Control of 3-Hydroxy-3-Methylglutaryl-CoA Reductase Activity in Cultured Human Fibroblasts by Very Low Density Lipoproteins of Subjects with Hypertriglyceridemia

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ABSTRACT Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) from human normolipemic plasma, and the VLDL, the intermediate density lipoprotein (IDL), and LDL from patients with Type III hyperlipoproteinemic plasma were tested for their abilities to suppress the activity of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase in cultured human fibroblasts from normal subjects and a Type III patient. Regulation of cholesterol synthesis in the fibroblasts of a patient with Type III hyperlipoproteinemia appears to be normal. VLDL from normal subjects, isolated by angle head ultracentrifugation ($d < 1.006$) or by gel filtration on BioGel A-5m, were about 5 times less effective than LDL in suppressing HMG-CoA reductase activity, based on protein content, in agreement with previous reports with normal fibroblasts. Zonal centrifugation of normal VLDL isolated by both methods showed that the VLDL contained IDL. Normal VLDL from the angle head rotor, refractionated by the zonal method, had little, if any, ability to suppress the HMG-CoA reductase activity in either normal or Type III fibroblasts. VLDL, IDL, and LDL fractionated by zonal ultracentrifugation from Type III plasma gave half-maximum inhibition at 0.2–0.5 μg of protein/ml, indistinguishable from the suppression caused by normal LDL. Type III VLDL did not suppress HMG-CoA reductase in mutant LDL receptor-negative fibroblasts. Zonally isolated VLDL obtained from one Type IV and one Type V patient gave half-maximal suppression at 5 and 0.5 μg of protein/ml, respectively. Molecular diameters and apoprotein compositions of the zonally isolated normal and Type III VLDL were similar; the major difference in composition was that Type III VLDL contained more cholesteryl esters and less triglyceride than did normal VLDL. The compositions and diameters of the Type IV and Type V VLDL were similar to normal VLDL. These findings show that the basic defect in Type III hyperlipoproteinemia is qualitatively different from the cellular defect found in familial hypercholesterolemia, since the regulation of HMG-CoA reductase activity is normal in Type III fibroblasts. The metabolic defect in hypertriglyceridemia is related to the triglyceride-rich lipoproteins which, free of other lipoproteins, have an enhanced ability to interact with cultured fibroblasts to regulate HMG-CoA reductase activity. These studies suggest that, in hypertriglyceridemia, there is a mechanism for direct cellular catabolism of VLDL which is not functional for normal VLDL.

INTRODUCTION

In Type III hyperlipoproteinemia (broad β-disease, or dysbetalipoproteinemia), plasma cholesterol and triglyceride levels are typically increased (1). One of the most striking aspects of the lipoprotein distribution in Type III is the presence of a lipoprotein fraction with a density intermediate between very low

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A preliminary report of this work has appeared in abstract form (1976, *Circulation*. **54:** II–55).

Doctors Jackson and Smith are Established Investigators of the American Heart Association.

Received for publication 31 December 1976 and in revised form 21 September 1977.

density lipoproteins (VLDL)[1] and low density lipoproteins (LDL) (2). VLDL are also elevated and their composition is abnormal. Compared with normal VLDL, Type III VLDL are reported to contain more arginine-rich protein and cholesteryl ester but less triglyceride (3). The metabolic defect which accounts for these abnormalities is unknown.

In normal cultured human fibroblasts, LDL decrease cholesterol biosynthesis by suppressing synthesis of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (4–6). By contrast, control of HMG-CoA reductase activity in fibroblasts derived from persons with the homozygous form of familial hypercholesterolemia is defective, since these cells cannot bind LDL normally because of an absent or defective LDL receptor. A similar receptor defect might exist in Type III hyperlipoproteinemia and account for the elevated cholesterol levels in plasma. However, if the regulation of HMG-CoA reductase proved to be normal in Type III fibroblasts, the Type III lipoproteins might show abnormal behavior as regulators of cellular cholesterol synthesis. Our studies show that the defect in Type III hyperlipoproteinemia is not the same cellular defect as in familial hypercholesterolemia. However, Type III VLDL is abnormal in that it is effective in suppressing HMG-CoA reductase activity. Zonally prepared VLDL from two patients with other forms of hypertriglyceridemia also suppressed, suggesting that this functional abnormality of VLDL may be a common feature of hypertriglyceridemia.

METHODS

Materials. DL-3-hydroxy-3-methyl[3-^{14}C]-glutaryl-CoA (26.2 mCi/mmol) was purchased from New England Nuclear (Boston, Mass.). Silica gel-coated plastic-backed thin-layer chromatography sheets without fluorescent indicator (No. 6601) were products of Eastman Kodak Co. (Rochester, N. Y.). NADP; D-glucose-6-phosphate, monosodium salt; and glucose 6-phosphate dehydrogenase (Type XV), sulfate-free, were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Cells. All cultures of human fibroblasts were established in our laboratory from skin biopsies, except for the receptor-negative mutant homozygous familial hypercholesterolemia fibroblasts. This mutant line, GM-361, purchased from the Human Genetic Mutant Cell Respository (Camden, N. J.), was established and characterized by Goldstein and Brown (7, 8). Normal cells were taken from adults whose plasma cholesterol and triglyceride levels and lipoprotein profiles were normal. Type III cells were from M. A., a 56-yr-old black male with tuberous xanthomas on his elbows and prominent bilateral corneal arcus. The Type III diagnosis was based on commonly used criteria (1). The fasting plasma was turbid; some chylomicrons were present. The cholesterol concentration was 310 mg/dl; triglyceride was 311 mg/dl of plasma. Lipoprotein electrophoresis of whole plasma showed a broad beta band. A beta-migrating band was present in the VLDL ($d < 1.006$); the ratio of VLDL cholesterol to plasma triglyceride concentration was above 0.30. Pedigree data are not available. A detailed metabolic study of this individual is presented elsewhere (9).

