

The Watanabe Heritable Hyperlipidemic (WHHL) rabbit is a widely studied animal model for the human genetic disorder familial hypercholesterolemia (FH). These rabbits have elevated serum lipids, predominantly as low density lipoproteins (LDL) and intermediate density lipoproteins, and develop spontaneous atherosclerotic disease that mimics the accelerated atherosclerosis seen in FH (for a review, see Reference 1). As in human FH homozygosity, LDL accumulation in the WHHL rabbit arises from a defect of the LDL receptor (also referred to as the apo B,E(LDL) receptor). Cultured fibroblasts,2 adrenal or liver membranes,3 cultured smooth muscle cells,4 and cultured hepatocytes5 from WHHL rabbits display low rates (<10% of normal) of LDL binding and uptake, and the plasma clearance of injected 125I-labeled LDL is impaired in the WHHL rabbit.6,7

In addition to the apolipoprotein (apo) B-mediated binding of LDL, the LDL receptor recognizes lipoproteins containing apo E (for a review, see Reference 8). Thus, a defect in LDL receptors should also inhibit the uptake of apo E-containing lipoproteins. However, WHHL rabbits efficiently catabolize certain apo E-containing lipoproteins, which may indicate the presence of additional LDL receptor-independent pathways of lipoprotein uptake. Because they lack LDL receptor activity, WHHL rabbits have been used in the search for lipoprotein receptors that are genetically distinct from the LDL receptor, particularly those with specificity for chylomicron remnants or β-very low density lipoproteins (β-VLDL). Chylomicron remnants transport dietary lipid to the liver, and their clearance from the plasma is mediated by apo E.9 In contrast to LDL, chylomicrons and chylomicron remnants do not accumulate in the plasma of WHHL rabbits or FH homozygotes,1 and chylomicron remnants injected into WHHL rabbits are taken up by the liver at normal rates.10 These data suggest that hepatic uptake of chylomicron remnants occurs independently of the LDL receptor.

The β-VLDL are cholesterol-enriched chylomicron remnant and very low density lipoprotein (VLDL) remnant lipoproteins that accumulate in the plasma of cholesterol-fed animals or of subjects with type III hyperlipoproteinemia.11 These lipoproteins bind to cellular lipoprotein receptors via
apo E and can induce massive accumulation of cholesterol esters in cultured macrophages. 

Degradation of \( \beta \)-VLDL has been demonstrated in cultured endothelial cells and in alveolar macrophages of WHHL rabbits. A specific \( \beta \)-VLDL receptor was thought to explain both the unique ability of \( \beta \)-VLDL to deliver cholesterol to macrophages and the mechanism of \( \beta \)-VLDL uptake by WHHL rabbit cells. However, recent studies indicate that macrophages from other species do not express unique receptors for \( \beta \)-VLDL. Rather, \( \beta \)-VLDL uptake is mediated exclusively by the LDL receptor in mouse macrophages and normal human monocyte-macrophages and does not occur in macrophages of LDL receptor-negative FH homozygotes. Thus, the mechanism of \( \beta \)-VLDL catabolism by cultured WHHL cells has not been established.

The characteristics of the defective LDL receptor in WHHL rabbits have been elaborated by Schneider et al., Yamamoto et al., and Hobbs et al., work that suggests that the possibility of the uptake of \( \beta \)-VLDL is mediated by the LDL receptor. Pulse-chase experiments have established that the intracellular processing, glycosylation, and transport of the receptor to the cell surface are impeded in the WHHL rabbit. Yamamoto et al. identified the mutation in the LDL receptor of WHHL rabbits: an in-frame deletion in the third cysteine-rich repeat region alters the proposed ligand binding domain of the receptor. The altered protein retains the capacity for binding of \( \beta \)-VLDL on ligand blots. Analogous mutations have been discovered in a subject with FH; in this mutation the sixth repeat of the ligand domain of the LDL receptor is deleted. The altered human receptor produced by this allele lacks LDL (apo B-mediated) binding, but displays normal \( \beta \)-VLDL (apo E-mediated) binding and uptake.

