Regulation of the Uptake and Degradation of \( \beta \)-Very Low Density Lipoprotein in Human Monocyte Macrophages*

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Brian J. Van Lenteren‡, Alan M. Fogelman‡§, Martha M. Hocom‡, Lee Benson‡, Margaret E. Haberland‡, and Peter A. Edwards‡¶

From the ‡Division of Cardiology, Department of Medicine, §Department of Biological Chemistry, and ¶Department of Pediatrics, School of Medicine, University of California at Los Angeles, Los Angeles, California 90024

In normal human monocyte macrophages \(^{125}\)I-labeled \( \beta \)-migrating very low density lipoproteins (\(^{125}\)I-\( \beta \)-VLDL), isolated from the plasma of cholesterol-fed rabbits, and \(^{125}\)I-human low density lipoprotein (LDL) were degraded at similar rates at protein concentrations up to 50 \( \mu \)g/ml. The high affinity degradation of \(^{125}\)I-labeled human LDL saturated at approximately 50 \( \mu \)g/ml; however, \(^{125}\)I-labeled rabbit \( \beta \)-VLDL high affinity degradation saturated at 100–120 \( \mu \)g/ml. The activity of the \( \beta \)-VLDL receptor was 3-fold higher than LDL receptor activity on freshly isolated normal monocyte macrophages, but with time-in-culture both receptor activities decreased and were similar after several days. The degradations of both \( \beta \)-VLDL and LDL were \( \mathrm{Ca}^{2+} \) sensitive, were markedly down regulated by sterols, and were up regulated by preincubation of the cells in a lipoprotein-free medium. The \( \beta \)-VLDL receptor is genetically distinct from the LDL receptor as indicated by its presence on monocyte macrophages from a familial hypercholesterolemic homozygote. Human thoracic duct lymph chylomicrons as well as lipoproteins of \( S_f \) 20–5000 from fat-fed normal subjects inhibited the degradation of \(^{125}\)I-labeled \( \beta \)-VLDL as effectively as normal canine lipoproteins, or LDL and the high density lipoproteins (HDL\(_a\), apo-E-HDL\(_a\), and apo-A-I-rich HDL\(_f\)) from cholesterol-fed dogs (3). Similar to the response of the LDL receptor (4) but unlike the scavenger receptor (5) and the receptor recognizing LDL-dextran sulfate complexes (6), the \( \beta \)-VLDL receptor activity in mouse peritoneal macrophages decreased as cellular cholesterol esters increased (3). Marine macrophages contain low levels of LDL receptor activity and the degradation of \(^{125}\)I-\( \beta \)-VLDL by these cells was poorly inhibited by increasing concentrations of nonradioactive LDL (3), implying that the \( \beta \)-VLDL and LDL receptors were distinctly different. There have been few studies, however, of the interaction of \( \beta \)-VLDL with human macrophages, which have highly active LDL (7) and \( \beta \)-VLDL (2) receptors.

In the present investigation we demonstrate that \( \beta \)-VLDL receptor activity in human monocyte macrophages, while similar to LDL receptor activity in its time course of development, requirement for \( \mathrm{Ca}^{2+} \), and the regulation of its expression by cholesterol in the medium, is genetically distinct from the LDL receptor activity. We further show that the receptor recognition of \( \beta \)-VLDL by these cells may be mediated via intestinally derived apolipoproteins.

**Experimental Procedures**

Materials—Sodium \[^{125}\]iodide (17 mCi/mg) and \[^{14}\]C-oleic acid (54 mCi/mmol) were obtained from Amersham Corp. and New England Nuclear, respectively. Phosphate-buffered saline and Dulbecco’s modified Eagle’s medium (catalogue no. 430-1600) were purchased from Grand Island Biological Co. Crystallized human albumin (catalogue no. A-8763) was purchased from Sigma; Picoll Hypaque was purchased from Pharmacia. Falcon (catalogue no. 3047) multiwell tissue culture plates were purchased from Van Waters and Rogers, Norwalk, CA. All other equipment and supplies were purchased from sources previously reported (8).

Subjects—Normal subjects were recruited from the staff and student body at UCLA. No one received drugs that might have affected serum cholesterol levels or lipid metabolism. All had normal hematocrits, white blood cell and differential counts, serum cholesterol, and triglycerides. Informed consent was obtained in writing from each person.