Monolayer cultures were maintained in a humidified incubator under 5% CO_2 at 37°C in 100-mm plastic tissue culture dishes (Lux Scientific Corp., Newbury Park, Calif.). Each dish contained 10 ml of complete medium consisting of Dulbecco's Modified Eagle's Medium (Grand Island Biological Co., Grand Island, N. Y., Cat. No. H21), supplemented with 10% (vol/vol) fetal calf serum (International Scientific Industries, Inc., Cary, Ill.), 2 mM glutamine, 36 mM $NaHCO_3$, 100 U/ml potassium penicillin G, and 100 μg/ml streptomycin sulfate. Cells were used between the third and seventh passage. The split ratio for cells in each passage was 1:4.

For experiments, confluent cell monolayers were dissociated from stock plates with trypsin (100 μg/ml; Grand Island Biological Co.) and the cells combined. Approximately 10^5 cells were then dispersed in 5 ml of complete medium into 60-mm plastic tissue culture dishes and placed in a 37°C humidified incubator under 5% CO_2 for this and subsequent incubations of cells. The medium was changed after 3 days. When the cells were 75–100% confluent (5–7 days), the medium was removed; the cells were washed with 3 ml of saline and were placed on 3 ml of medium containing 5% human lipoprotein-deficient serum (LPDS) for 24 h to allow HMG-CoA reductase activity to increase to unsuppressed levels. Measured quantities of lipoproteins were then added to duplicate dishes for 16 h. The medium was then removed, the cell monolayers washed twice at room temperature 2 ml of saline, and the cells removed with a rubber policeman into 2.0 ml of 0.15 M NaCl, 50 mM Tris-HCl, pH 7.4. The cells were sedimented by centrifugation, the supernate was discarded, and the cell pellets stored at −80°C.

HMG-CoA reductase assay. HMG-CoA reductase activities were determined by a modification of the method of Brown et al. (5). Thawed cell pellets were incubated for 10 min at 37°C with 0.1 ml of 50 mM K_2HPO_4, pH 7.4, containing 5 mM dithiothreitol, 1 mM EDTA, and 0.25% Kyro EOB (Proctor & Gamble, Cincinnati, Ohio). After centrifugation for 5 min at 5,000 rpm at room temperature, the clear supernates were assayed in duplicate for HMG-CoA reductase activity. Cell extracts (20 μl) were incubated at 37°C in a final volume of 35 μl with 3 mM NADP; 22 mM glucose 6-phosphate; 14 mM Tris-HCl, pH 7.5; 5 mM dithiothreitol; 0.15 U of glucose 6-phosphate dehydrogenase; and 43 μM DL-3-hydroxy-3-methyl[3-^{14}C]-glutaryl-CoA. After 2 h, 10 μl of 2.5 N HCl containing 60 mM mevalonolactone as carrier was added. After 20 min at 37°C, 15 μl of the acidified reaction mixture, in 5-μl aliquots, was streaked on silica thin-layer chromatograms. The chromatograms were air-dried and developed in acetone-benzene (1:1, vol/vol) until the solvent front had moved 5.5 cm above the origin. Areas of the chromatogram containing mevalonolactone (R_f 0.63) and residual HMG-CoA (origin) were counted with Triton X-100:toluene scintillation fluid (10). To calculate the picomoles of mevalonate formed during the incubations, the fraction of total radioactivity which appeared in the mevalonolactone region was multiplied by the picomoles of HMG-CoA initially present in the assay. For figures, each HMG-CoA reductase activity

[1] *Abbreviations used in this paper:* HMG-CoA reductases, 3-hydroxy-3-methylglutaryl-CoA reductase; IDL, intermediate density lipoprotein; LDL, low density lipoproteins; LPDS, lipoprotein-deficient serum; VLDL, very low density lipoproteins.

data point is the average of values from duplicate dishes; the variation between duplicate values in each experiment is stated in each figure legend. Protein content of extracts was determined by the method of Lowry et al. (11), with bovine serum albumin as a standard.