Because these recent observations suggest that WHHL rabbits could display apo E-mediated uptake via the mutant LDL receptor, WHHL rabbits may not be an ideal system for studying LDL receptor-independent uptake of \( \beta \)-VLDL or chylomicron remnants. The goal of the current study was to determine whether the expression of the altered LDL receptor on fibroblasts and smooth muscle cells cultured from WHHL rabbits could contribute to apo E-mediated lipoprotein uptake. Fibroblasts were selected because previous work established that they take up apo E-containing lipoproteins via the LDL receptor, and not by additional receptors specific for apo E-containing lipoproteins. We report that receptor-mediated uptake and metabolism of \( \beta \)-VLDL occur in cultured WHHL fibroblasts and smooth muscle cells and that apo E-mediated uptake by the altered LDL receptor must be considered a potential metabolic pathway for apo E-containing lipoproteins in the WHHL rabbit.

**Methods**

### Materials and Animals

Sodium \( ^{125} \)Iodide and \( ^{14} \)C-oleic acid were purchased from Amersham (Arlington Heights, IL). Dulbecco’s modified Eagle’s medium (DMEM), penicillin, and streptomycin were obtained from GIBCO Laboratories (Grand Island, NY). Fetal bovine serum was purchased from HyClone (Logan, UT) and was heat-inactivated for 30 minutes at 56°C before use. An anti-apo E monoclonal antibody, 1D7, was supplied by Yves Marcel and Ross Milne of the Clinical Research Institute of Montreal. The 25-hydroxycholesterol was obtained from Steraloids, Inc. (Wilton, NH). New Zealand White rabbits were purchased from Hazleton Research Products Inc. (Denver, PA). The WHHL rabbits were raised at The Gladstone Foundation Laboratories from a mating pair supplied by Alan Fogelman, University of California, Los Angeles, California. The guinea pig was obtained from Elm Hill Breeding Labs (Chelmsford, MA). Except in preparation for isolation of hypercholesterolemic lipoproteins, all animals were maintained on commercial animal chow.

**Lipoprotein Preparation**

Human LDL (d=1.02 to 1.05 g/ml) were isolated by sequential ultracentrifugation from the plasma obtained from normal volunteers who had fasted overnight. Canine \( \beta \)-VLDL (d<1.006 g/ml) and apo E high density lipoproteins (apo E HDLc) (d=1.006 to 1.02 g/ml) were obtained from the plasma of hypercholesterolemic dogs fed a semisynthetic diet containing cholesterol and coconut oil and were purified by Pevikon block electrophoresis. Rabbit \( \beta \)-VLDL (d<1.006 g/ml) were isolated from the plasma of hypercholesterolemic rabbits. Protein concentrations were determined by the method of Lowry et al. Lipoproteins were labeled with \( ^{125} \)I by the McFarlane method to specific activities of 100 to 400 cpm/ng of protein. For canine \( \beta \)-VLDL, the distribution of \( ^{125} \)I was 82% on apo B and 18% on apo E and the other apolipoproteins. The incorporation of \( ^{125} \)I is not proportional to the relative mass of each apolipoprotein because apo E is iodinated less efficiently by the McFarlane procedure. Reductive methylation of \( \beta \)-VLDL was performed with formamide and sodium borohydride as described.

The lipoproteins were fluorescently labeled by incorporation of 1,1’-dioctadecyl-3,3’,3’,3’-tetramethylindocarboxyanine perchlorate (DiI) from a dimethylsulfoxide stock solution during incubation at 37°C for 8 to 15 hours and were purified by ultracentrifugation (d=1.063 g/ml, 49 000 rpm, 15 to 20 hours, 4°C) as described. The efficiency of DiI incorporation was assessed by the ability of human fibroblast cultures to accumulate DiI from the labeled lipoproteins as described below.

