The role of ApoCI, LPL and CETP in plasma lipoprotein metabolism - studies in mice

Hoogt, C.C. van der

4 The Hepatic Clearance of VLDL in the Absence of the Three Major ApoE-Recognizing Receptors is Dependent on LPL

Caroline C. van der Hoogt[1,2], Sonia M.S. Espirito Santo[1,2], Kyriakos E. Kypreos[5], Bart J.M. van Vlijmen[1,2], Louis M. Havekes[1,2,3], Ko Willems van Dijk[2,4], Patrick C.N. Rensen[1,2]

[1]The Netherlands Organization for Applied Scientific Research-Quality of Life, Gaubius Laboratory, P.O. Box 2215, 2301 CE Leiden, The Netherlands; Departments of [2]General Internal Medicine, Endocrinology, and Metabolic Diseases, [3]Cardiology, and [4]Human Genetics, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands; [5]Boston University School of Medicine, Boston, MA, USA

Submitted

Objective - Lipoprotein lipase (LPL) activity plays an important role in preceding the remnant clearance via the three major apoE-recognizing receptors, the LDL receptor (LDLr), LDLr related protein (LRP), and VLDL receptor (VLDLr). We recently showed that mice deficient for these three receptors do have elevated fasted plasma total cholesterol (TC) and triglyceride (TG) levels, mainly present as VLDL. However, since VLDL is continuously produced by the liver, their remnants must thus still be cleared to attain steady state lipid levels in plasma. Therefore, the aim of this study was to determine whether LPL is important in the VLDL clearance irrespective of these receptors.

Methods and Results - Administration of an adenovirus expressing LPL (Ad*LPL*) into *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice, reduced both plasma TG (6.8-fold) and TC (1.2-fold), mainly in VLDL. Conversely, administration of an adenovirus expressing apoCI (Ad*APOC1*), leading to inhibition of LPL, resulted in increased plasma TG (5.0-fold) and TC (1.6-fold), also specific for VLDL. Metabolic studies with glycerol tri[^{3}H]oleate and [^{14}C]cholesteryl oleate-double-labeled VLDL-like emulsion particles showed an 7.0-fold increased hepatic association of remnants after administration of Ad*LPL*, whereas the association was completely abolished after administration of Ad*APOC1*. *In vitro* studies demonstrated that VLDL-like emulsion particles avidly bound to the cell-surface of primary hepatocytes that lack the LDLr and LRP, followed by internalization. This illustrates that uptake of emulsion particles occurs even in the absence of these receptors.

Conclusion - Collectively, we conclude that the hepatic remnant clearance in absence of the three major apoE-recognizing receptors depends on LPL.

Lipoprotein lipase (LPL) is the key enzyme responsible for hydrolysis of triglycerides (TG) in TG-rich lipoproteins such as chylomicrons and VLDL.[1,2] During lipolysis, the lipoproteins are reduced in size and enriched with apoE. Subsequently, their core remnants are taken up mainly by the liver via apoE-recognizing receptors, *i.e.* the LDL receptor (LDLr) and the LDLr related protein (LRP).[2] Therefore, mice deficient for the LDLr and hepatic LRP show marked accumulation of TG-rich lipoprotein remnants.[3] Although core remnants may be directly internalized via the LDLr, the binding and internalization via the LRP is thought to involve previous binding of core remnants to heparan sulfate proteoglycans (HSPG) in the space of Disse via heparin-binding proteins such as apoE.[4,5] In addition, it has been reported that apoE-enriched TG-rich particles can be directly taken up via HSPG, either alone or in combination with LRP, both *in vitro*[6] and *in vivo*.[7]

The third major apoE-recognizing receptor, the VLDL receptor (VLDLr), is expressed abundantly in tissues active in fatty acid metabolism (*i.e.* heart, skeletal muscle and white adipose tissue), and functions as a peripheral lipoprotein remnant receptor. As for the LDLr and LRP, the VLDLr binds TG-rich lipoproteins via apoE and this binding is modulated by LPL.[8,9] VLDLr-deficient mice have normal plasma lipoprotein levels when fed a chow diet.[10] However, when TG metabolism was stressed by feeding a high fat diet or by cross-breeding on an *ob/ob* or *ldlr*$^{-/-}$ background, the VLDLr deficiency did result in moderate accumulation of plasma TG-rich lipoproteins.[11,12] Recently, we demonstrated that the VLDLr plays a major role in postprandial lipoprotein metabolism by facilitating LPL-mediated TG hydrolysis.[13] Therefore, additional deletion of the VLDLr from LDLr- and hepatic LRP-double deficient mice aggravates their phenotype upon stressing TG metabolism, either by high-fat feeding or by giving an intragastric olive oil bolus.[14] On a chow diet, the steady state fasted plasma TG and total cholesterol (TC) levels are 8-9-fold increased in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice as compared to wild-type mice.[14] However, because these mice have continuous lipid input into their circulation via the production of VLDL (4.5±1.2 mM TG/h), which is similar to wild-type mice (3.8±0.7 mM TG/h), their remnants must thus still be cleared from plasma to attain steady state lipid levels. This indicates that, although less efficient than via the classical receptors, additional pathways can indeed play a role in lipoprotein remnant clearance *in vivo*.

Sehayek *et al.*[15] showed that the lipolytic activity of LPL (*i.e.* hydrolysis of TG within the lipoprotein core) is a requisite for apoE-dependent uptake of lipoprotein remnants via the LDLr and possibly the LRP *in vitro*. In addition LPL is involved in remnant uptake via its bridging function.[8,9] However, it remains unclear whether modulation of LPL also affects the catabolism of TG-rich particles in the absence of the three major apoE-recognizing receptors *in vivo*. Therefore, the aim of this study was to determine the role of LPL in hepatic VLDL metabolism in mice that lack the LDLr, hepatic LRP and the VLDLr. Hereto, we either increased LPL by adenovirus-mediated overexpression[16] or decreased LPL by adenovirus-mediated expression of the LPL-inhibitor apoCI.[17] From these studies, we conclude that the receptor-independent hepatic clearance of VLDL *in vivo* depends on LPL.