Lipoproteins and lipoprotein-deficient serum. Normal lipoprotein fractions and LPDS were isolated from the plasma of fasting adult normolipemic males. Diagnoses of hyperlipoproteinemia types were based on commonly used criteria (1). Secondary causes of hyperlipoproteinemia were excluded for each patient from whom lipoproteins were obtained. None of the patients was on any special diet or medication at the time plasma was taken for lipoprotein isolation. Type III patients M. A. and W. M. had been on no special diet or medication for 1 mo before plasma was obtained. Type III patient J. H. and Type IV patient Y. K. had never received lipid-lowering drugs. Type V patient M. V. had received Atromid S and was on a restricted carbohydrate diet. Blood was collected in 0.1% EDTA from fasting subjects; erythrocytes were removed by low-speed centrifugation. To prepare LPDS, lipoproteins were removed by flotation at d 1.21 (KBr) in a Beckman preparative ultracentrifuge (L3-50) using a 60 Ti rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 45,000 rpm at 14°C for 60 h. LPDS was dialyzed against two changes of 150 vol of 0.15 M NaCl containing 50 mM Tris-HCl, pH 7.4, and 0.3 mM EDTA at 4°C for 36–48 h. After clotting with thrombin (4), LPDS was sterilized by filtration through a 0.2-μm filter unit (Nalge Co., Rochester, N. Y.) and stored at −20°C. Normal VLDL and LDL for the experiment in Fig. 2 were isolated from the lipoprotein fraction, obtained from the ultracentrifugation of plasma at d = 1.21, by chromatography over Bio-Gel A-5m, 200–400 mesh, 6 × 60 cm (Bio-Rad Laboratories, Richmond, Calif.) (12). All other lipoprotein fractions were isolated from plasma according to standard techniques by sequential flotation in a 60 Ti rotor at 45,000 rpm and 14°C for 18–22 h (13). VLDL were isolated in a single centrifugation without adjusting the density of plasma ($d < 1.006$); intermediate density lipoproteins (IDL) were isolated at d = 1.006–1.020, and LDL at d = 1.020–1.063. Each lipoprotein class was further purified by rate zonal ultracentrifugation in a Beckman Ti-14 zonal rotor. A linear gradient in the density range of 1.000–1.300 (NaBr) was used for ultracentrifugation at 15°C and 42,000 rpm for 140 min (14). All lipoproteins were concentrated by ultrafiltration at <7 psi N_2 at 4°C in an Amicon Cell (model 8MC) fitted with an XM-100 A membrane (Amicon Corp., Scientific Systems Div., Lexington, Mass.). Lipoprotein fractions were dialyzed at 4°C against two changes of 150 vol of 0.15 M NaCl containing 50 mM Tris-HCl, pH 7.5, and 0.3 mM EDTA, sterilized by filtration, and stored at 4°C. Cholesterol and triglyceride contents of the lipoprotein fraction were determined in the Core Laboratory of the Baylor Lipid Research Clinic (15).

Free and total cholesterol were determined by an enzymatic colorimetric method (16). The difference between free and total cholesterol was multiplied by a factor of 1.68 to calculate cholesteryl ester content. Lipid phosphorus was determined by the method of Bartlett (17). A factor of 25 was used to convert lipid phosphorus into phospholipid concentration.

Total protein contents of the lipoproteins were determined by a modification of the Lowry method; sodium dodecyl sulfate, at a final concentration of 0.1%, was included to prevent interference by opalescence and light scattering (18). The major protein component of VLDL and LDL, apoB, was determined as the difference between the total protein and the protein soluble in 4.2 tetramethylurea (19).

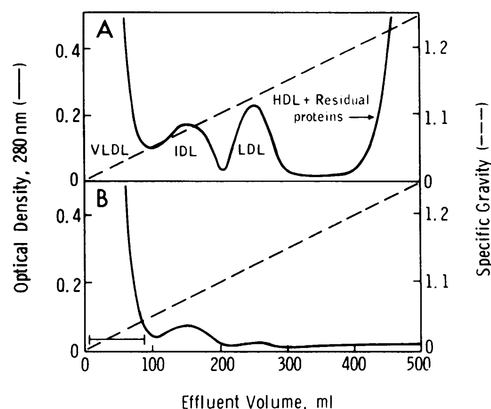

FIGURE 1 (A) Zonal rotor profile of plasma lipoproteins of 10 ml of plasma from a Type III patient (M. A.). (B) Zonal rotor profile of the $d < 1.006$ top fraction. Plasma from the Type III patient (10 ml) was centrifuged in an angle head rotor at 1.0 × 10^8 g/min. The top fraction ($d < 1.006$) was subjected to zonal ultracentrifugation under identical conditions as the whole plasma.

Soluble apoprotein composition was determined after delipidation with ether:ethanol, 3:1 (vol/vol). The apoproteins were separated by electrophoresis on 7.5% polyacrylamide gels containing 8 M urea, stained with Amido-Schwartz, and quantitated by scanning densitometry (19).

Diameters of the lipoproteins were determined by electron microscopy after negative staining with phophotungstic acid (20).

