Addition of oleic acid to Type IV VLDL (without LPL) did not cause any increase in cellular cholesterol ester. Oleic acid alone produced a markedly enhanced cellular triglyceride content, whereas coincubation of Type IV VLDL (without LPL) and oleic acid did not result in a further increment in cellular triglyceride mass. Thus, the increased media-free fatty acid concentration alone was not sufficient, while lipolytic modification by LPL was necessary and sufficient to promote uptake of Type IV S<sub>3</sub> 60–400 VLDL by HepG2 cells. Although the LDL receptor has been shown to be the primary mechanism for the uptake of intermediate density lipoprotein by HepG2 cells (43), the LDL receptor-related protein, known to be expressed by HepG2 cells and to bind lipoproteins enriched in apoE (60, 61) may be involved. Beiiegel et al. (62) have shown that chylomicrons bind to the LDL receptor-related protein of HepG2 cells, mediated by exogenous bovine milk lipoprotein lipase. The relative roles of these receptors and ligands in the uptake of the lipolyzed Type IV VLDL by HepG2 cells remains to be elucidated.

In summary, our data shows that lipolysis of Type IV S<sub>3</sub> 60–400 VLDL by milk LPL is a prerequisite in order for these lipoproteins to induce lipid accumulation in HepG2 cells and that HepG2 cell-secreted apoE rather than apoE associated with VLDL as isolated from plasma, is the ligand involved in uptake. This suggests that hepatocytes lack the ability to directly catabolize large VLDL and may only be involved in uptake of Type IV VLDL following partial lipolysis of VLDL by LPL in vivo. It is possible that nonparenchymal liver cells, such as Kupffer cells, may secrete the LPL activity necessary to facilitate the uptake of large VLDL by hepatocytes.

Acknowledgments—We thank Sandra Kleinstiver for expert technical assistance and Camilla Vezina of Dr. Philip Connelly's laboratory at the University of Toronto for performing the enzyme-linked immunoassortent assays. We are grateful to B. M. Wolfe, T. J. McDonald, and I. Hramiak for supplying plasma from hyperlipidemic patients used in these studies.

REFERENCES
1. Barbir, M., Wile, D., Trayner, I., Aber, V. R., and Thompson, G. R. (1988) Br. Heart J. 60, 397–403
2. Carlson, L. A., and Bottiger, L. E. (1985) Acta Med. Scand. 218, 207–211
3. Freedman, D. S., Gruchow, H. W., Anderson, A. J., Rimm, A. A., and Barboriak, J. J. (1988) Am. J. Epidemiol. 127, 1118–1130
4. Huf, M. W., and Telford, D. E. (1984) Biochim. Biophys. Acta 796, 251–261
5. Evans, A. J., Huf, M. W., and Wolfe, B. M. (1989) J. Lipid Res. 30, 1699–1707
6. Sheppard, J., and Packard, C. J. (1987) Am. Heart J. 113, 505–508
7. Reardon, M. F., Fidge, N. H., and Nestel, P. J. (1978) J. Clin. Invest. 61, 850–860
8. Dashti, N., and Wolfbauer, G. (1986) Biochim. Biophys. Acta 875, 473–486
9. Krempler, F., Kostner, G. M., Friedl, W., Paulsweber, B., Bauer, H., and Sandhofer, F. (1987) J. Clin. Invest. 80, 401–408
10. Gustafson, S., Ostlund-Lindqvist, A., and Vessey, E. (1985)
Uptake of Large VLDL by HepG2 Cells