**Cell Cultures**

Rabbit fibroblast cultures were established from abdominal skin explants and maintained in DMEM with 10% heat-inactivated fetal bovine serum, 100 units of penicillin/ml, and 100 \( \mu \)g of streptomycin/ml. Rabbit fibroblasts were passaged by trypsinization and reseeded at a dilution ratio of 1:6. Rabbit smooth muscle cell cultures were initiated from aortic explants, maintained in DMEM with 10% fetal bovine serum, penicillin, and streptomycin (as above), and passaged at a dilution of 1:4. Two New Zealand White rabbits and two WHHL rabbits were used, and the following cell strains were produced: New Zealand White fibroblasts, NFB1 and N2FB; New Zealand White smooth muscle cells, NSMC and N2SMC; WHHL fibroblasts, WFb and W2Fb; and WHHL smooth muscle cells, WSMC and W2SMC. Experiments were performed on fibroblasts between passages 3 through 12 or on smooth
muscle cells between passages 4 through 10. Human skin fibroblasts were cultured as previously described.\textsuperscript{28} Rabbit skin fibroblasts (RABS) were obtained from the American Type Culture Collection (Rockville, MD) and were used at passages 32 through 35. Rabbit aortic smooth muscle cells (SMC3) were provided by Lisa Minor and George Rothblat (Medical College of Pennsylvania, Philadelphia, PA). Unless otherwise indicated, the fetal bovine serum was replaced with human lipoprotein-deficient serum (LPDS) for 2 days before each experiment to induce the maximal expression of LDL receptors.

**Lipoprotein Binding and Degradation Assays**

Cell-surface binding was determined at 4°C as described.\textsuperscript{26} Lipoprotein degradation was measured as the release of trichloroacetic acid-soluble radioactivity (\(^{125}\)I-tyrosine) after the cells were incubated with \(^{125}\)I-labeled lipoproteins for 6 to 16 hours as described.\textsuperscript{23} All data were the average of duplicate determinations. The amounts of nonspecific binding or degradation, as determined in the presence of a 50- to 100-fold excess of the corresponding unlabeled lipoprotein, were similar for normal or WHHL rabbit cells and were 5% to 25% of the binding (at 4°C) or degradation (at 37°C) for the normal cells. Scatchard analysis\textsuperscript{29} was performed to calculate the apparent \(K_m\) and maximal degradation rates for lipoprotein degradation experiments and to calculate the \(K_m\) and \(B_{max}\) values for equilibrium binding experiments. Values are reported in the Results section as the means \(\pm\) SD, with the number of determinations given in parentheses.

**Cellular Cholesterol Esterification Assays**

Cells were incubated for 16 to 18 hours at 37°C with 0.2 mM \(^{14}\)C-oleate (20 to 40 dpm/pmol) in the presence or absence of lipoproteins, and the incorporation of \(^{14}\)C-oleate into cellular cholesteryl \(^{14}\)C-oleate was determined as described.\textsuperscript{28} All data are the average of duplicate determinations and are presented as the amount of lipoprotein-induced cholesteryl \(^{14}\)C-oleate formation after correction for cholesteryl \(^{14}\)C-oleate synthesis in the absence of added lipoprotein.

**Uptake of Fluorescently Labeled Lipoproteins**

Cultured fibroblasts were incubated for 36 hours in lipoprotein-deficient medium, then for 16 hours at 37°C in lipoprotein-deficient medium supplemented with Dil-labeled lipoproteins. The cell monolayers were washed with DMEM to remove extracellular Dil-labeled lipoproteins, and the fluorescence of intracellular Dil was examined with epifluorescent illumination on a Zeiss Universal microscope by using a rhodamine filter package. The photomicrographs were taken with Kodak TriX panchromatic film, ASA 400.

**Production of Antibody to Rabbit Liver Low Density Lipoprotein Receptor**

Male New Zealand White rabbits were injected with 17 \(\alpha\)-ethinylestradiol (5 mg/kg, s.c.) once a day for 10 days to induce LDL receptor expression.\textsuperscript{30} The livers were collected, and their membranes were prepared by the method of Kovanen et al.\textsuperscript{31} The LDL receptor was purified by DEAE-Sephadex chromatography and immunoaffinity chromatography as described for the bovine adrenal LDL receptor,\textsuperscript{32} with the following modifications. The receptors were solubilized from liver membranes with 3-[\(\beta\)-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS) detergent and were applied to the DEAE-Sephadex column.\textsuperscript{33} The CHAPS was exchanged for octyl glucoside during the DEAE-Sephadex chromatography, and the receptors were eluted in octyl glucoside.\textsuperscript{34} The immunoaffinity column was constructed with the monoclonal antibody produced by the 9D9 hybridoma cell line (American Type Culture Collection), which recognizes rabbit LDL receptors.\textsuperscript{35} Final purification of the receptor was achieved by electofugation from nonreduced polyacrylamide gels by the method of Hunkapiller et al.\textsuperscript{36}