Materials and Methods

Transgenic Animals
Male MX1Cre^{+}*lrp*$^{lox/lox}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice,[14] 4-6 months of age were used in experiments. Mice were obtained from our breeding colony at the Institutional Animal Facility and housed under standard conditions in conventional cages and were fed regular chow *ad libitum*. LRP deficiency was induced by intraperitoneal injection of polyinosinic: polycytidylic ribonucleic acid (pI:pC, Sigma, St Louis, MO, USA), which results in the complete absence of LRP protein in liver membrane extracts.[14] The mice will be further referred to as *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$. Experiments were performed after 4 h of fasting at 12:00 pm with food withdrawn at 8:00 am, unless indicated otherwise. The Institutional Ethical Committee on Animal Care and Experimentation has approved all experiments.

Adenoviruses and Administration to Mice
The generation of an adenovirus expressing human apoCI (Ad*APOC1*) has recently been described in full detail elsewhere.[17] An adenovirus expressing human LPL (Ad*LPL*)[16] was a kind gift of Dr. Silvia Santamarina-Fojo. An adenovirus expressing ß-galactosidase (Ad*LacZ*) was used as control. Viruses were grown and purified by standard procedures and typical titers of $1x10^{10}$ – $1x10^{11}$ pfu/ml were obtained. Virus was stored in aliquots at –80°C until use. Basal serum lipid levels were measured 4 weeks after pI:pC injection, at least 3 days before adenovirus injection into mice. At day 0, mice were injected into the tail vein with either Ad*APOC1*, Ad*LPL* or Ad*LacZ* ($1x10^{9}$ pfu/mouse), diluted with PBS to a total volume of 200 µl. To prevent sequestration of low doses of virus by Kupffer cells, mice were pre-injected with $0.5x10^{9}$ pfu Ad*LacZ* at 3 h before injection of the adenoviruses of interest.[18]

Plasma Lipid and Lipoprotein Analysis
In all experiments blood was collected from the tail vein into chilled paraoxon (Sigma, St. Louis, MO, U.S.A.)-coated capillary tubes to prevent ongoing *in vitro* lipolysis.[19] The tubes were placed on ice, centrifuged at 4°C, and the obtained plasma was assayed for TC and TG, using the commercially available enzymatic kits 236691 and 11488872 (Roche Molecular Biochemicals, Indianapolis, IN, USA), respectively. For determination of the distribution of lipids over plasma lipoproteins by fast-performance liquid chromatography (FPLC), 50 µl of pooled plasma per group was injected onto a Superose 6 column (Äkta System; Amersham Pharmacia Biotech, Piscataway, NJ, USA), and eluted at a flow rate of 50 µl/min with PBS, 1 mM EDTA, pH 7.4. Fractions of 50 µl were collected and assayed for TC and TG as described above.

Post-Heparin Plasma LPL Levels
Blood was collected 10 min after tail vein injection of heparin dissolved in PBS (0.1 U/g body weight; Leo Pharmaceutical Products B.V., Weesp, The Netherlands). Plasma was isolated, snap-frozen and stored at –80°C until analysis for total LPL activity as previously described.[20]

Preparation of VLDL-Like Emulsion Particles
TG-rich VLDL-like emulsion particles (80 nm) were prepared as described.[21] Radiolabeled emulsions were obtained by adding 200 μCi of glycerol tri[^{3}H]oleate (triolein, TO) and 20 μCi of [^{14}C]cholesteryl oleate (CO) to 100 mg of emulsion lipids before sonication. Alternatively, fluorescently labeled emulsions were prepared by adding 500 μg of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanide perchlorate (DiI) (Molecular Probes, Leiden, The Netherlands) to 100 mg of emulsion lipids before sonication.

In vivo *Clearance of VLDL-Like Emulsion Particles*
Five days after virus administration, mice were injected with the radiolabeled emulsion particles (1.0 mg TG) at 8:00 am into the vena cava inferior. At indicated timepoints after injection, blood was taken from the vena cava inferior, to determine the serum decay of [^{3}H]TO and [^{14}C]CO by scintillation counting (Packard Instruments, Dowers Grove, IL, USA). At 30 minutes after injection, mice were sacrificed and tissues were collected. Tissues were weighed and dissolved over night in Solvable (Packard Bioscience, Meriden, CT, U.S.A.), whereafter ^{3}H- and ^{14}C-activities were determined in Ultima Gold (Packard Bioscience). The total plasma volumes of the mice were calculated from the equation V (ml) = 0.04706 x body weight (g), as determined from ^{125}I-BSA clearance studies as previously described.[22]

In vitro *Uptake of VLDL-Like Emulsion Particles by Mouse Hepatocytes*
Mouse hepatocytes were isolated from anesthetized wild-type or *lrp*$^{-}$*ldlr*$^{-/-}$ mice and subjected to Percoll® gradient centrifugation to discard nonviable cells.[23] The cells (viability >99% as judged from 0.2% trypan blue exclusion) were attached to collagen S-coated (3.87 μg/cm^{2}) 2.5 cm glass cover slips in 9.6 cm^{2} 6-well dishes (0.8 × 10^{6} cells/well) by culturing in DMEM+10% fetal calf serum (3-4 h at 37 °C). The cover slips were washed to remove unbound cells and incubated (2 h at 4°C) with DiI-labeled VLDL-like emulsion particles (100 μg TG/ml). The cover slips were washed twice with DMEM+2% BSA to remove unbound particles, and transferred to a Zeiss IM-35 inverted microscope (Oberkochen, Germany) with a Zeiss plan apochromatic 63×/1.4 NA oil objective and fitted with a temperature-controlled incubation chamber, which was equipped with a Bio-Rad 600 MRC confocal laser scanning microscopy system. The cells were further incubated (30 min at 37 °C) in DMEM+2% BSA, after which the (intra)cellular localization of DiI was visualized (λ_{ex} 543 nm).

Statistical Analysis
All data are presented as means ± SD. Data were analyzed using the Mann-Whitney U-test unless indicated otherwise. *P*-values less than 0.05 were regarded as statistically significant.