Clinical Data—Cultured monocyte macrophages were also obtained from a subject exhibiting the homozygous form of FH. Patient M.W. (2 years old) is an FH homozygote possessing 5% of the normal number of LDL receptors. Her LDL cholesterol concentration exceeded 1000 mg/dl on a low fat-low cholesterol diet. After the administration of maximum doses of cholestyramine and nicotinic acid her cholesterol ester synthesis by \( \beta \)-VLDL in murine macrophages showed saturation kinetics consistent with receptor-mediated uptake and processing of \( \beta \)-VLDL (2, 3). Canine \( \beta \)-VLDL stimulated cholesteryl ester synthesis in murine macrophages much more effectively than did normal canine lipoproteins, or LDL and the high density lipoproteins (HDL\(_a\), apo-E-HDL\(_a\), and apo-A-I-rich HDL\(_f\)) from cholesterol-fed dogs (3).

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‡ The abbreviations used are: VLDL, very low density lipoprotein that has \( \beta \)-mobility on electrophoresis; \( \beta \)-VLDL, very low density lipoprotein that has \( \beta \)-mobility on electrophoresis; LDL, low density lipoprotein; HDL, high density lipoprotein; HDL\(_a\), cholesterol-injected high density lipoprotein; apo, apolipoprotein; FH, familial hypercholesterolemia; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

‡ Joseph L. Goldstein, personal communication.

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LDL cholesterol was 624 mg/dl. Following a portacaval shunt and while still receiving maximal doses of cholesterolamine and nicotinic acid, her LDL cholesterol was 480 mg/dl.

Separation of Cells—Five hundred ml of blood were taken from adult subjects after an overnight fast and the monocytes were separated from 300 ml of blood using counterflow centrifugation (method BB in Reference 9). Twenty ml of blood were taken from the child and donor monocytes were separated using the same methods except that a 7-ml receiving chamber was used and the monocytes appeared in earlier fractions.

Cell Culture—Unless otherwise specified the cells were suspended in 30% autologous serum in Dulbecco’s modified Eagle’s medium supplemented with NaHCO3 (24 mM), Heps (10 mM), insulin (8 μg/ml), glucose (2 mg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml), hereafter referred to as medium B in order to be consistent with our previous publications (8, 9). One ml samples of the cell suspension containing 10,000 cells were transferred to plastic tissue culture dishes (35 x 10 mm) or 0.5 ml samples of the cell suspension containing 0.44 x 106 cells were transferred to 2.0 cm2 polystyrene wells and incubated at 37 °C in a humidified incubator with 5% CO2. Unless otherwise stated, the medium was aspirated and replaced with fresh medium of the same composition twice weekly.

Classification and Viability of Cells—The cells were classified and their viability determined as described previously (10). Because of media changes and the washes prior to beginning each experiment, the cells were 99% monocyte macrophages before the radioactive lipoproteins were added. More than 95% of the cells were viable at the end of the incubations.

Rabbit Lipoproteins—Young adult male New Zealand white rabbits weighing 2.5 kg were placed on a diet of either standard Purina Rabbit Chow (Ralston Purina Co.) or rabbit chow supplemented with 3% w/w of cholesterol and 10% w/w of corn oil. On this latter diet a plasma cholesterol of 4200 mg/dl was reached. The d < 1.006 g/ml fraction was isolated at plasma density from cholesterol-fed and chow-fed rabbits that were fasted for 24 h. The lipoproteins were radioiodinated according to the method of McFarlane (11) as modified by Bilheimer et al. (12). Greater than 97% of the total 125I-radioactivity of the 125I-labeled rabbit β-VLDL was trichloroacetic acid-preparable and less than 3% of the total 125I radioactivity was extracted into chloroform/methanol. Distribution of the radioactivity among the apolipoproteins of rabbit β-VLDL was: 78% in apo-B (of which 90% was in the high molecular weight form), 20% in apo-E, and 2% in apo-C. The d < 1.006 g/ml fraction of cholesterol-fed rabbits that were fasted for 24 h. The lipoproteins were washed by recentrifugation in isotonic saline at 40,000 rpm for 18 h at 15 °C in an SW 41 rotor (Beckman Instruments), resuspended in the buffer solution, and then recentrifuged at d = 1.063 g/ml was isolated by centrifugation at 40,000 rpm for 24 h and then recentrifuged at d = 1.063 g/ml for 24 h. The lipoproteins were radioiodinated according to the method of McFarlane (11) as modified by Bilheimer et al. (12). Greater than 97% of the total 125I-radioactivity of the 125I-labeled rabbit β-VLDL was trichloroacetic acid-preparable and less than 3% of the total 125I radioactivity was extracted into chloroform/methanol. Distribution of the radioactivity among the apolipoproteins of rabbit β-VLDL was: 78% in apo-B (of which 90% was in the high molecular weight form and 10% was in the lower molecular weight form), 20% in apo-E, and 2% in apo-C. The d < 1.006 g/ml fraction from cholesterol-fed rabbits showed a single β-migrating band on agarose electrophoresis.