A guinea pig was injected intradermally with approximately 5 \(\mu\)g of the purified LDL receptor protein obtained from rabbit liver and was suspended in Freund's complete adjuvant. Additional injections of approximately 2 \(\mu\)g of the protein in Freund's incomplete adjuvant were given at 4 and 6 weeks. At 8 weeks, after a total of three injections, the immune serum was collected by exsanguination. Pooled guinea pig sera were used as the nonimmune control. Immunoglobulin G (IgG) was purified from the sera by Protein A-Sepharose chromatography\textsuperscript{36} and was dialyzed with phosphate-buffered saline.

**Results**

**Characterization of Low Density Lipoprotein Degradation by Cultured Rabbit Fibroblasts**

Cultures of fibroblasts were initiated from abdominal skin explants of New Zealand White or WHHL rabbits, and the degradation of human \(^{125}\)I-labeled LDL by the fibroblast cultures was measured (Figure 1). The degradation of \(^{125}\)I-labeled LDL by New Zealand White fibroblasts displayed high affinity and saturability. The apparent \(K_m\) for LDL uptake by these cells was 35 \(\mu\)g of protein/ml, and the maximal rate of degradation was 3.8 \(\mu\)g of protein degraded/mg of cell protein/16 hours. These rates are consistent with previous reports for cultured rabbit cells (0.8 to 3 \(\mu\)g degraded/mg of cell protein/4 hours).\textsuperscript{2,4,18} In contrast, the rate of degradation of \(^{125}\)I-labeled LDL by fibroblasts from the WHHL rabbit was less than 5% of the corresponding rate in normal rabbit fibroblasts (Figure 1). These values are consistent with previously published results\textsuperscript{2,3} and verify that the cells used for these studies were homozygous for the allele for the defective LDL receptor in WHHL rabbits.

**Metabolism of \(\beta\)-Very Low Density Lipoproteins by Rabbit Fibroblasts**

The degradation of canine \(^{125}\)I-labeled \(\beta\)-VLDL by cultured rabbit fibroblasts is shown in Figure 2. Saturable, high-affinity degradation was observed for both normal and WHHL fibroblasts. The apparent \(K_m\) for \(\beta\)-VLDL uptake in normal rabbit fibroblasts was 15 \(\pm\) 7 \(\mu\)g of protein/ml (\(n=8\)), and the maximal rate of degradation was 0.86 \(\pm\) 0.11 \(\mu\)g of protein/mg of cell protein/6 hours (\(n=5\)). The WHHL fibroblasts also degraded canine \(\beta\)-VLDL with an apparent \(K_m\) of 29 \(\pm\) 18 \(\mu\)g of protein/ml (\(n=11\),
Degradation of 125I-labeled low density lipoproteins (LDL) by fibroblasts from New Zealand White (NZW) (●) or Watanabe Heritable Hyperlipidemic (WHHL) (○) rabbits. Rabbit fibroblasts, cultured in 22-mm dishes, were incubated with 0.5 ml of Dulbecco's modified Eagle's medium containing 10% human lipoprotein-deficient serum (LPDS) and the indicated concentrations of 125I-labeled LDL for 16 hours at 37°C. Lipoprotein degradation was measured as described and corrected for nonspecific degradation in the presence of a 50-fold excess of unlabeled LDL. The experiment shown is representative of six similar experiments.

Figure 2. Degradation of 125I-labeled β-very low density lipoproteins (β-VLDL) by fibroblasts from New Zealand White (●) or Watanabe Heritable Hyperlipidemic (WHHL) (○) rabbits. Rabbit fibroblasts were incubated with the indicated concentrations of 125I-labeled β-VLDL. Lipoprotein degradation was measured as in Figure 1 and was corrected for nonspecific degradation. Each point is the average ±SEM of five experiments. The values for each point differed by less than 10%.