Results

LPL Modulates VLDL-Cholesterol Levels in lrp-ldlr-/-vldlr-/- Mice

To study the impact of LPL on the clearance of cholesterol in the absence of the apoE-recognizing receptors, we used mice deficient for the LDLr, hepatic LRP, and VLDLr, as previously described.[14] Upon deletion of hepatic LRP from MX1Cre+*lrp*$^{lox/lox}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice, their plasma lipid levels were determined and the mice were assigned to three groups, matched for TC and TG plasma levels (Table 1). The mice received Ad*LPL* to increase LPL levels,[16] Ad*APOC1* to inhibit LPL,[20] or Ad*LacZ* as a control group. The effect of these interventions on plasma lipid levels was assessed at 5 days after injection.

Ad*LPL* administration resulted in a 3.0-fold increase in post-heparin LPL plasma activity (33.7±5.4 vs. 11.4±1.6 μmol FFA generated/h/ml; $P<0.05$), with a concomitant 6.8-fold reduction in plasma TG (1.1±0.1 vs. 7.5±0.9 mM; $P<0.01$) (Table 1). Additionally, Ad*LPL* decreased plasma TC 1.2-fold (15.7±3.9 vs. 19.0±1.9 mM; $P<0.05$) (Table 1).

Table 1. Effect of adenovirus administration on plasma lipid levels and post-heparin LPL plasma activity in *lrp-ldlr-/-vldlr-/-* mice

	LPL activity (μmol FFA generated/h/ml)	TG (mM)	TC (mM)
Before adenovirus			
Ad*LPL*	n.d.	3.9±0.7	19.5±3.0
Ad*LacZ*	n.d.	4.0±0.8	19.4±3.3
Ad*APOC1*	n.d.	3.8±0.6	19.5±8.1
After adenovirus			
Ad*LPL*	33.7±5.4*	1.1±0.1**	15.7±3.9*
Ad*LacZ*	11.4±1.6	7.5±0.9	19.0±1.9
Ad*APOC1*	11.5±7.6	37.6±10.1**	30.1±6.7**

Plasma was obtained from fasted *lrp-ldlr-/-vldlr-/-* mice before and after administration of Ad*LacZ*, Ad*LPL* or Ad*APOC1*, and assayed for triglycerides (TG) and total cholesterol (TC). After the second blood withdrawal, heparin was injected and post-heparin plasma was obtained, and assayed for lipoprotein lipase (LPL) activity. Values are expressed as means ± S.D. n.d., not determined. Statistical differences were assessed with respect to Ad*LacZ*. *$P<0.05$. **$P<0.01$.

From FPLC fractionation of pooled plasma it was apparent that the decrease in plasma TG and TC was confined to the VLDL fractions (Fig. 1).

Ad*APOC1* administration did not result in altered post-heparin LPL plasma activity (11.5±7.6 vs. 11.4±1.6 μmol FFA/h/ml) (Table 1). This is in accordance with previous findings that human *APOC1* transgenic mice do not show a change in plasma total LPL activity as compared to wild-type mice,[20] rather apoCI acts by modulating local LPL activity. Administration of Ad*APOC1* resulted in 5.0-fold increased plasma TG levels (37.6±10.1 vs. 7.5±0.9 mM; $P<0.01$) in addition to 1.6-fold increased plasma TC-levels (30.1±6.7 vs. 19.0±1.9 mM; $P<0.01$) as compared to Ad*LacZ* control mice (Table 1). The increased plasma TG and TC levels were due to increased VLDL levels, as was shown after FPLC fractionation of pooled plasma (Fig. 1). Taken together, these results suggest that LPL not only regulates the clearance of VLDL-TG, but also determines the clearance of VLDL-cholesterol in absence of the LRP, LDLr and VLDLr *in vivo*.

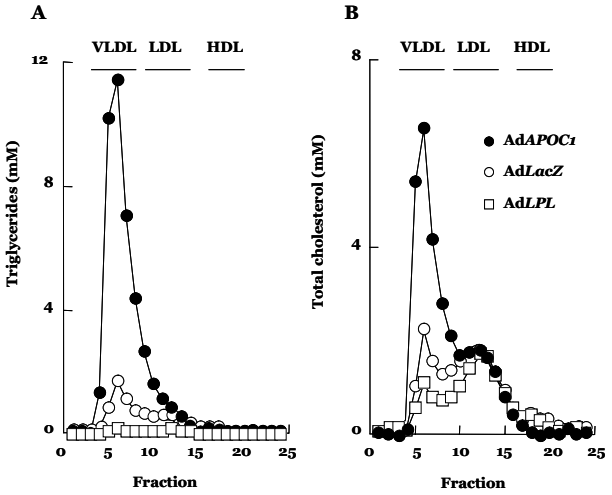


Figure 1. Plasma TG and TC distribution. *Lrp-ldlr-/-vldlr-/-* mice were injected with Ad*LacZ* (open circles), Ad*LPL* (open squares), or Ad*APOC1* (closed circles) (1x10⁹ pfu). 4 Days after injection 4h fasted plasma samples were drawn, pooled, and subjected to FPLC fractionation. Fractions were analyzed for TG (A) and TC (B).

LPL Modulates the Liver Association of VLDL-Like Emulsion Core Remnants in lrp-ldlr-/-vldlr-/- Mice

To provide direct *in vivo* evidence that LPL determines the clearance of VLDL-TC, [^{3}H]TO and [^{14}C]CO double-labeled TG-rich VLDL-like emulsion particles were injected into *lrp-ldlr-/-vldlr-/-* mice 5 days after Ad*LPL*, Ad*APOC1*, or Ad*LacZ* administration. The clearance of [^{3}H]TO was substantially accelerated in Ad*LPL* treated mice as compared to control mice, as evidenced by a 2.5-fold decreased serum half-life of ^{3}H-activity ($t_{1/2}$=18±7 vs. 45±11 min) (Fig. 2A). On the other hand, mice that were treated with Ad*APOC1* showed a 1.7-fold decreased serum half-life ($t_{1/2}$=77±13). Thus, the increase in LPL is positively correlated with the serum half-life of ^{3}H-activity ($P<0.05$). This was accompanied by a significantly increased uptake of TO-derived ^{3}H-activity in LPL-expressing organs as heart ($P<0.05$), muscle ($P<0.05$), and white adipose tis-