RESULTS

The zonal ultracentrifugal lipoprotein profile in Type III plasma is abnormal (Fig. 1 A), as previously described (2, 3). The most striking feature of this pattern is the appearance of a distinct peak between VLDL and LDL. This lipoprotein is present in such large amounts that it is found in both the VLDL and LDL fractions isolated from Type III plasma by sequential flotation in an angle head rotor. Analytical zonal ultracentrifugation shows that the $d < 1.006$ fraction, prepared from Type III plasma in an angle head rotor, contains VLDL, IDL,[2] and LDL (Fig. 1 B). However, Type III VLDL in the initial 100-ml effluent from the zonal rotor contained only pre-β migrating lipoproteins on Geon Pevikon electrophoresis (9). The lipoproteins producing the broad-β band, IDL and LDL, were removed in the zonal isolation. The effects of the Type III lipoproteins on the cells were tested after each lipoprotein class was freed of contaminating lipoproteins by rate zonal ultracentrifugation.

VLDL, IDL, and LDL from Type III plasma (M. A.) were just as effective as normal LDL in suppressing

[2] Whether IDL from normal subjects and IDL from Type III individuals, "LP-III," are identical in all respects is not known.

HMG-CoA reductase activity; approximately 10 μg of protein/ml of each gave complete suppression (Fig. 2). Similarly, there was no difference in the inhibition by Type III lipoproteins and normal LDL when compared on the basis of cholesterol content. There was, however, a difference in the suppression caused by the different VLDL fractions. Type III VLDL were at least 5 times more effective than the normal VLDL. The finding that normal VLDL suppressed less efficiently than did LDL is consistent with the reports of Brown et al. (4, 5); the degree of suppression exhibited by Type III VLDL was unexpected. The Type III VLDL, IDL, and LDL from a second patient with Type III (J. H.) were also as effective as normal LDL in suppression (Fig. 3). VLDL from a third patient (W. M.) with Type III was also inhibitory (Fig. 7). Thus, the functional abnormality was found in all three of the Type III patients available for study.

The Type III lipoprotein fractions used in Fig. 2 were isolated by the zonal method, while the normal VLDL and LDL were prepared by gel filtration. Normal VLDL were fractionated by zonal centrifugation to determine if the difference observed in the effects of normal VLDL and Type III VLDL on HMG-CoA reductase activity were due to the method of lipoprotein isolation.

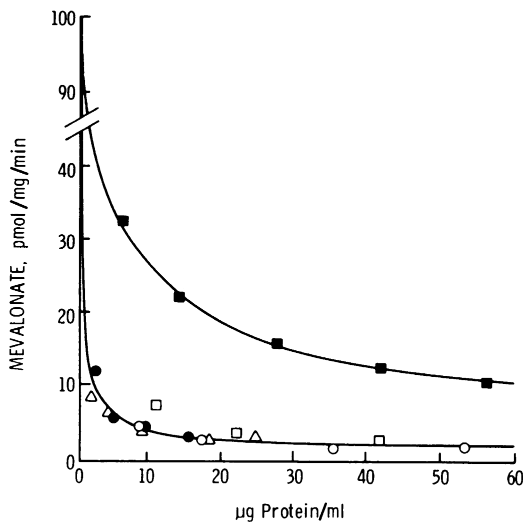

FIGURE 2 Effects of Type III and normal lipoproteins on HMG-CoA reductase activity in normal fibroblasts. Normal fibroblasts were grown to confluency in complete medium containing 10% fetal calf serum, washed, and then placed on 3 ml of medium containing 5% LPDS for 24 h, as described in Methods. Indicated amounts of lipoproteins, in 0.3 ml, were added to duplicate dishes for 16 h before the cells were washed and harvested. Each data point is an average of HMG-CoA reductase activity in fibroblasts from duplicate dishes, which differed by less than 5%. Normal lipoproteins, LDL (● —— ●) and VLDL (■ —— ■), were isolated by gel filtration. Type III lipoproteins (M. A.), VLDL (○ —— ○), IDL (△ —— △), and LDL (□ —— □), were isolated by rate zonal ultracentrifugation.

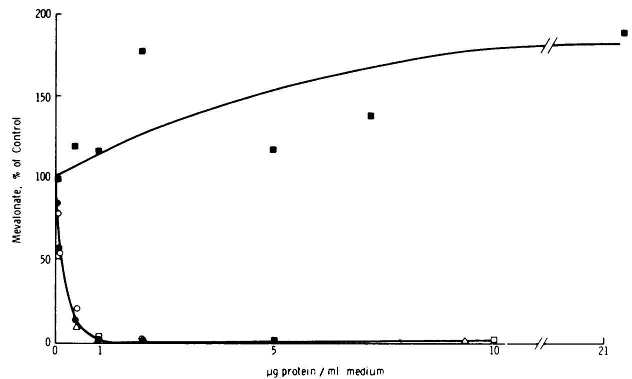

FIGURE 3 Effects of Type III and normal lipoproteins on HMG-CoA reductase activity in normal fibroblasts. Normal fibroblasts were grown to approximately 75% confluency in complete medium containing 10% fetal calf serum, washed, and then placed on 3 ml of medium containing 5% LPDS for 24 h, as described in Methods. The indicated quantities of lipoproteins, isolated by zonal ultracentrifugation, were added in 0.2 ml to duplicate dishes for incubation for 16 h before the cells were washed and harvested. Values from duplicate dishes, which differed by less than 10%, were averaged and expressed as a percentage of mevalonate formed in control cells that received no lipoproteins. For the experiment with normal VLDL, this value was 54.2 pmol of mevalonate formed/mg per min; for the experiment with Type III lipoproteins (J. H.), the control value was 37.0 pmol mevalonate formed/mg per min. Normal VLDL (■ —— ■); normal LDL (● —— ●); Type III VLDL (○ —— ○); Type III IDL (△ —— △); Type III LDL (□ —— □).