The apolipoprotein specificity of β-VLDL uptake was determined with antibodies to apo E or apo B that inhibit recognition by LDL receptors. Pre-incubation of 125I-labeled β-VLDL with the IgG fraction of antibody 1D7, a monoclonal antibody to apo E that inhibits apo E binding to receptors, inhibited β-VLDL degradation in normal and WHHL fibroblasts (Figure 4). In contrast, an apo B-specific monoclonal antibody that inhibits LDL binding had no effect on β-VLDL degradation by rabbit cells (data not shown). These results demonstrate that WHHL rabbit fibroblasts possess a capacity for degradation of β-VLDL despite the absence of LDL degradation, and that the degradation of β-VLDL is mediated by apo E and not by apo B-100.

Lipoprotein Binding to Cell-Surface Receptors
Surface binding sites for 125I-labeled LDL and 125I-labeled β-VLDL were measured directly at 4°C. The binding of
Figure 4. Ability of the apolipoprotein E monoclonal antibody, 1D7, to inhibit the degradation of ¹²⁵I-labeled β-very low density lipoproteins (β-VLDL) in fibroblasts from Watanabe Heritable Hyperlipidemic rabbits. The indicated concentrations of immunoglobulin G (IgG) were preincubated for 14 hours with Dulbecco's modified Eagle's medium containing 10% lipoprotein-deficient serum and 10 μg of ¹²⁵I-labeled β-VLDL/ml. The medium was incubated with the cells for 10 hours at 37°C. The lipoprotein degradation was measured as described and was corrected for nonspecific degradation in the presence of a 30-fold excess of unlabeled β-VLDL.

human ¹²⁵I-labeled LDL to rabbit fibroblasts is shown in Figure 5A. Scatchard analysis of these binding data indicated that, in New Zealand White rabbit fibroblasts, the K_d for LDL was 5.3±2.2 μg of protein/ml and the maximal binding of LDL was 85±15 ng of protein/mg of cell protein (n=4). The LDL bound to normal rabbit fibroblasts with a lower affinity than that typically observed for human LDL binding to human fibroblasts (K_d ~2 μg of protein/ml), but the affinity was similar to or greater than that typically observed for human LDL binding to other, nonhuman cell types, i.e., mouse peritoneal macrophages, rat fibroblasts, mouse fibroblasts, J774.2 cells, and Fu5AH cells. The maximal binding of LDL to the New Zealand White rabbit fibroblasts indicates that these cells have a relatively low number of receptors. For comparison, mouse peritoneal macrophages bound similar amounts of LDL per milligram of cell protein. Human fibroblasts bound approximately two- to threefold greater amounts of LDL per milligram of cell protein than normal rabbit fibroblasts. The surface binding of LDL to WHHL fibroblasts was less than 8% of control and was too low for accurate Scatchard analysis (Figure 5A).

Binding of ¹²⁵I-labeled β-VLDL was of high affinity and was saturable for both normal and WHHL fibroblasts (Figure 5B). The apparent K_d values were similar for New Zealand White and WHHL fibroblasts [0.6±0.3 (n=4) vs. 0.7±0.5 μg of protein/ml, (n=7)] and typical of β-VLDL binding to the LDL receptor. The capacity of β-VLDL binding to normal rabbit fibroblasts (72±12 ng of protein/mg of cell protein, n=4) is consistent with the extent of expression of the LDL receptor as determined by LDL binding. At receptor saturation (B_max), β-VLDL binding to WHHL fibroblasts was only about 9% that of binding to normal rabbit cells.

**Lipoprotein-Induced Cholesteryl Oleate Synthesis**

The incorporation of ¹⁴C-oleate was measured to confirm that the β-VLDL degradation by WHHL fibroblasts was the result of receptor-mediated uptake of β-VLDL. In normal rabbit fibroblasts, both LDL and β-VLDL induced cholesterol esterification (Figure 6). At low concentrations of lipoprotein, β-VLDL induced greater esterification than LDL because of the higher affinity of β-VLDL for the LDL receptor and the greater content of cholesterol per β-VLDL particle. LDL induced little or no cholesteryl ester formation in the WHHL fibroblasts (Figure 6), in agreement with the rates of degradation of LDL by normal or WHHL cells (Figure 2). However, β-VLDL uptake stimulated cholesterol ¹⁴C-oleate synthesis in the WHHL fibroblasts (Figure 6). The relative rates of β-VLDL–induced cholesteryl ester synthesis (23% to 69%) paralleled the relative rates of β-VLDL degradation in normal and WHHL fibroblasts (Figure 2). These results demonstrate that β-VLDL degradation by WHHL fibroblasts is accompanied by cholesterol delivery and cholesteryl ester formation.