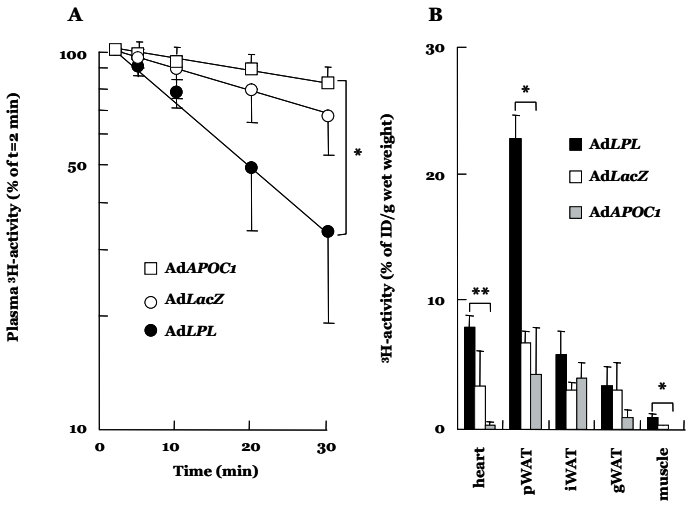


Figure 2. Serum decay and organ distribution of [³H]TO-labeled VLDL-like emulsion particles. *Lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice were injected with Ad*LacZ*, Ad*LPL* or Ad*APOC1* (1x10^{9} pfu). 5 Days after injection mice were anaesthetized and received 200 µl of [³H]TO and [¹⁴C]CO double-labeled emulsion particles (1 mg TG). Serum samples were collected at indicated times and measured for ³H-activity (A). At t=30 min the animals were sacrificed and tissues collected. Tissues were solved in Solvable (overnight, 60°C) and measured for ³H-activity (B). Asterisks indicate a statistically significant trend (*P<0.05; **P<0.01) as analyzed by one-way ANOVA. ID, injected dose; pWAT, perirenal white adipose tissue; iWAT, intestinal WAT; gWAT, gonadal WAT.

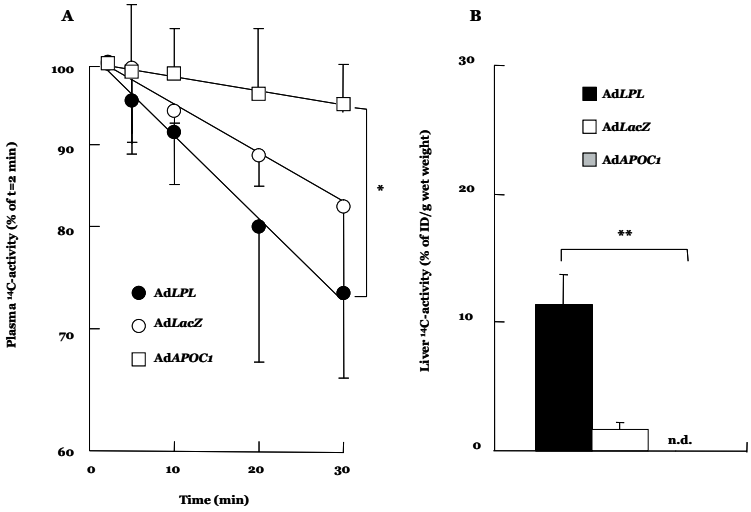


Figure 3. Serum decay and liver association of [¹⁴C]CO-labeled VLDL-like emulsion particles. *Lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice were injected with Ad*LacZ*, Ad*LPL*, or Ad*APOC1* (1x10^{9} pfu). 5 Days after injection mice were anaesthetized and received 200 µl of [³H]TO and [¹⁴C]CO double-labeled emulsion particles (1 mg TG). Serum samples were collected at indicated times and measured for ¹⁴C (A). At t=30 min the animals were sacrificed and the liver was collected. Tissues were solved in Solvable (O/N 60°C) and measured for ¹⁴C (B). n.d., not detectable. Asterisks indicate a statistically significant trend (*P<0.05; **P<0.01) as analyzed by one-way ANOVA.

sue (WAT) (P<0.05 for perirenal WAT) (Fig. 2B). Serum [^{14}C]CO decay was slower as compared to the [^{3}H]TO decay, but also the [^{14}C]CO serum half-life was dependent on LPL. This was evidenced by a 1.4-fold decreased half-life of ^{14}C-label in Ad*LPL* treated mice ($t_{1/2}$=71±24 min) and a 3.7-fold increased half-life in Ad*APOC1* treated animals ($t_{1/2}$= 364±241 min) as compared to controls ($t_{1/2}$= 99±8 min) (P<0.05) (Fig. 3A). In addition, the association of ^{14}C-activity to the liver was 7.0-fold increased in Ad*LPL* animals as compared to control mice, and decreased to zero by Ad*APOC1* (P<0.01) (Fig. 3B). Further analysis of the distribution of ^{14}C-activity over cholesterol and cholesteryl esters in the liver revealed that the ^{14}C-activity was almost exclusively recovered in the cholesteryl esters (data not shown), which indicates that particles are indeed associated with the liver, however, they have not entered the lysosomal degradation pathway yet. Taken together, these results show that LPL positively correlates with liver association of core remnants.