Human Lipoproteins—Human LDL (d = 1.019-1.063 g/ml) was prepared and radioiodinated as described previously (7). Lipoproteins of S, 20-5000 were isolated from the plasma of a subject 2 h after the ingestion of a liquid meal containing 189 g of total fat and 3 g of cholesterol. For the isolation of S, 400-5000 lipoproteins fresh serum was overlayed with a phosphate-buffered saline solution (pH 7.4, d = 1.006 g/ml) and centrifuged for 30 min at 17,500 rpm at 15 °C in an SW 41 rotor. Lipoproteins of S, 100-400 were isolated by centrifugation of the above infranate for 60 min at 35,000 rpm. Lipoproteins of S, 20-100 were isolated by centrifugation of the infranate from the previous infranate at 40,000 rpm for 24 h. The top layer of each isolated fraction was removed by a tube slicer (Beckman Instruments), resuspended in buffer solution, washed with centrifugation 3 times under conditions identical with the initial isolations.

Thoracic duct lymph chylomicrons were isolated by overlaying the lymph (generously provided by Dr. Jay Fish, University of Texas Medical Center at Galveston, Galveston, TX) with a d = 1.006 g/ml phosphate-buffered saline solution of pH 7.4 and centrifuging the sample at 27,000 rpm for 85 min at 15 °C in an SW 41 rotor. Fractions were removed with a tube slicer and washed by recentrifugation under identical conditions. All isolated animal and human lipoprotein samples were dialyzed against isotonic saline of pH 7.4, stored at 0-8 °C, and were used within 1 month from time of initial isolation from the donor.

Assays—Prior to the addition of radioactive lipoproteins, the cells were washed three times with 1.5 ml (cells in dishes) or 1.0 ml (cells in a medium D containing 100 mM Heps (medium C). Unless otherwise stated radioactive lipoproteins were added in the same medium supplemented with 24 mM NaHCO3 and glucose, 2 mg/ml (medium D).

The proteolytic degradation of 125I-LDL and 125I-labeled rabbit β-VLDL was measured by assaying the amount of 125I-labeled trichloroacetic acid-soluble (noniodide) material formed by the cells and excreted into the culture medium as described by Goldstein and Brown (13). Corrections were made for the small amounts (<0.01% of total radioactivity added) of 125I-labeled acid-soluble material that was found in parallel incubations without cells. Acyl-CoA cholesterol acyltransferase activity in whole cells was determined by measuring the incorporation of [1-14C]oleate/albumin into cholesteryl [1-14C] oleate as described by Brown et al. (14). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15) was used to evaluate the apolipoprotein composition of the lipoprotein fractions. The protein contents of the cells and lipoproteins were determined by the method of Lowry et al. (16). Statistical analyses were carried out using the student t distribution and a p value of <0.05 was considered significant.

RESULTS

Degradation of 125I-labeled Lipoproteins—In Fig. 1 the degradation of 125I-labeled rabbit β-VLDL by human monocyte macrophages is compared to that of 125I-labeled human LDL. The degradation of both these lipoproteins was via a saturable high affinity process, as reported previously (3, 7). Whereas degradation of 125I-labeled rabbit β-VLDL paralleled that of 125I-labeled human LDL up to 50 μg/ml of protein, the apparent saturation of LDL high affinity degradation, 125I-labeled rabbit β-VLDL high affinity degradation was saturated at approximately 100-120 μg/ml of protein (Fig. 1). The 125I-labeled rabbit β-VLDL was not being degraded to any substantial degree via the scavenger receptor of the monocyte.

Fig. 1. Degradation of 125I-labeled rabbit β-VLDL (□) and 125I-labeled human LDL (○) by human monocyte macrophages.