A comparison of lipoprotein-induced cholesteryl ester synthesis by smooth muscle cells is shown in Figure 7. There was no significant degradation of LDL by WHHL smooth muscle cells and, correspondingly, no stimulation of cholesterol esterification. In contrast, β-VLDL induced ¹⁴C-oleate incorporation into cholesteryl esters of the WHHL smooth muscle cells (Figure 7).
Comparison of Cholesteryl Ester Synthesis in WHHL and Control Cells

The rates of lipoprotein-induced cholesteryl 
$^{14}$C-oleate formation for LDL and $\beta$-VLDL were compared in a number of rabbit cell strains: fibroblasts and aortic smooth muscle cells derived from New Zealand White and WHHL rabbits, a rabbit skin fibroblast culture obtained from the American Type Culture Collection (RAB9), and a normal rabbit aortic smooth muscle cell culture obtained from Lisa Minor and George Rothblat (SMC3). The average results from three experiments with several cell strains are summarized in Figure 8. In each case, the cells from WHHL rabbits displayed little (5% to 15% of normal), if any, LDL-induced cholesteryl ester formation. However, $\beta$-VLDL consistently induced much more cholesteryl esterification in cells from WHHL rabbits (23% to 69% of normal). For comparison, human fibroblasts were examined in parallel experiments. The average rates of cholesteryl $^{14}$C-oleate synthesis for human fibroblasts were 79 pmol/mg of cell protein in the presence of LDL and 97 pmol/mg of cell protein in the presence of $\beta$-VLDL. Thus, all rabbit cells expressed substantially lower receptor activity than human fibroblasts, with RAB9 cells expressing the greatest receptor activity among the rabbit cells tested.

Uptake of Dil-labeled Lipoproteins

The fluorescent lipid-soluble marker, Dil, is readily incorporated into lipoproteins. Upon uptake and degradation of the Dil-labeled lipoprotein, the Dil moiety is released and accumulates intracellularly without further metabolism. When fibroblasts from a New Zealand White rabbit were incubated with either Dil-labeled LDL or Dil-labeled $\beta$-VLDL, accumulation of the fluorescent label was observed (Figures 9A and 9B). No accumulation of Dil was detected in WHHL cells incubated with Dil-labeled LDL (Figure 9C), consistent with the lack of LDL uptake. However, there was substantial uptake of Dil-labeled $\beta$-VLDL in the WHHL fibroblasts (Figure 9D). These studies confirm that $\beta$-VLDL uptake, as measured by accumulation of the fluorescent adduct Dil, was present in cells of WHHL rabbits.

Down-regulation of $\beta$-Very Low Density Lipoprotein Uptake in Rabbit Fibroblasts

A characteristic property of LDL receptors is sensitivity of receptor expression to cellular cholesterol content. Sterol-
induced down-regulation of β-VLDL degradation was compared in fibroblasts from New Zealand White and WHHL rabbits (Figure 10A). Pre-incubation with cholesterol (15 μg/ml) and 25-hydroxycholesterol (1 μg/ml) inhibited the degradation of 125I-labeled β-VLDL by normal rabbit fibroblasts. This result is in agreement with previous observations that the number of LDL receptors on rabbit fibroblasts, as measured by monoclonal antibody binding, was down-regulated by sterols.34 Van Lenten et al.18 have shown that β-VLDL uptake by WHHL alveolar macrophages is also inhibited by sterols. Similarly, sterol pre-incubation resulted in down-regulation of β-VLDL degradation by WHHL fibroblasts (Figure 10B), which is consistent with β-VLDL uptake through the mutant LDL receptor of WHHL cells.