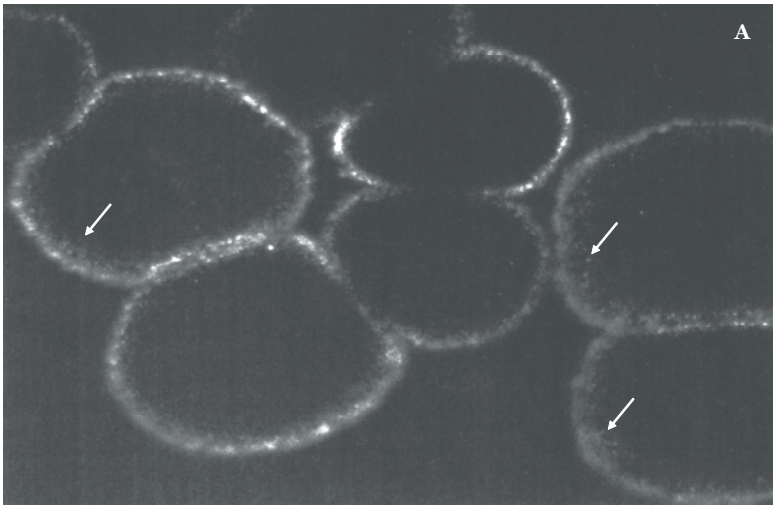
Association of VLDL-Like Emulsion Particles with Hepatocytes from lrp^{-}ldlr$^{-/-}$ Mice is Followed by Slow Internalization

We have previously shown that the hepatic binding and uptake of TG-rich lipoproteins and VLDL-like emulsion particles is mainly exerted by hepatocytes.[24] To establish whether binding of VLDL-like emulsion particles to hepatocytes that lack the LDLr and LRP can still lead to (receptor-independent) internalization, we incubated freshly isolated hepatocytes with DiI-labeled emulsion particles. The emulsion particles avidly bound to the cell surface upon incubation at 4°C. LDLr- and LRP-independent internalization of cell-bound emulsion particles was observed on further incubation at 37°C, as evidenced by the detection of fluorescence in compartments below the cell surface after 30 min of incubation (Fig. 4A). However, the rate of internalization was slower as compared to that of wild-type hepatocytes, which internalized the majority of cell-associated particles within the same time period (Fig. 4B).

Discussion

Previous studies have shown that apoE plays an important role in the uptake of lipoprotein remnants via the LDLr, LRP, and VLDLr, and that the apoE-mediated clearance is dependent on LPL-mediated hydrolysis of TG.[1,2,15] In addition, LPL is involved in remnant uptake via its bridging function.[8,9] Strikingly, in the absence of these three major apoE-recognizing receptors, lipoprotein remnants are still cleared from the circulation.[14] The aim of the current study was to investigate the role of LPL in the receptor-independent clearance of lipoprotein remnants, by modulating LPL in *lrp^{-}ldlr$^{-/-}$vldlr$^{-/-}$* mice. We indeed demonstrate that LPL is involved in the subsequent hepatic clearance of VLDL remnants independent of the three main apoE-recognizing receptors *in vivo*.

It is intriguing to speculate about the mechanism underlying the LPL-dependent clearance in absence of the three major apoE-receptors. It was previously shown that lactoferrin inhibits the clearance of chylomicron remnants and ß-VLDL by binding to HSPG[25] and to LRP *in vitro*,[26] probably because of its structural homology with the



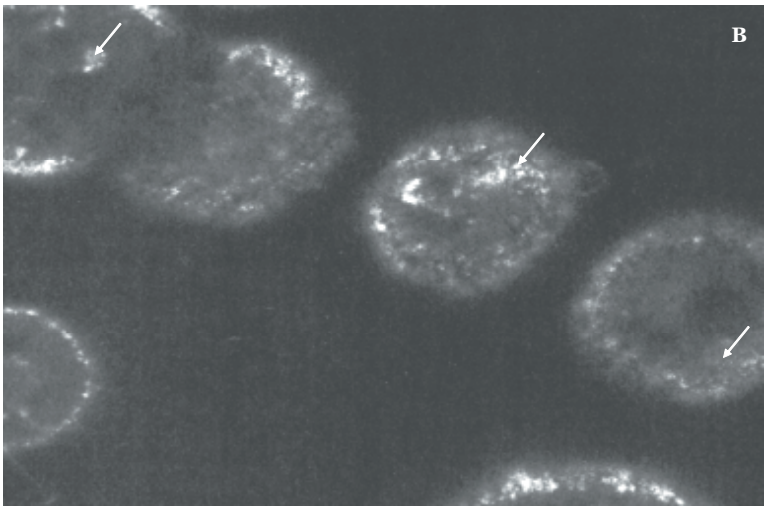


Figure 4. Uptake of VLDL-like emulsion particles by isolated mouse hepatocytes. Freshly isolated hepatocytes from *lrp*$^{-}$*ldlr*$^{-/-}$ mice (A) or wild-type mice (B) were cultured (3-4 h at 37 °C) in DMEM with 10% foetal calf serum and incubated (2 h at 4°C) in DMEM with 2% BSA with 50 nm sized DiI-labeled emulsion particles (100 µg of TG/ml). The cells were washed to remove unbound particles and further incubated at 37°C. After 30 min, localization of DiI was determined by confocal laser scanning microscopy. Intracellular fluorescently labeled compartments are indicated by arrows. Under the applied conditions, autofluorescence was negligible.

heparin binding site of apoE and its overall positive charge.[27] In addition, lactoferrin reduces the uptake of VLDL-like emulsion particles by 90% *in vivo*,[21] indicating that hepatic uptake of these particles requires HSPG and/or LRP. Protamine, like lactoferrin, binds to HSPG by electrostatic interaction, thereby reducing remnant binding.[28] Initial studies indicated that protamine administration to mice that lack both the LDLr and hepatic LRP completely inhibited the liver-association of the emulsion particles (unpublished observations by G. Gerritsen, P.C.N.R., C.C.H *et al.*). This underscores the involvement of HSPG in the hepatic uptake of VLDL-like emulsion particles. It is possible that HSPG may contribute to the internalization of the VLDL-like emulsion particles either directly[6,7] or indirectly via their docking function and the subsequent uptake via receptors such as LRP5,[29] apoB-48 receptor,[30] LR11[31] or scavenger receptor BI.[32,33] Either way, the uptake of remnant particles in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice appears to involve an HSPG-dependent pathway.

How could LPL affect the hepatic uptake of lipoprotein remnants? Binding of core-remnants to HSPG in the space of Disse involves heparin-binding proteins such as LPL, hepatic lipase (HL) and apoE.[2,34] Altered activity or levels of these proteins may thus affect the hepatic uptake in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice. LPL protein increases the binding of remnant lipoproteins to cells[35] and induces the catabolism of chylomicrons and VLDL *in vitro*.[35-40] Furthermore, inactive LPL can mediate hepatic lipoprotein clearance by bridging between lipoproteins of the LDL receptor family and/or HSPG.[41,42] In addition, the non-enzymatic function of LPL is responsible for the uptake and degradation of LDL independent of classical lipoprotein receptor pathways *in vitro*.[43] Such a mechanism may attribute to the VLDL-modulating effects of LPL in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice. As would be expected, Ad*LPL* administration to these mice increased plasma LPL activity levels (3-fold), suggesting a role for both the lipolytic activity and the bridging function of LPL.