Normal monocytes were cultured in 0.5 ml of 30% autologous serum in medium B. After 7 days the medium was removed and the cells were washed 3 times with 1 ml of medium C. Then 0.5 ml of medium D containing 125I-labeled rabbit β-VLDL (99 cpm/ng of protein) or 125I-labeled human LDL (285 cpm/ng of protein) at the protein concentration shown on the abscissa was added. After 4 h of incubation at 37 °C the medium was removed and the 125I-labeled acid-soluble content was determined. The values shown are the mean ± 1 S.D. of quadruplicate wells.
macrophage, since in the presence of a 25-fold excess of nonradioactive malondialdehyde-treated low density lipoprotein, a particle shown to be taken up by the scavenger receptor (17), the degradation of 125I-labeled rabbit β-VLDL was not different from its degradation in the absence of added competitor (746.5 ± 33.9 versus 731.3 ± 27.8 ng of lipoprotein degraded·h⁻¹·mg of protein⁻¹, respectively).

To compare the abilities of rabbit β-VLDL and human LDL to compete for receptor-mediated degradation by human monocyte macrophages the experiments in Fig. 2 were carried out. Addition of both nonradioactive rabbit β-VLDL and human LDL to the medium progressively inhibited the degradation of 125I-labeled human LDL (Fig. 2A) and 125I-labeled rabbit β-VLDL (Fig. 2B). However, nonradioactive rabbit β-VLDL inhibited the degradation of both radioactive lipoproteins more readily than did nonradioactive human LDL.

The results of the experiments in Fig. 2 demonstrated the difficulty of studying LDL receptor activity in a cell that also has substantial LDL receptor activity. The data from Fig. 2 suggest that the LDL receptor has a greater affinity for rabbit β-VLDL than for LDL itself. Therefore, a significant portion of the total 125I-labeled rabbit β-VLDL degradation may be attributed to uptake via the LDL receptor at low protein concentrations. To reduce the contribution of the LDL receptor to the degradation of 125I-labeled rabbit β-VLDL a protocol was designed based on the results of the experiments in Figs. 1 and 2. At 50 μg/ml of LDL protein the LDL receptor is largely saturated while the β-VLDL receptor is not (Fig. 1). Moreover, LDL has a relatively poor affinity for the β-VLDL receptor (Fig. 2B). As shown in Fig. 3, 50 μg/ml (a 25-fold excess) of nonradioactive human LDL inhibited the high affinity degradation of 2 μg/ml of 125I-rabbit β-VLDL by only 30% (Fig. 3A) while effectively inhibiting the degradation of the same concentration of 125I-labeled human LDL (Fig. 3B). Therefore, subsequent experiments utilized this protocol in order to assess β-VLDL receptor activity independently of LDL receptor activity.

The expression of LDL receptor activity on human monocyte macrophages had been shown previously to be a function of time-in culture (9), Ca²⁺ concentration (17), and sterol content of the medium (9). The following experiments compare β-VLDL receptor activity to LDL receptor activity as a function of these variables. In Fig. 4 it can be seen that in 1-day-old cells the activity of the β-VLDL receptor was significantly higher than that of the LDL receptor. After 1 day, however, both receptor activities decreased and remained similar throughout the duration of the time course. Additionally, the degradation of both β-VLDL and LDL are equally dependent upon the concentration of Ca²⁺ in the medium (Fig. 5). Table I shows that both β-VLDL and LDL receptor activities responded similarly to the concentration of cholesterol provided in the medium. When cells were incubated in a lipoprotein-free medium (0.1% human serum albumin) for 48 h prior to their incubation with either 125I-labeled rabbit β-VLDL or 125I-labeled human LDL, the rate of degradation of both lipoproteins was greater than that observed in cells preincubated in 30% autologous serum. Preincubation of cells with sterols for 72 h resulted in a marked down regulation of both β-VLDL and LDL receptor activities.

β-VLDL Receptor Activity in PH Cells—The data above demonstrated that the β-VLDL and the LDL receptor activi-

![Fig. 2. Ability of nonradioactive human LDL (●) and nonradioactive rabbit β-VLDL (○) to inhibit the degradation of 125I-labeled human LDL and 125I-labeled rabbit β-VLDL by human monocyte macrophages. Normal monocytes were cultured in 30% autologous serum in 0.5 ml of medium B. After 7 days the medium was removed and the cells were washed 3 times with 1 ml of medium. Then 0.5 ml of medium D containing 20 μg/ml of 125I-labeled human LDL (285 cpm/ng of protein) (A) or 20 μg of 125I-labeled rabbit β-VLDL (99 cpm/ng protein) (B) was added together with the concentration of nonradioactive lipoprotein-protein shown on the abscissa. The 100% values for the degradation of the 125I-lipoproteins in the absence of competing lipoproteins were 0.489 μg of degraded·h⁻¹·mg protein⁻¹ and 0.473 μg·h⁻¹·mg of protein⁻¹ for 125I-labeled rabbit β-VLDL, respectively. Values shown are the mean ± 1 S.D. of quadruplicate wells. *p < 0.05.](image)