Inhibition of β-Very Low Density Lipoprotein Degradation by Antibody to Low Density Lipoprotein Receptor

A polyclonal antiserum to purified rabbit liver LDL receptor was produced in a guinea pig. The immune serum specifically reacted with rabbit liver LDL receptors on immunoblots (Figure 11, inset), and IgG purified from this antiserum inhibited 125I-labeled LDL binding to cultured human fibroblasts (data not shown). The anti-LDL receptor IgG prevented the degradation of β-VLDL in New Zealand White rabbit fibroblasts (Figure 11A). Similarly, the anti-LDL receptor IgG blocked β-VLDL degradation in WHHL fibroblasts (Figure 11B), indicating that the receptor-dependent uptake of β-VLDL in WHHL cells is mediated by the LDL receptor.

Discussion

The WHHL rabbit has been very useful for studying lipoprotein metabolism and atherosclerosis. The virtual absence of receptor-mediated LDL metabolism in this animal, as assessed by turnover studies6,7 and in various types of cell cultures,2-5 results in elevated serum cholesterol concentrations and eventual atherosclerosis. The genetic mutation responsible for this defect is located in one of the proposed ligand binding domains of the LDL receptor.21 The mutation causes delayed intracellular processing of the receptor, as indicated by pulse-chase studies in cultured fibroblasts20 and by the accumulation of the partially processed precursor form of the LDL receptor in adrenal glands of WHHL rabbits.34 Direct studies of binding to WHHL fibroblasts with a monoclonal antibody that cross-reacts with the rabbit LDL receptor...
also suggested that few receptors were expressed on the cell surface. Because they lack LDL receptors, WHHL rabbits have been used as a model in the search for additional receptors with specificity for apo E-containing lipoproteins.

Initial studies on mouse peritoneal macrophages suggested that a unique receptor might mediate the uptake of β-VLDL, resulting in massive lipid accumulation and a morphology similar to that of foam cells in atherosclerotic lesions. This proposal was supported by studies on monocytes from a subject with FH and on endothelial cells or alveolar macrophages from WHHL rabbits. However, later studies showed that the uptake of β-VLDL by mouse peritoneal macrophages is mediated by the LDL receptor on these cells, not by a unique β-VLDL receptor. Studies on human monocyte-derived macrophages have extended this conclusion: the uptake of β-VLDL in human monocyte-macrophages is mediated exclusively by the LDL receptor, and there was no β-VLDL metabolism by macrophage-monocytes from seven FH subjects. Since no evidence of a unique β-VLDL receptor has been established in other species, the demonstration of β-VLDL degradation in WHHL rabbit cells required further investigation.

The identification of the deletion mutation in the LDL receptor gene in WHHL rabbits and its similarity to a deletion mutation in the LDL receptor gene from an FH subject offered a possible alternative explanation for the uptake of β-VLDL by WHHL cells: expression of an LDL receptor that lacks LDL binding capacity but retains the ability to bind β-VLDL. Our data support this hypothesis. Fibroblasts from WHHL rabbits display a receptor-mediated catabolism of β-VLDL and of other apo E-containing lipoproteins that has properties identical to the metabolism promoted by the LDL receptor: affinity for β-VLDL, sensitivity to apo E modification or an apo E antibody, down-regulation in response to sterols, and inhibition by a specific antibody to the rabbit LDL receptor. Previous studies with human cells established that β-VLDL uptake in fibroblasts is mediated exclusively by the LDL receptor. The binding or degradation of β-VLDL is inhibited by LDL receptor antibody, decreases upon down-regulation of the LDL receptor, and is absent in fibroblasts from an FH subject in whom there was no detectable LDL receptor mRNA.

The results of β-VLDL catabolism by WHHL fibroblasts differ from those observed for WHHL endothelial cells only in that the fibroblasts demonstrated approximately 50% of normal β-VLDL degradation, whereas the endothelial cells degraded β-VLDL at nearly normal rates. The use of different types of β-VLDL may have contributed to this difference. Nevertheless, the demonstration of β-VLDL metabolism by WHHL fibroblasts indicates that this process is not unique to certain specialized cells and suggests that the uptake of β-VLDL previously observed for WHHL endothelial cells and macrophages was mediated by the mutant LDL receptor rather than a genetically distinct β-VLDL receptor.