37. Gianturco, S. H., Gotto, A. M., Hwang, S. C., Karlin, J. B., Lin, A. H. Y., Prasad, S. C., and Bradly, W. H. (1983) J. Biol. Chem. 258, 4526-4533
38. Gianturco, S. H., Broome, W. A., Gotto, A. M., Jr., Morissett, J. D., and Peavy, D. L. (1982) J. Clin. Invest. 70, 168-178
39. Sacks, F. M., and Breslow, J. L. (1987) Arteriosclerosis 7, 35-46
40. Jovin, T. N. (1990) FASEB J. 4, 161-168
41. Ranganathan, S., and Kottke, B. A. (1989) Hepatology 9, 547-551
42. Craig, W. Y., Nutik, R., and Cooper, A. D. (1988) J. Biol. Chem. 263, 13880-13889
43. Craig, W. Y., and Cooper, A. D. (1988) J. Lipid Res. 29, 299-308
44. Schafer, E. J., and Levy, R. I. (1985) N. Engl. J. Med. 312, 1300-1310
45. Markwell, M. K., Hsia, S. M., and Tolbert, N. E. (1978) Anal. Biochem. 87, 206-210
46. Novak, M. (1965) J. Lipid Res. 6, 431-433
47. Buri, J., and Rossenou, M. (1985) Clin. Chem. 31, 247-251
48. Ordovas, J. M., Peterson, J. P., Snaitenello, P., Cohn, J. S., Wilson, P. W. F., and Schaefer, E. J. (1987) J. Lipid Res. 28, 1216-1224
49. Huff, M. W., Evans, A. J., Sawyee, C. G., Wolfe, B. M., and Nestel, P. J. (1991) Arteriosclerosis Thromb. 11, 221-233
50. Milne, R. W., and Marcel Y. L. (1988) FEBS Lett. 146, 97-100
51. Weiggraber, K. H., Innerarity, T. L., Harder, K. J., Mahley, R. W., Milne, R. W., Marcel, Y. L., and Sparrow, J. T. (1983) J. Biol. Chem. 258, 12433-12554
52. Milne, R. W., Theolis, R., Jr., Verdeny, R. B., and Marcel, Y. L. (1983) Arteriosclerosis 3, 23-30
53. Marcel, Y. L., Hogue, M., Weech, P. K., Davignon, J., and Milne, R. W. (1988) Arteriosclerosis 8, 522-544
54. Rudling, M. J., and Peterson, C. O. (1985) Biochim. Biophys. Acta 834, 395-396
55. Neri, B. P., and Frings, C. S. (1973) Clin. Chem. 19, 1201-1202
56. Brown, M. S., Ho, H. K., and Goldstein, J. L. (1980) J. Biol. Chem. 255, 9344-9352
57. Blache, D., Bouthillier, D., and Davignon, J. (1983) Clin. Chem. 29, 154-158
58. Soccorro, L., and Jackson, R. L. (1985) J. Biol. Chem. 260, 6524-6528
59. Huff, M. W., Evans, A. J., Wolfe, B. M., Connelly, P. W., Maguire, G. F., and Strong, W. L. P. (1990) J. Lipid Res. 31, 385-396
60. Innerarity, T. L., Hui, D. Y., Berset, T. P., and Mahley, R. W. (1986) in Lipoprotein Deficiency Syndromes (Angel, A., and Frohlich, J., eds) pp 273-278, Plenum Publishing Co., New York
61. Huff, M. W., Breckenridge, W. C., Strong, W. L. P., and Wolfe, B. M. (1988) Arteriosclerosis 8, 471-479
62. Gianturco, S. H., Brown, S. A., Via, D. P., and Bradley, W. A. (1986) J. Lipid Res. 27, 415-420
63. Gianturco, S. H., Lin, A. H. Y., Hwang, S. C., Young, J., Brown, S. A., Via, D. P., and Bradley, W. A. (1988) J. Clin. Invest. 82, 1833-1843
64. Shelburne, F., Hanks, J., Meyers, W., and Varoquard, S. (1980) J. Clin. Invest. 65, 652-656
65. Persoon, N. L. M., Sips, H. J., and Jansen, H. (1986) Life Sci. 38, 1029-1033
66. Busch, S. J., Krustenansky, J. L., Owen, T. J., and Jackson, R. L. (1989) Life Sci. 45, 615-622
67. Mulsinger, T. A., Herbert, P. N., and Kingston, M. J. (1979) Biochim. Biophys. Acta 575, 277-288
68. Mahley, R. W., Hui, D. Y., Innerarity, T. L., and Beisiegel, U. (1989) Arteriosclerosis 9, Suppl. I, 14-18
69. Mudler, M., de Wit, E., and Havekes L. M. (1991) Biochim. Biophys. Acta 1081, 308-314
70. Schneider, W. J., Kovanen, P. T., Brown, M. S., Goldstein, J. L., Utermann, G., Weber, W., Havel, R. J., Kotite, L. Kane, J. P., Innerarity, T. L., and Mahley, R. W. (1981) J. Clin. Invest. 68, 1075-1085
71. Tabas, I., Boykow, G. C., and Tall, A. R. (1987) J. Clin Invest. 79, 418-426
72. Bradley, W. A., Hwang, S. C., Karlin, J. B., Lin, A. H. Y., Prasad, S. C., Gotto, A. M., and Gianturco, S. H. (1984) J. Biol. Chem. 259, 14728-14735
73. Krul, E. S., Tikkanen, M. J., Cole, T. G., Davie, J. M., and Schoenfeld, G. (1984) J. Clin. Invest. 75, 361-369
74. Faust, R. A., Cheung, M. C., and Albers, J. J. (1984) Atherosclerosis 77, 77-82
75. Zannis, V. I., Breslow, J. L., SanGiacomo, T. R., Aden, D. P., and Knowles, B. B. (1981) Biochemistry 20, 7089-7096
76. Erickson, S. K., and Fielding P. E. (1986) J. Lipid Res. 27, 875-883
77. Eiseberg, S., Friedman, G., and Vogel, T. (1988) Arteriosclerosis 8, 480-487
78. Schayek, E., Lewin-Veltur, V., Chajok-Shaul, T., and Eiseberg, S. (1991) J. Clin. Invest. 88, 553-560
79. Windler, E. T., Chao, Y., and Havel, R. J. (1980) J. Biol. Chem. 255, 5475-5480
80. Windler, E., and Havel, R. J. (1986) J. Lipid Res. 26, 566-565
81. Oswald, B., and Quartfordt, S. (1987) J. Lipid Res. 28, 798-809
82. Friedman, G., Gavish, D., Vogel, T., and Eisenberg, S. (1990) Biochim. Biophys. Acta 1044, 118-126
83. Hamilton, R. L., Wong, J. S., Guo, L. S. S., Kriaus, S., and Havel, R. J. (1990) J. Lipid Res. 31, 1589-1603
84. Mahley, R. W., and Hussain, M. M. (1991) Curr. Opin. Lipidology 2, 170-176
85. Yen, P. T., Bihain, B. E., Glessen, A. M., Vogel, T., Gorecki, M., and Deckelbaum, R. J. (1989) Circulation 80, 11-487 (abstr.)
86. Kuo, P., Weinfield, M., and Loscalzo, J. (1990) Biochem. 29, 6626-6632
87. Beisiegel, U., Weber, W., Irbeke, G., Herz, J., and Stanley, K. K. (1989) Nature 341, 162-164
88. Beisiegel, U., Weber, W., and Bengtsson-Olivecrona, G. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8342-8347