When both normal VLDL and Type III VLDL were isolated using the zonal rotor, the difference in the effects of normal and Type III VLDL on HMG-CoA reductase activity was greatly enhanced. At a level of 21 μg of VLDL protein/ml medium, normal VLDL isolated by the zonal method did not suppress HMG-CoA reductase activity in normal cells (Fig. 3). Type III VLDL caused a 50% suppression at less than 0.5 μg of protein/ml medium.

Normal VLDL isolated by gel filtration or by angle head ultracentrifugation suppressed HMG-CoA reductase activity in normal cells; half-maximal inhibition of enzyme synthesis was obtained with approximately 4 and 5 μg of VLDL cholesterol/ml medium, respectively, when the lipoproteins were isolated by angle head ultracentrifugation and gel filtration. The profiles obtained from zonal centrifugation showed that normal VLDL prepared either by angle head ultracentrifugation (Fig. 4 A) or by gel filtration (Fig. 4 B) contained IDL. The percentage contamination of VLDL by IDL in four different angle head preparations of $d < 1.006$ from normal plasma ranged from 7% to 50%, on the basis of Lowry protein recovered as VLDL and IDL after zonal ultracentrifugation. Opti-

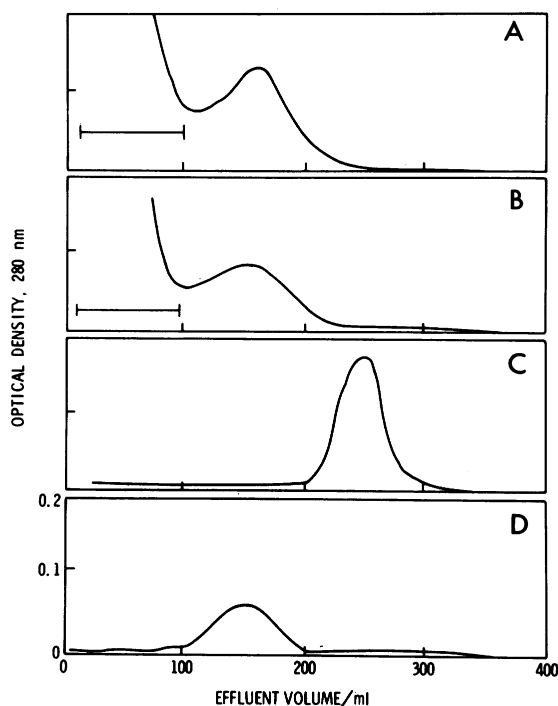

FIGURE 4 Analysis and isolation of lipoproteins by rate zonal ultracentrifugation. Normal VLDL isolated at $d < 1.006$ in an angle head rotor (A) and by gel filtration over BioGel A-5m (B) contain IDL. C shows the relative position of LDL (angle head rotor, $d = 1.02-1.063$), and D shows the relative position of IDL (angle head rotor $d = 1.006-1.02$) when these lipoproteins are recentrifuged in the zonal rotor. Fractions in the barred area of A were pooled and used as zonally isolated normal VLDL. Only the first 400 ml of the total rotor volume (665 ml) was collected. Details of the zonal preparation are found in Methods.

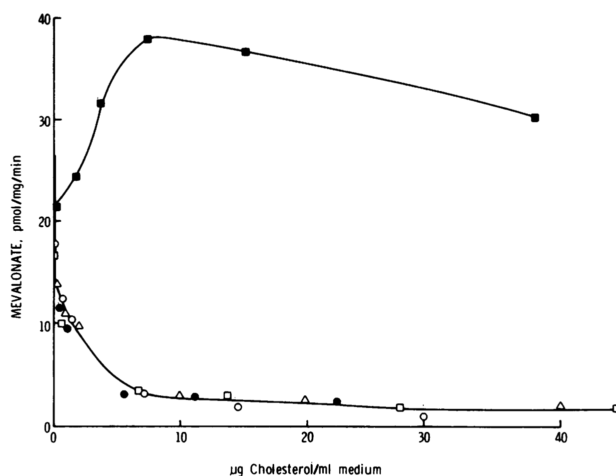

FIGURE 5 Effects of Type III and normal lipoproteins on HMG-CoA reductase activity in Type III fibroblasts. Growth of Type III fibroblasts (M. A.) and experimental design were the same as described in the legend to Fig. 2, except that all lipoproteins were isolated by zonal ultracentrifugation. Each data point is the average of the HMG-CoA reductase activity in cells from duplicate dishes, which differed by less than 10%. Normal LDL (● —— ●), normal VLDL (■ —— ■), Type III VLDL (M. A.) (○ —— ○), IDL (△ —— △), and Type III LDL (□ —— □).

cal densities recorded as a function of effluent volume are misleading because of the light scattering caused by the large particles present in the VLDL region of the zonal profile.