![Fig. 3. The effect of a 25-fold excess of nonradioactive rabbit β-VLDL (solid bars) and normal human LDL (crosshatched bars) on the degradation of 125I-labeled rabbit β-VLDL (A) and 125I-labeled human LDL (B). Normal monocytes were cultured in 30% autologous serum in 0.5 ml of medium B. After 7 days the medium was removed and the cells were washed 3 times with 1 ml of medium. Then 0.5 ml of medium D containing protein concentrations of 2 μg/ml of 125I-labeled rabbit β-VLDL (234 cpm/ng of protein) (A) or 2 μg/ml of 125I-labeled human LDL (300 cpm/mg protein) (B) was added together with 50 μg/ml of nonradioactive lipoprotein or without competing lipoproteins (open bars). After 4 h of incubation at 37 °C the medium was removed and the 125I-labeled acid-soluble content determined. The values shown are the mean ± 1 S.D. of quadruplicate wells. *p < 0.05, significantly different from wells without competing lipoprotein. **p < 0.05, significantly different from wells without competing lipoprotein and from the other competing lipoprotein.](image)
VLDL was significantly higher than that of LDL in cells from an FH homozygote known to have only 5% of the normal number of LDL receptors (Fig. 4). Therefore, we investigated whether or not the P-VLDL receptor is genetically distinct from the LDL receptor. The degradation of \( ^{125}\text{I}-\text{labeled rabbit } \beta\text{-VLDL} \) and \( ^{125}\text{I}-\text{labeled human LDL} \) (Fig. 5) by human monocyte macrophages. Normal monocytes were cultured in 30% autologous serum in medium B. After 7 days the medium was removed and the cells were washed three times with 1 ml of medium C. Then 0.5 ml of medium D containing protein concentrations of 2 \( \mu g/ml \) of \(^{125}\text{I}-\text{labeled rabbit } \beta\text{-VLDL} \) (234 cpm/ng of protein) plus 80 \( \mu g/ml \) of nonradioactive human LDL or \(^{125}\text{I}-\text{labeled human LDL} \) (360 cpm/ng of protein) with EDTA to give the unbound Ca\(^{2+}\) concentration shown on the abscissa. After 4 h at 37°C, the medium was removed and the \(^{125}\text{I}-\text{labeled acid-soluble content was determined}. The 100% values for the degradation of the \(^{125}\text{I}-\text{labeled rabbit } \beta\text{-VLDL} \) and \(^{125}\text{I}-\text{labeled human LDL} \) were 0.337 ± 0.007 \( \mu g \) and 0.350 ± 0.025 \( \mu g \) of degraded \( \cdot 4 \) h\(^{-1}\)·mg of protein\(^{-1}\), respectively.

\[ \begin{array}{c|cc|c}
\text{Preparation of cells} & ^{125}\text{I}-\text{labeled rab}- & ^{125}\text{I}-\text{labeled hu}- \\
& \beta\text{-VLDL} & \text{man LDL} \\
\text{100 \% autologous serum} & 0.99 ± 0.07 & 0.90 ± 0.02 \\
0.1 \% human serum albumin & 1.56 ± 0.08\( ^{\text{a}} \) & 1.85 ± 0.19\( ^{b} \) \\
Ethanol alone & 0.59 ± 0.09 & 0.57 ± 0.07 \\
16 \( \mu g/ml \) of cholesterol and 1 \( \mu g/ml \) of 25-OH cholesterol in ethanol & 0.027 ± 0.006\( ^{\text{a}} \) & 0.006 ± 0.003\( ^{b} \)
\end{array} \]

\( ^{a} \) \( p < 0.05 \), significantly different from control.