However, mice that were treated with Ad*APOC1* had similar post-heparin LPL plasma levels (Table 1), in addition to similar levels of LPL protein (295±40 vs. 254±53 ng/ml), as compared to control mice. This is in agreement with previous findings that plasma total LPL activity is not affected in human *APOC1* transgenic mice as compared to wild-type mice, rather apoCI causes hypertriglyceridemia by modulating local LPL activity.[20] Our results thus suggest that the bridging capacity of LPL has not changed by Ad*APOC1*. On the other hand, apoCI has been reported to inhibit the apoE-dependent hepatic uptake of lipoprotein remnants by the LDLr and LRP.[44-47] Thus, if receptors are involved in the clearance of VLDL remnants, apoCI might also interfere with the uptake via these receptors. We speculate that the enzymatic activity of LPL is the primary requisite for the clearance of VLDL-remnants in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice and that potential receptor-inhibiting effects of apoCI thus play a minor role. However, this will be subject of future studies.

Huff *et al*.[48] showed that HL activity enhanced the uptake of remnants *in vitro*, which is in accordance with studies of Ji *et al*.[49] Administration of Ad*APOC1* to *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice slightly reduced the HL activity in post-heparin plasma (21±3 vs. 30±6 μmol FFA generated/h/ml; $P<0.05$) as compared to control mice. On the other hand, HL activity was not altered in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice that received Ad*LPL* (32±5 μmol

FFA generated/h/ml). Taken together, although HL might play a minor role in the VLDL remnant clearance, it is unlikely that HL is responsible for the positive correlation between LPL and hepatic VLDL remnant clearance.

ApoE leads to sequestration of remnants on the hepatocyte surface.[5,49-51] In the absence of the LDLr, the subsequent catabolism of remnant particles via an apoE-dependent pathway is slow, probably due to slow internalization of the remnants. This pathway appears to require HSPG as shown by binding experiments using either lactoferrin or heparinase. Apolipoprotein-free TG-rich emulsion particles, as we used in these experiments, have been shown to rapidly acquire apolipoproteins (among which apoE) after injection,[21,52] which would allow apoE to play a role in the subsequent uptake of emulsion particles. In addition, mice deficient for the LDLr have elevated plasma apoE levels as compared to wild-type mice, which are maintained upon the deletion of LRP[3,53] and the VLDLr.[14] Also, in the space of Disse, the local apoE concentration is high,[2] thereby affecting hepatic remnant uptake via the secretion-capture role for apoE that was suggested by Ji *et al*.[5] Finally, the LPL-mediated TG hydrolysis causes the formation of smaller remnant-particles that become enriched in apoE.[54] Thus, the affected hepatic uptake of VLDL remnants is likely to involve apoE as a ligand.

In conclusion, we have demonstrated that in the absence of the three major apoE-recognizing receptors, remnant-cholesterol clearance by the liver depends on LPL *in vivo*. The underlying main mechanism probably involves modulation of LPL-mediated TG hydrolysis, which causes the formation of remnant particles that are reduced in size and become enriched in apoE. Subsequently, these particles are sequestered by HSPG, via apoE as a heparin-binding molecule, and finally they maybe internalized via HSPG, either directly or indirectly through the docking function of HSPG.

Acknowledgements

This work was performed in the framework of the Leiden Center for Cardiovascular research LUMC-TNO, and supported by the Leiden University Medical Center (Gisela Thier Fellowship to P.C.N.R.), the Netherlands Organization for Scientific Research (NWO grant 908-02-097 and NWO VIDI grant 917.36.351), and the Netherlands Heart Foundation (NHS grant 2003B136).

References

1. Goldberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J Lipid Res. 1996;37:693-707.
2. Mahley RW and Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. J Lipid Res. 1999;40:1-16.
3. van Vlijmen BJ, Rohlmann A, Page ST, Bensadoun A, Bos IS, van Berkel TJ, Havekes LM, Herz J. An extrahepatic receptor-associated protein-sensitive mechanism is involved in the metabolism of triglyceride-rich lipoproteins. J Biol Chem. 1999;274:35219-35226.
4. Ji ZS, Brecht WJ, Miranda RD, Hussain MM, Innerarity TL, Mahley RW. Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. J Biol Chem.

1993;268:10160-10167.