VLDL isolated by zonal centrifugation from the plasma of five fasting normolipemic males were studied with four normal cell lines. In each experiment, zonally isolated normal VLDL had little, if any, ability to suppress HMG-CoA reductase activity in the range of cholesterol or protein concentrations that gave complete suppression by LDL (Fig. 3). IDL isolated by the zonal method were as effective as LDL in suppressing HMG-CoA reductase. An increase in HMG-CoA reductase activity in the presence of low concentrations of VLDL was found with three of five preparations.

The control of HMG-CoA reductase activity in Type III fibroblasts was studied with both normal and Type III lipoproteins isolated by the zonal method (Fig. 5). Normal LDL efficiently suppressed HMG-CoA reductase in Type III fibroblasts, comparable to the effects found in normal fibroblasts. All of the Type III lipoproteins tested (VLDL, IDL, and LDL) suppressed just as effectively as did normal LDL, with 50% suppression at 1 μg of lipoprotein cholesterol/ml medium. At a level of 38 μg of VLDL cholesterol/ml medium, normal VLDL isolated by the zonal method failed to suppress HMG-CoA reductase activity in Type III cells. When the abilities of normal LDL and the Type III lipoprotein fractions to suppress HMG-CoA reductase in normal and Type III fibroblasts were compared on the basis of lipoprotein-protein content, they were still equally effective.

Neither LDL nor Type III VLDL suppressed HMG-CoA reductase in receptor-negative cells from a homozygous familial hypercholesterolemic individual, even at 20 μg of protein/ml medium, a level 40-fold higher than the level sufficient to give half-maximal suppression in normal cells (Fig. 6). Normal VLDL had no effect in the mutant cells.

VLDL were prepared by zonal centrifugation from the plasma of two patients with other types of hypertriglyceridemia (Fig. 7). The VLDL from a Type V patient were as suppressive as normal LDL, with half-maximal suppression occurring at approximately 0.5 μg of protein/ml. The VLDL from a Type IV individual also suppressed, with half-maximal suppression at about 5 μg of protein/ml. The VLDL contained no IDL yet were inhibitory. The ability of VLDL to suppress HMG-CoA reductase in cultured

fibroblasts may be a common characteristic of hypertriglyceridemia.

The chemical compositions of the lipoproteins studied are given in Table I. The apoprotein contents of the normal VLDL and the Type III VLDL were similar. The normal VLDL protein was 58% apoB, 9% arginine-rich apoprotein, and 33% apoC (the small molecular weight apoproteins of VLDL), whereas the Type III VLDL contained 62% apoB, 11% arginine-rich apoprotein, and 27% apoC. The Type III VLDL were approximately 30% cholesteryl ester by weight, compared with the 6–14% cholesteryl ester content of normal VLDL. Type III VLDL contained less triglyceride than normal VLDL, 31–42% and 50–62%, respectively. The lipid and apoprotein compositions of the Types IV and V VLDL were similar to those of normal VLDL. The ranges of diameters of all of the hypertriglyceridemic VLDL were the same as those of normal VLDL, as determined by negative staining. There are no differences in the mean diameters of the hypertriglyceridemic and normal VLDL that could account for the differences in effects on HMG-CoA reductase activity.

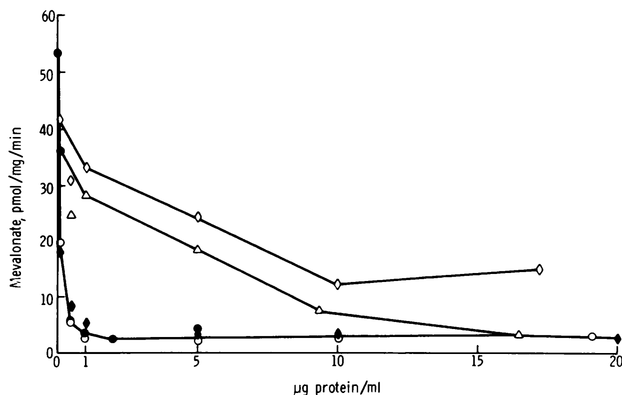

FIGURE 7 Effects of VLDL from plasma of patients with Types III, IV, and V hyperlipoproteinemia on HMG-CoA reductase activity in normal cells. Growth of the normal fibroblasts and the experimental design were the same as described in the legend to Fig. 3. Each data point is the average of the HMG-CoA reductase activity in cells from duplicate dishes, which differed by less than 10%. Normal LDL (● —— ●); Type III (J. H.) VLDL (○ —— ○); Type III (W. M.) VLDL (△ —— △); Type IV (J. K.) VLDL (◇ —— ◇); Type V (M. V.) VLDL (◆ —— ◆).

DISCUSSION

Our results indicate that the regulation of HMG-CoA reductase by plasma lipoproteins in Type III fibroblasts is similar to that in normal fibroblasts. Although we did not measure LDL binding to the specific receptor or cholesterol synthesis directly, it is reasonable to assume that these processes are also normal, since HMG-CoA reductase suppression in Type III fibroblasts was indistinguishable from that of normal fibroblasts. Brown and Goldstein (6) have shown that the activity of this enzyme accurately reflects LDL-receptor function and the entire process of internalization and degradation which are necessary for regulation of cholesterol synthesis. Our experiments suggest that the principal abnormality in Type III is associated with the plasma lipoproteins, since there is no apparent cellular defect in the regulation of HMG-CoA reductase in the Type III fibroblasts.