All subjects (Fig. 6A). Whereas the degradation curve for \( \beta\text{-VLDL} \) for the FH homozygote is not significantly different from the normal curve, LDL degradation by the cells from the FH subject was much lower than in normals and was linearly related to the LDL concentration throughout the entire concentration range. From these results we conclude that the \( \beta\text{-VLDL} \) receptor is genetically distinct from the LDL receptor. Fig. 6B shows that a 25-fold excess of nonradioactive LDL did not significantly suppress \(^{125}\text{I}-\text{LDL} \) degradation by the cells from the FH homozygote. The degradation of \(^{125}\text{I}-\text{LDL} \) in the FH homozygote, on the other hand, was suppressed by a 25-fold excess of nonradioactive \( \beta\text{-VLDL} \). Lipo-proteins Induced by Fat Feeding in Normal Subjects—A recent study by Gianturco et al. (18) showed that VLDL from hypertriglyceridemic patients but not normal VLDL stimulated triglyceride synthesis and accumulation in macrophages. This process occurred primarily via a receptor that recognized \( \beta\text{-VLDL} \). To determine if lipoproteins recognized by the \( \beta\text{-VLDL} \) receptor could be induced in normal subjects, the following experiment was conducted. Two h after a high fat-high cholesterol meal, we isolated lipoproteins of S\(_20\) 20–1000 from a subject’s plasma and tested the abilities of the lipoproteins to compete with rabbit \( \beta\text{-VLDL} \) for \(^{125}\text{I}-\text{labeled
Fig. 6. The degradation of $^{125}$I-labeled rabbit $\beta$-VLDL and $^{125}$I-labeled human LDL by monocyte macrophages from 10 normal subjects and a homozygous FH subject. Monocytes were prepared from each subject and cultured in 0.5 ml of medium B. After 1 h the medium was removed and replaced with 0.5 ml of medium B containing (A) either $^{125}$I-labeled rabbit $\beta$-VLDL (136 cpm/ng of protein) with 50 $\mu$g/ml of nonradioactive human LDL (---) or $^{125}$I-labeled human LDL (213 cpm/ng of protein) (-----) at the protein concentration shown on the abscissa. In B the medium was removed and replaced with 0.5 ml of medium B containing 2 $\mu$g/ml of $^{125}$I-$\beta$-VLDL (136 cpm/ng of protein) (•) or $^{125}$I-LDL (213 cpm/ng of protein) (□) or 2 $\mu$g/ml of $^{125}$I-$\beta$-VLDL in the presence of a 25-fold excess of nonradioactive $\beta$-VLDL (■) or 2 $\mu$g/ml of $^{125}$I-LDL in the presence of a 25-fold excess of nonradioactive LDL (□). After 4 h at 37 °C the medium was removed and the $^{125}$I-labeled acid-soluble content was determined.

Fig. 7. The effects of a 25-fold excess of nonradioactive human $d < 1,006$ g/ml of lipoproteins, human LDL, and rabbit $\beta$-VLDL on the degradation of $^{125}$I-labeled rabbit $\beta$-VLDL in the absence (A) or presence (B) of 50 $\mu$g/ml of nonradioactive human LDL. Normal human monocytes were cultured in 0.5 ml of 30% autologous serum in medium B. After 7 days of culture the medium was removed and the cells were washed 3 times with 1 ml of medium C and incubated with 0.5 ml of medium D containing protein concentrations of 2 $\mu$g of protein/ml of $^{125}$I-labeled rabbit $\beta$-VLDL (153 cpm/ng of protein) alone (□) or together with 50 $\mu$g of protein/ml of the following nonradioactive lipoproteins: ■, chylomicrons Sf 400-5000 taken from a normal subject (fasting values for plasma cholesterol and triglyceride were 196 mg/dl and 96 mg/dl, respectively) 2 h after feeding a liquid meal containing 189 g of fat and 3 g of cholesterol; □, chylomicrons Sf 100-400 from the same subject; □, VLDL Sf 20-100 from the same subject; □, rabbit $\beta$-VLDL; □, human LDL. After 4 h of incubation at 37 °C the medium was removed and the $^{125}$I-labeled acid-soluble content was determined. The values shown are the mean ± 1 S.D. of quadruplicate wells. * $p < 0.05$, significantly different from control.
rabbit β-VLDL degradation (Fig. 7). At equivalent protein concentrations each of the postprandial lipoproteins from the fat-fed subject was as effective as nonradioactive rabbit β-VLDL in suppressing the degradation of [125I]-labeled rabbit β-VLDL by human monocyte macrophages, whereas normal human LDL was not. A separate experiment using two different normal subjects yielded identical results (data not shown). VLDL (d < 1.006 g/ml) from one subject fasted for 14 h was only 15% more effective than human LDL in suppressing [125I]-β-VLDL degradation; in contrast, his d < 1.06 g/ml lipoproteins isolated after fat feeding were as effective as rabbit β-VLDL in suppressing [125I]-β-VLDL degradation (data not shown). These postprandial lipoproteins were triglyceride-rich (Sf 400–5000 = 76.6 mg of triglyceride/mg of protein; Sf 100–400 = 8.32 mg of triglyceride/mg of protein; Sf 20–100 = 4.45 mg of triglyceride/mg of protein).