5. Ji ZS, Fazio S, Lee YL, Mahley RW. Secretion-capture role for apolipoprotein E in remnant lipoprotein metabolism involving cell surface heparan sulfate proteoglycans. J Biol Chem. 1994;269:2764-2772.
6. Al Haideri M, Goldberg IJ, Galeano NF, Gleeson A, Vogel T, Gorecki M, Sturley SL, Deckelbaum RJ. Heparan sulfate proteoglycan-mediated uptake of apolipoprotein E-triglyceride-rich lipoprotein particles: a major pathway at physiological particle concentrations. Biochemistry. 1997;36:12766-12772.
7. Ji ZS, Sanan DA, Mahley RW. Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulfate proteoglycans. J Lipid Res. 1995;36:583-592.
8. Niemeier A, Gafvels M, Heeren J, Meyer N, Angelin B, Beisiegel U. VLDL receptor mediates the uptake of human chylomicron remnants in vitro. J Lipid Res. 1996;37:1733-1742.
9. Takahashi S, Sakai J, Fujino T, Hattori H, Zenimaru Y, Suzuki J, Miyamori I, Yamamoto TT. The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. J Atheroscler Thromb. 2004;11:200-208.
10. Frykman PK, Brown MS, Yamamoto T, Goldstein JL, Herz J. Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. Proc Natl Acad Sci U S A. 1995;92:8453-8457.
11. Goudriaan JR, Tacken PJ, Dahlmans VE, Gijbels MJ, van Dijk KW, Havekes LM, Jong MC. Protection from obesity in mice lacking the VLDL receptor. Arterioscler Thromb Vasc Biol. 2001;21:1488-1493.
12. Tacken PJ, Teusink B, Jong MC, Harats D, Havekes LM, van Dijk KW, Hofker MH. LDL receptor deficiency unmasks altered VLDL triglyceride metabolism in VLDL receptor transgenic and knockout mice. J Lipid Res. 2000;41:2055-2062.
13. Goudriaan JR, Espirito Santo SM, Voshol PJ, Teusink B, van Dijk KW, van Vlijmen BJ, Romijn JA, Havekes LM, Rensen PC. The VLDL receptor plays a major role in chylomicron metabolism by enhancing LPL-mediated triglyceride hydrolysis. J Lipid Res. 2004;45:1475-1481.
14. Espirito Santo SM, Rensen PC, Goudriaan JR, Bensadoun A, Bovenschen N, Voshol PJ, Havekes LM, van Vlijmen BJ. Triglyceride-rich lipoprotein metabolism in unique VLDL receptor, LDL receptor, and LRP triple-deficient mice. J Lipid Res. 2005;46:1097-1102.
15. Sehayek E, Lewin-Velvert U, Chajek-Shaul T, Eisenberg S. Lipolysis exposes unreactive endogenous apolipoprotein E-3 in human and rat plasma very low density lipoprotein. J Clin Invest. 1991;88:553-560.
16. Kobayashi J, Applebaum-Bowden D, Dugi KA, Brown DR, Kashyap VS, Parrott C, Duarte C, Maeda N, Santamarina-Fojo S. Analysis of protein structure-function in vivo. Adenovirus-mediated transfer of lipase lid mutants in hepatic lipase-deficient mice. J Biol Chem. 1996;271:26296-26301.
17. van der Hoogt CC, Berbee JF, Espirito Santo SM, Gerritsen G, Krom YD, van der ZA, Havekes LM, van Dijk KW, Rensen PC. Apolipoprotein CI causes hypertriglyceridemia independent of the very-low-density lipoprotein receptor and apolipoprotein CIII in mice. Biochim Biophys Acta. 2006;1761:213-220.
18. Tao N, Gao GP, Parr M, Johnston J, Baradet T, Wilson JM, Barsoum J, Fawell SE. Sequestration of adenoviral vector by Kupffer cells leads to a nonlinear dose response of transduction in liver. Mol Ther. 2001;3:28-35.
19. Zambon A, Hashimoto SI, Brunzell JD. Analysis of techniques to obtain plasma for measurement of levels of free fatty acids. J Lipid Res. 1993;34:1021-1028.
20. Berbee JF, van der Hoogt CC, Sundararaman D, Havekes LM, Rensen PC. Severe hypertriglyceridemia in human APOC1 transgenic mice is caused by apoC-I-induced inhibition of LPL. J Lipid Res. 2005;46:297-306.
21. Rensen PC, Herijgers N, Netscher MH, Meskers SC, van Eck M, van Berkel TJ. Particle size determines the specificity of apolipoprotein E-containing triglyceride-rich emulsions for the LDL receptor versus hepatic remnant receptor in vivo. J Lipid Res. 1997;38:1070-1084.

22. Jong MC, Rensen PC, Dahlmans VE, van der BH, van Berkel TJ, Havekes LM. Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. J Lipid Res. 2001;42:1578-1585.
23. Rensen PC, Sliedregt LA, Ferns M, Kieviet E, van Rossenberg SM, van Leeuwen SH, van Berkel TJ, Biessen EA. Determination of the upper size limit for uptake and processing of ligands by the asialoglycoprotein receptor on hepatocytes in vitro and in vivo. J Biol Chem. 2001;276:37577-37584.
24. Rensen PC, van Dijk MC, Havenaar EC, Bijsterbosch MK, Kruijt JK, van Berkel TJ. Selective liver targeting of antivirals by recombinant chylomicrons--a new therapeutic approach to hepatitis B. Nat Med. 1995;1:221-225.
25. van Barlingen HH, de Jong H, Erkelens DW, de Bruin TW. Lipoprotein lipase-enhanced binding of human triglyceride-rich lipoproteins to heparan sulfate: modulation by apolipoprotein E and apolipoprotein C. J Lipid Res. 1996;37:754-763.
26. Ji ZS and Mahley RW. Lactoferrin binding to heparan sulfate proteoglycans and the LDL receptor-related protein. Further evidence supporting the importance of direct binding of remnant lipoproteins to HSPG. Arterioscler Thromb. 1994;14:2025-2031.
27. Huettinger M, Retzek H, Hermann M, Goldenberg H. Lactoferrin specifically inhibits endocytosis of chylomicron remnants but not alpha-macroglobulin. J Biol Chem. 1992;267:18551-18557.
28. Zeng BJ, Mortimer BC, Martins IJ, Seydel U, Redgrave TG. Chylomicron remnant uptake is regulated by the expression and function of heparan sulfate proteoglycan in hepatocytes. J Lipid Res. 1998;39:845-860.
29. Magoori K, Kang MJ, Ito MR, Kakuuchi H, Ioka RX, Kamataki A, Kim DH, Asaba H, Iwasaki S, Takei YA, Sasaki M, Usui S, Okazaki M, Takahashi S, Ono M, Nose M, Sakai J, Fujino T, Yamamoto TT. Severe hypercholesterolemia, impaired fat tolerance, and advanced atherosclerosis in mice lacking both low density lipoprotein receptor-related protein 5 and apolipoprotein E. J Biol Chem. 2003;278:11331-11336.
30. Brown ML, Ramprasad MP, Umeda PK, Tanaka A, Kobayashi Y, Watanabe T, Shimoyamada H, Kuo WL, Li R, Song R, Bradley WA, Gianturco SH. A macrophage receptor for apolipoprotein B48: cloning, expression, and atherosclerosis. Proc Natl Acad Sci U S A. 2000;97:7488-7493.
31. Taira K, Bujo H, Hirayama S, Yamazaki H, Kanaki T, Takahashi K, Ishii I, Miida T, Schneider WJ, Saito Y. LR11, a mosaic LDL receptor family member, mediates the uptake of ApoE-rich lipoproteins in vitro. Arterioscler Thromb Vasc Biol. 2001;21:1501-1506.
32. Out R, Kruijt JK, Rensen PC, Hildebrand RB, de Vos P, van Eck M, van Berkel TJ. Scavenger receptor BI plays a role in facilitating chylomicron metabolism. J Biol Chem. 2004;279:18401-18406.
33. Out R, Hoekstra M, de Jager SC, de Vos P, van der Westhuyzen DR, Webb NR, van Eck M, Biessen EA, van Berkel TJ. Adenovirus-mediated hepatic overexpression of scavenger receptor class B type I accelerates chylomicron metabolism in C57BL/6J mice. J Lipid Res. 2005;46:1172-1181.
34. Kolset SO and Salmivirta M. Cell surface heparan sulfate proteoglycans and lipoprotein metabolism. Cell Mol Life Sci. 1999;56:857-870.
35. Eisenberg S, Sehayek E, Olivecrona T, Vlodavsky I. Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. J Clin Invest. 1992;90:2013-2021.
36. Chappell DA, Fry GL, Waknitz MA, Muhonen LE, Pladet MW, Iverius PH, Strickland DK. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor in vitro. A process facilitated by cell-surface proteoglycans. J Biol Chem. 1993;268:14168-14175.
37. Mulder M, Lombardi P, Jansen H, van Berkel TJ, Frants RR, Havekes LM. Heparan sulphate proteoglycans are involved in the lipoprotein lipase-mediated enhancement of the cellular binding of very low density and low density lipoproteins. Biochem Biophys Res Commun. 1992;185:582-587.