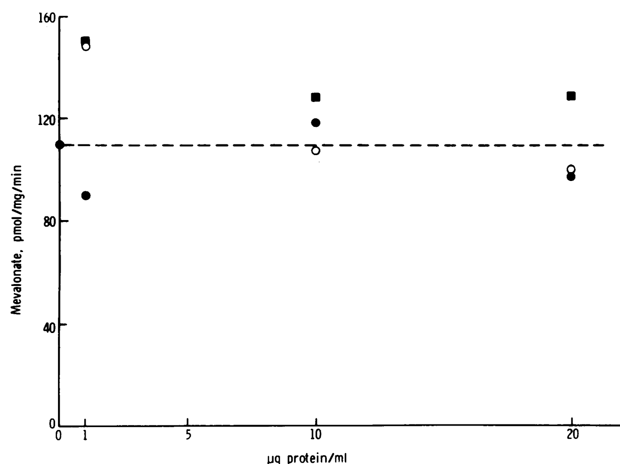

FIGURE 6 Effects of normal VLDL, Type III VLDL, and normal LDL on HMG-CoA reductase activity in homozygous familial hypercholesterolemia fibroblasts. Growth of the mutant cells and experimental design were the same as described in the legend to Fig. 3. Each data point is the average of values from duplicate dishes, which differed by less than 15%. Normal LDL (● —— ●); normal VLDL (■ —— ■); Type III (J. H.) VLDL (○ —— ○).

Analytical zonal ultracentrifugation of Type III plasma showed a distinct lipoprotein distribution, characteristic of this disorder. Analysis of the optical density profile indicated that high resolution separation in a continuous density gradient was the method of choice to prepare lipoproteins which were free of other lipoproteins with only slightly different densities. Isolation of all lipoprotein fractions by zonal ultracentrifugation was adopted as our standard method for these experiments, with the single exception of the experiment in Fig. 2.

Normal VLDL isolated in an angle head rotor or by gel filtration (Fig. 2) suppressed HMG-CoA reductase, as previously reported by others (4, 5); however, zonal ultracentrifugation analysis indicated that IDL was present in this fraction (Fig. 4). The amount of IDL in various VLDL preparations ranged from 7% to 50%, based on protein recovery. After removal of IDL by zonal ultracentrifugation, normal VLDL from the plasma of five individuals did not suppress HMG-CoA reductase in confluent Type III fibroblasts, or in

TABLE I

Composition and Size of Zonal Lipoproteins

Lipoprotein	Percentage weight of component*						
	Protein‡						
	Soluble	Insoluble	Free cholesterol	Esterified cholesterol	Phospho-lipid	Triglyceride	Mean diameter§
							Å
Type III							
VLDL (M. A.)	2.7	4.4	9.6	29.6	20.3	33.4	455±167
VLDL (J. H.)	2.8	3.8	4.0	31.1	16.2	42.1	606±160
VLDL (W. M.)	2.2	4.9	12.4	27.4	22.0	31.1	625±209
IDL (M. A.)	1.0	11.3	10.3	33.3	23.0	21.1	288±36
LDL (M. A.)	1.3	25.3	11.0	39.1	13.0	10.3	243±36
Type IV							
VLDL (Y. K.)	4.0	3.2	7.0	7.5	18.5	59.8	537±176
Type V							
VLDL (M. V.)	4.1	3.7	5.4	11.9	16.2	58.7	697±216
Normal							
VLDL (L. S.)	4.4	6.1	4.1	5.6	18.1	61.7	647±110
VLDL (R. J.)	7.3	5.0	6.3	13.9	18.0	49.5	607±165
VLDL (M. D.)	4.6	4.3	6.1	10.2	20.2	54.6	645±200
LDL (L. S.)	0	21.3	9.9	35.6	23.0	10.2	246±35

* The total weight of the particle was taken as the sum of the amounts of free cholesterol, esterified cholesterol, triglyceride, phospholipid, and total Lowry protein.

‡ Protein content is given as that soluble and insoluble in 4.2 M tetramethylurea; the latter is an estimate of apoB content (19).

§ ±1 SD.

either confluent or nonconfluent normal fibroblasts. It appears that the presence of IDL in normal VLDL isolated by anglehead ultracentrifugation or gel filtration can account for most, if not all, of the suppression caused by these preparations.

In contrast to normal VLDL, VLDL isolated by zonal ultracentrifugation from the plasma of three Type III patients suppressed HMG-CoA reductase activity as effectively as did LDL, suggesting that the type III VLDL interacts with the LDL receptor before suppression. As a control, the effects of Type III VLDL were tested in receptor-negative fibroblasts. The lack of suppression of HMG-CoA reductase activity in LDL receptor-negative cells by Type III VLDL and LDL (Fig. 6) indicates that suppression by Type III VLDL in normal and Type III fibroblasts is a result of binding to the LDL receptor.