![Fig. 8. Apolipoprotein composition of rabbit β-VLDL, human LDL, and human thoracic duct lymph chylomicrons (CHYLOS), analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis utilizing 3.5% acrylamide gels. The two major components of apo-B, B-100 and B-48, are labeled. 30 μg of apolipoprotein were applied to each gel.](image)

![Fig. 9. Ability of nonradioactive human thoracic duct lymph chylomicrons (○) and nonradioactive rabbit β-VLDL (△) to inhibit the degradation of [125I]-labeled rabbit β-VLDL by human monocyte macrophages. Normal monocytes were cultured in 30% autologous serum in 0.5 ml of medium B. After 7 days the medium was removed and the cells were washed 3 times with 1 ml of medium C. Then 0.5 ml of medium D was added containing 20 μg of [125I]-labeled rabbit β-VLDL (298.3 cpm/n  

### Table II

| Lipoproteins | [1-14C]Oleate incorporation | Cholesterol/mg protein ratio |
|--------------|----------------------------|-----------------------------|
| Human LDL    | 5.09 ± 0.44                | 2.5                         |
| Sf 20-100    | 2.11 ± 0.01                | 1.7                         |
| Rabbit β-VLDL| 25.18 ± 4.50               | 10.2                        |
| Lymph chylomicrons | 0.22 ± 0.01          | 0.5                         |
| None         | 0.11 ± 0.01                | 0.1                         |

**Ability of Human Thoracic Duct Lymph Chylomicrons to Compete for β-VLDL Receptor**—Kane et al. (19) reported that chylomicrons from human thoracic duct lymph contained a greater proportion of the B-48 form of apo-B than of the B-100 form. We have confirmed these findings (Fig. 8) and have further demonstrated that these lipoproteins competed as effectively as rabbit β-VLDL for the β-VLDL receptor (Fig. 9).

**Stimulation of Cholesteryl Ester Synthesis by Lipoproteins**—Rabbit β-VLDL produced a 5-fold greater stimulation of [1-14C]oleate incorporation into cholesteryl [1-14C]oleate than normal human LDL (Table II). Human thoracic duct lymph chylomicrons, containing very little cholesteryl ester, showed almost no capacity to stimulate cholesteryl ester formation. Also ineffective in this regard were the Sf 20–100 lipoproteins from fat-fed subjects.

**DISCUSSION**

We have shown in the present study that the receptor on human monocyte macrophages recognizing β-VLDL from cholesterol-fed rabbits is similar to the LDL receptor in several ways: 1) the degradation of β-VLDL and LDL is dependent upon Ca2+: 2) the activities of the receptors are highest in the early days of cell culture and then tend to decline with time in culture; 3) LDL and β-VLDL are degraded by high affinity processes; 4) both receptor activities increase in cells incubated in the absence of lipoproteins and can be markedly down regulated by preincubation of the cells with sterol. However, the β-VLDL receptor has a greater capacity to degrade lipoprotein than does the LDL receptor. This was particularly apparent in freshly isolated normal human monocyte macrophages in which the activity of the β-VLDL...
receptor was several-fold higher than the LDL receptor activity. In cells from an FH homozygote, with only 5% of the normal number of LDL receptors, the activity of the β-VLDL receptor was not significantly reduced from that of normal cells. Thus, it is evident, as has been suggested by Goldstein et al. (3), that the receptor recognizing β-VLDL is a different receptor from the LDL receptor and as we have shown in Fig. 6, is genetically distinct.

Particles rich in triglyceride from hypertriglyceridemics are recognized by the β-VLDL receptor (18). We found that 2 h after a high fat-high cholesterol meal the plasma of normal individuals contained particles that effectively competed with rabbit β-VLDL for uptake by the macrophage. These particles were triglyceride-rich and not cholesteryl ester-rich, and similar to β-VLDL (20), they contained apo-E and both the B-100 and B-48 forms of apo-B (19). This suggested that some combination of these apolipoproteins is required for recognition and uptake by the β-VLDL receptor. As reported by Kane et al. (19) human thoracic duct lymph chylomicrons did not contain detectable apo-E and contained primarily the B-48 form of apo-B. Yet, these latter lipoproteins also competed effectively with rabbit β-VLDL for recognition by the β-VLDL receptor. The uptake of these particles appears to depend on their apolipoprotein composition and not their lipid content; the lipid that would accumulate within the macrophage would be a function of the particles' lipid composition. Thus, cholesteryl ester-rich lipoproteins would cause cholesteryl ester accumulation (Table II) whereas triglyceride-rich lipoproteins would cause triglyceride accumulation, as shown by Gian turco et al. (18).