The extent of β-VLDL degradation, as well as of β-VLDL-induced cholesterol esterification, in WHHL cells does not appear to correlate with the expression of mature LDL receptors on the cell surface. Pulse-chase studies indicate no complete maturation of the WHHL LDL receptor, and little receptor protein is detected on WHHL fibroblasts.

Figure 10. Sterol-induced down-regulation of 125I-labeled β-very low density lipoprotein (β-VLDL) degradation in fibroblasts from New Zealand White (NZW) (A) or Watanabe Heritable Hyperlipidemic (WHHL) (B) rabbits. Fibroblasts were cultured in the presence of lipoprotein-deficient serum (LPDS) or in LPDS with cholesterol (15 μg/ml) and 25-hydroxycholesterole (1 μg/ml) as described in Methods. Degradation was then assayed after incubation of the fibroblasts with the indicated concentrations of 125I-labeled β-VLDL for 6 hours at 37°C and was corrected for nonspecific degradation.

Figure 11. Inhibition of 125I-labeled β-very low density lipoprotein (β-VLDL) degradation in fibroblasts from New Zealand White (NZW) (A) or Watanabe Heritable Hyperlipidemic (WHHL) (B) rabbits by low density lipoprotein (LDL) receptor antibodies. Rabbit fibroblasts were incubated for 6 hours at 37°C with the indicated concentrations of immunoglobulin G (IgG) purified from nonimmune or anti-LDL receptor serum.
lower than the corresponding rates for β-VLDL degradation or cholesteryl ester formation would suggest (see Figures 2 and 5). The explanation for these differences is unknown; one possibility is that some aspect of the intracellular process, such as receptor recycling, is more efficient in WHHL than in normal cells. In addition, these studies demonstrate that even though most of the newly synthesized WHHL LDL receptors are not fully processed, a number of them reach the cell surface and become functional, at least in regard to the uptake of β-VLDL.

Recent studies using the LDL receptor-deficient strain of Chinese hamster ovary cells demonstrate that precursor forms of the LDL receptor can be expressed on the cell surface. In these cells, which are defective in protein glycosylation, a partially glycosylated precursor of the LDL receptor is transported to the cell surface but is very susceptible to degradation there.44

The possibility of LDL receptor-mediated uptake of apo E-containing lipoproteins in the WHHL rabbit requires a reconsideration of whether this animal is the ideal model for studying LDL receptor-independent metabolism of β-VLDL and clearance of chylomicron remnants. Our study indicates that the altered LDL receptor participates in receptor-mediated uptake of apo E-containing lipoproteins in cultured cells from WHHL rabbits. Fibroblasts from WHHL rabbits display normal affinity but reduced capacity for β-VLDL. The capacity of the LDL receptor in WHHL rabbits apparently is not sufficient for normal clearance of endogenous apo E-containing lipoproteins in vivo since the turnover of VLDL is substantially delayed,45 and intermediate density lipoproteins accumulate in plasma. The contribution of LDL receptor-dependent metabolism of apo E-containing lipoproteins in vivo will depend on the number of receptors expressed in the WHHL rabbit, and this contribution is difficult to estimate. Nevertheless, this study demonstrates that the altered LDL receptor can catabolize β-VLDL and thus cannot be excluded as a possible route of apo E-mediated lipoprotein metabolism.

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References


21. Yamamoto T, Bishop RW, Brown MS, Goldstein JL, Russell DW. Deletions in cysteine-rich region of LDL receptor

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29. Scatchard G. The attractions of proteins for small molecules and ions. Ann NY Acad Sci 1949;51:660–672
39. Pitas RE, Innerarity TL, Arnold KS, Mahley RW. Rate and equilibrium constants for binding of apo-E HDL (a cholesterol-induced lipoprotein) and low density lipoproteins to human fibroblasts: evidence for multiple receptor binding of apo-E HDL. Proc Natl Acad Sci USA 1979;76:2311–2315
44. Kozarsky K, Kingsley D, Krilger M. Use of a mutant cell line to study the kinetics and function of O-linked glycosylation of low density lipoprotein receptors. Proc Natl Acad Sci USA 1988;85:4335–4339
45. Kitta T, Brown MS, Bilhelmer DW, Goldstein JL. Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoprotein in WHHL rabbits. Proc Natl Acad Sci USA 1982; 79:5693–5697

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