VLDL isolated by zonal ultracentrifugation from the plasma of patients with Type IV and Type V hyperlipoproteinemia were also studied to see if the ability to supress HMG-CoA reductase activity could also be demonstrated in these disorders. Both Type IV and Type V VLDL suppressed enzyme activity in normal cells. These initial studies indicate that these types of hypertriglyceridemia are, like Type III, characterized by functionally abnormal VLDL.

Our studies document the abnormal ability of triglyceride-rich lipoproteins present in the plasmas of patients with Types III, IV, and V hyperlipoproteinemias to suppress the activity of HMG-CoA reductase of cultured fibroblasts. Moreover, there appear to be similar mechanisms involved in the suppression by LDL and by the VLDL from hypertriglyceridemic patients. Binding to the LDL cell surface receptor of normal fibroblasts is thought to involve apoB (21) or the arginine-rich protein (22). These apoproteins are present in normal VLDL isolated both by the zonal method and by conventional techniques (14, 23, 24). We found that the arginine-rich apoprotein content of zonally isolated normal and Type III VLDL were similar, in contrast to the observations of Havel and Kane (24) and Utermann et al. (25) that the amount of arginine-rich apoprotein is elevated two-to threefold in VLDL from Type III patients. These differences may be due to differences in VLDL isolation used in the two laboratories. The inability of normal VLDL, which contain both apoB and the arginine-rich protein, to suppress remains to be explained. There appears to be no single determinant, such as apoB, cholesterol or cholesteryl ester content, or lipoprotein diameter, which produces an obligatory interaction that results in suppression. The

lipid and apoprotein components of zonally isolated normal VLDL and VLDL from the plasmas of subjects with hyperlipoproteinemia are qualitatively the same (Table I). Within the constraints of the present analytical methods, the differences in apoprotein content of the various VLDL isolated by the zonal method cannot be considered significant. The major quantitative difference in composition between normal and Type III VLDL is that the Type III VLDL are unusually rich in cholesteryl esters, with a reciprocal decrease in triglyceride content. However, elevated cholesteryl ester content cannot account for the suppression by Types IV and V VLDL, which are similar to normal VLDL in cholesteryl ester content. The total protein content is somewhat lower in all the hypertriglyceridemic VLDL than in normal VLDL. There appear to be no differences in the range of sizes found in the various VLDL. Differences in the composition of the surface film or in size which could account for the ability of hypertriglyceridemic VLDL, but not normal VLDL, to suppress HMG-CoA reductase activity are not apparent by these analytical criteria. Conceivably, the proportions of surface film components or the extent of aggregation of the apoproteins could determine which lipoproteins produce suppression of HMG-CoA reductase.

These studies are the first to demonstrate in vitro that there is a mechanism for VLDL catabolism in Types III, IV, and V hyperlipoproteinemia, that (*a*) does not function with normal VLDL and (*b*) does not involve conversion of VLDL to IDL or LDL. Although the physiological conditions under which the LDL receptors are functionally important are not known, an enhanced interaction of VLDL with the tissue could suppress cholesterol synthesis while simultaneously causing abnormal intracellular accumulations of cholesterol and fatty acid produced by lysosomal degradation of the triglyceride-rich lipoproteins.

The different clinical manifestations of these disorders and the heterogeneity of the VLDL (26) remain as sources of speculation about the cellular process by which these various lipoproteins effect suppression of HMG-CoA reductase. The abnormal suppressiveness of hypertriglyceridemic VLDL may be due to chylomicron remnants, known to be present in VLDL from plasma of subjects with Type III and Type IV hyperlipoproteinemia (27). Although the present study adds strong confirmation to the observation that hypertriglyceridemia and vascular disease are in some way linked, the mechanisms of this relationship are still unknown. Future studies are required to define further these important, complex mechanisms involved in the regulation of cellular cholesterol synthesis.

In summary, these findings show that the basic defect in Type III hyperlipoproteinemia is qualitatively different from the cellular defect found in familial hypercholesterolemia, since the regulation of HMG-CoA reductase activity is normal in Type III fibroblasts. The metabolic defect in hypertriglyceridemia is related to the triglyceride-rich lipoproteins which, free of other lipoproteins, have an enhanced ability to interact with cultured fibroblasts to regulate HMG-CoA reductase activity. These studies suggest that, in hypertriglyceridemia, there is a mechanism for direct cellular catabolism of VLDL which is not functional for normal VLDL.

ACKNOWLEDGMENTS

Ms. Flora Brown, Ms. Sandy Floores, Ms. Pat Overton, and Mr. Ennah Manson provided excellent technical assistance. Kyro EOB was a generous gift of Dr. D. H. Hughes, Miami Valley Research Laboratories, Proctor & Gamble, Cincinnati, Ohio. We thank Mrs. Phyllis Gutierrez for typing the manuscript, Dr. Alberico Catapano for performing the apoprotein electrophoresis, and Mike Gann for electron microscopy.

This research was supported, in part, by grants from the American Heart Association 73-778; U.S. Public Health Service grants HL-15648, HL-17269, and RR-00350; National Heart, Lung, and Blood Institute Lipid Research Clinic Contract 71-2156; and the American Heart Association, Texas Affiliate.

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