Lusk et al. (21) observed that in cholesterol-fed rhesus monkeys two VLDL were produced; one was cholesteryl ester-rich, had mainly the B-48 form of apo-B, and was intestinal in origin, and the other VLDL resembled triglyceride-rich VLDL and was thought to be heptatically produced. In our study the B-48 apolipoprotein was present in each lipoprotein recognized by the β-VLDL receptor, but apo-E was virtually absent from the human thoracic duct lymph chylomicrons. Since these latter particles contain other minor proteins it would be premature to conclude that the B-48 apolipoprotein itself was responsible for receptor recognition by the β-VLDL receptor. Nevertheless it would seem likely from our studies that apo-E alone is not the major recognition factor for uptake by the β-VLDL receptor and that apolipoproteins of intestinal origin may be of primary importance in this mechanism.

The hallmark of the atheromatous lesion is a cholesteryl ester-laden foam cell (22). Zilversmit has postulated (23) that lipoproteins of dietary origin or their remnants may be prime factors in producing foam cells. It would be important to determine if cholesteryl ester-rich lipoproteins recognized by the β-VLDL receptor exist or can be produced in normal humans. The presence of such particles may well constitute a risk factor for the development of coronary heart disease.

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REFERENCES
1. Mahley, R. W. (1978) in Disturbances in Lipid and Lipoprotein Metabolism (Dietschy, J. M., Goto, A. M., Jr., and Omtko, J. A., eds), pp. 181-197 American Physiological Society, Bethesda, MD
2. Mahley, R. W., Innerarity, T. L., Brown, M. S., Ho, Y. K., and Goldstein, J. L. (1980) J. Lipid Res. 21, 970-980
3. Goldstein, J. L., Ho, Y. K., Brown, M. S., Innerarity, T. L., and Mahley, R. W. (1980) J. Biol. Chem. 255, 1839-1848
4. Brown, M. S., and Goldstein, J. L. (1976) Science 191, 150-154
5. Goldstein, J. L., Ho, Y. K., Basu, S. K., and Brown, M. S. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 335-357
6. Basu, S. K., Brown, M. S., Ho, Y. K. and Goldstein, J. L. (1978) J. Biol. Chem. 254, 7141-7146
7. Fogelman, A. M., Schechter, I., Seager, J., Hokom, M., Child, J. S., and Edwards, P. A. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2214-2218
8. Fogelman, A. M., Seager, J., Hokom, M., and Edwards, P. A. (1979) J. Lipid Res. 20, 379-388
9. Fogelman, A. M., Haberland, M. E., Seager, J., Hokom, M., and Edwards, P. A. (1981) J. Lipid Res. 22, 1131-1141
10. Fogelman, A. M., Edmond, J., Seager, J., and Popjak, G. (1979) J. Biol. Chem. 254, 2045-2055
11. McFarlane, A. S. (1958) Nature (Lond.) 182, 53
12. Bilheimer, D. W., Eisenberg, S., and Levy, R. I. (1972) Biochem. Biophys. Acta 260, 212-221
13. Goldstein, J. L., and Brown, M. S. (1974) J. Biol. Chem. 249, 5153-5162
14. Brown, M. S., Ho, Y. K., and Goldstein, J. L. (1980) J. Biol. Chem. 255, 9344-9352
15. Shapiro, A. L., Viñuela, E., and Maizel, J. V., Jr. (1967) Biochem. Biophys. Res. Commun. 28, 815-820
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
17. Schechter, I., Fogelman, A. M., Haberland, M. E., Seager, J., Hokom, M., and Edwards, P. A. (1981) J. Lipid Res. 22, 63-71
18. Gian turco, S. H., Bradley, W. A., Goto, A. M., Jr., Morrisett, J. D., and Peavy, D. L. (1982) J. Clin. Invest. 70, 168-178
19. Kane, J. P., Hardman, D. A., and Paulus, H. E. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2465-2469
20. Fainaru, M., Mahley, R. W., Hamilton, R. L., and Innerarity, T. L. (1982) J. Lipid Res. 23, 702-714
21. Lusk, L., Chang, J., and Scanz, A. M. (1982) Biochim. Biophys. Acta 710, 134-142
22. Small, D. M. (1977) N. Engl. J. Med. 297, 873-877
23. Zilversmit, D. B. (1979) Circulation 60, 473-485