38. Mulder M, Lombardi P, Jansen H, van Berkel TJ, Frants RR, Havekes LM. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. J Biol Chem. 1993;268:9369-9375.
39. Saxena U, Klein MG, Vanni TM, Goldberg IJ. Lipoprotein lipase increases low density lipoprotein retention by subendothelial cell matrix. J Clin Invest. 1992;89:373-380.
40. Williams KJ, Fless GM, Petrie KA, Snyder ML, Brocia RW, Swenson TL. Mechanisms by which lipoprotein lipase alters cellular metabolism of lipoprotein(a), low density lipoprotein, and nascent lipoproteins. Roles for low density lipoprotein receptors and heparan sulfate proteoglycans. J Biol Chem. 1992;267:13284-13292.
41. Heeren J, Niemeier A, Merkel M, Beisiegel U. Endothelial-derived lipoprotein lipase is bound to postprandial triglyceride-rich lipoproteins and mediates their hepatic clearance in vivo. J Mol Med. 2002;80:576-584.
42. Merkel M, Kako Y, Radner H, Cho IS, Ramasamy R, Brunzell JD, Goldberg IJ, Breslow JL. Catalytically inactive lipoprotein lipase expression in muscle of transgenic mice increases very low density lipoprotein uptake: direct evidence that lipoprotein lipase bridging occurs in vivo. Proc Natl Acad Sci U S A. 1998;95:13841-13846.
43. Rumsey SC, Obunike JC, Arad Y, Deckelbaum RJ, Goldberg IJ. Lipoprotein lipase-mediated uptake and degradation of low density lipoproteins by fibroblasts and macrophages. J Clin Invest. 1992;90:1504-1512.
44. Jong MC, Dahlmans VE, van Gorp PJ, van Dijk KW, Breuer ML, Hofker MH, Havekes LM. In the absence of the low density lipoprotein receptor, human apolipoprotein C1 overexpression in transgenic mice inhibits the hepatic uptake of very low density lipoproteins via a receptor-associated protein-sensitive pathway. J Clin Invest. 1996;98:2259-2267.
45. Jong MC, van Dijk KW, Dahlmans VE, van der BH, Kobayashi K, Oka K, Siest G, Chan L, Hofker MH, Havekes LM. Reversal of hyperlipidaemia in apolipoprotein C1 transgenic mice by adenovirus-mediated gene delivery of the low-density-lipoprotein receptor, but not by the very-low-density-lipoprotein receptor. Biochem J. 1999;338 (Pt 2):281-287.
46. Sehayek E and Eisenberg S. Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway. J Biol Chem. 1991;266:18259-18267.
47. Weisgraber KH, Mahley RW, Kowal RC, Herz J, Goldstein JL, Brown MS. Apolipoprotein C-I modulates the interaction of apolipoprotein E with beta-migrating very low density lipoproteins (beta-VLDL) and inhibits binding of beta-VLDL to low density lipoprotein receptor-related protein. J Biol Chem. 1990;265:22453-22459.
48. Huff MW, Miller DB, Wolfe BM, Connelly PW, Sawyez CG. Uptake of hypertriglyceridemic very low density lipoproteins and their remnants by HepG2 cells: the role of lipoprotein lipase, hepatic triglyceride lipase, and cell surface proteoglycans. J Lipid Res. 1997;38:1318-1333.
49. Ji ZS, Lauer SJ, Fazio S, Bensadoun A, Taylor JM, Mahley RW. Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. J Biol Chem. 1994;269:13429-13436.
50. Hamilton RL, Wong JS, Guo LS, Krisans S, Havel RJ. Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections. J Lipid Res. 1990;31:1589-1603.
51. Mortimer BC, Beveridge DJ, Martins IJ, Redgrave TG. Intracellular localization and metabolism of chylomicron remnants in the livers of low density lipoprotein receptor-deficient mice and apoE-deficient mice. Evidence for slow metabolism via an alternative apoE-dependent pathway. J Biol Chem. 1995;270:28767-28776.
52. Mortimer BC, Simmonds WJ, Cockman SJ, Stick RV, Redgrave TG. The effect of monostearoylglycerol on the metabolism of chylomicron-like lipid emulsions injected intravenously in rats. Biochim Biophys Acta. 1990;1046:46-56.
53. Rohlmann A, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. J Clin Invest. 1998;101:689-695.

54. Rensen PC, de Vrueh RL, Kuiper J, Bijsterbosch MK, Biessen EA, van Berkel TJ. Recombinant lipoproteins: lipoprotein-like lipid particles for drug targeting. Adv Drug Deliv Rev. 2001;47:251-276.