In vivo removal of β-VLDL, chylomicron remnants, and α_2-macroglobulin in the rat

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Abstract The low density lipoprotein receptor-related protein (LRP)/α_2-macroglobulin (α_2M) receptor has been suggested as a potential chylomicron remnant receptor. To investigate the involvement of LRP in chylomicron remnant metabolism in vivo, cross-competition experiments with chylomicron remnants, β-VLDL, and receptor-active α_2M, complexed with trypsin (α_2M-trypsin), were performed in rats. Saturating concentrations of unlabeled β-VLDL failed to inhibit the plasma clearance and hepatic uptake of ^{125}I-labeled α_2M-trypsin and, vice versa, α_2M-trypsin failed to retard the removal of ^{125}I-labeled chylomicron remnants. It has been demonstrated previously that bovine lipoprotein lipase (LPL) strongly enhances the binding of apolipoprotein E-containing lipoproteins to LRP (U. Beisiegel, W. Weber, and G. Bengtsson-Olivecrona. 1991. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346). Therefore, β-VLDL were enriched with isolated LPL or heparin was injected simultaneously with β-VLDL to increase the concentration of endogenous LPL bound to β-VLDL. Yet, no inhibition of the plasma elimination and the hepatic uptake of ^{125}I-labeled α_2M-trypsin was observed after injection of saturating amounts of β-VLDL enriched with LPL. We conclude that in the rat triglyceride-rich lipoproteins and α_2M-trypsin bind in vivo either to different binding domains of LRP or to a different receptor protein. **—Jäckle, S., C. Huber, S. Moestrup, J. Gliemann, and U. Beisiegel.** In vivo removal of β-VLDL, chylomicron remnants, and α_2-macroglobulin in the rat. *J. Lipid Res.* 1993. **34:** 309–315.

Supplementary key words low density lipoprotein receptor-related protein • α_2-macroglobulin receptor

Chylomicron remnants are cleared rapidly from the plasma and are chiefly taken up by the liver (1, 2). The clearance of chylomicron remnants is mediated by apolipoprotein (apo) E but is independent of apoB (3, 4). The low density lipoprotein (LDL) receptor contributes to the removal of chylomicron remnants in vitro (5–9) and in vivo (10–13). Yet, tissue culture and in vivo experiments have shown that chylomicron remnants are, in addition, removed by a mechanism distinct from the LDL receptor (5, 14–17). The LDL receptor-related protein (LRP) (18) has been shown to bind apoE-containing lipoproteins in vitro (19–22); binding of apoE to LRP can be highly stimulated by addition of lipoprotein lipase (23).

Recently, Strickland et al. (24) and Kristensen et al. (25) discovered that LRP is identical with the α_2-macroglobulin (α_2M) receptor. α_2M is capable of inhibiting a wide variety of proteolytic enzymes (26). α_2M proteinase complexes are rapidly removed from the circulation, primarily by receptor-mediated endocytosis in hepatocytes (27–29).

Hussain et al. (30) demonstrated that receptor-active α_2M partially inhibited the plasma clearance of chylomicron remnants and, vice versa, apoE-enriched chylomicron remnants partially inhibited the removal of α_2M in mice (30). In the present study we performed in vivo cross-competition experiments with rat chylomicron remnants, rabbit β-very low density lipoproteins (β-VLDL), and human α_2M-trypsin in rats. To increase the affinity of β-VLDL to LRP, bovine lipoprotein lipase (LPL) was added to β-VLDL.

The results suggest that triglyceride-rich lipoproteins and α_2M-trypsin bind to different and mutually independent binding domains on the same receptor protein or alternatively to different receptors.

MATERIALS AND METHODS

Reagents

[^{125}I]iodide (carrier-free) was from Amersham-Buchler (Braunschweig, Germany); heparin was from Hoffman La Roche (Basel, Switzerland).

Abbreviations: α_2M, α_2-macroglobulin; α_2M-trypsin, α_2-macroglobulin complexed with trypsin; apo, apolipoprotein; β-VLDL, β-very low density lipoprotein; LDL, low density lipoprotein; LRP, low density lipoprotein receptor-related protein; LPL, bovine lipoprotein lipase.

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Animals

Male Spraque-Dawley rats (200–300 g) were maintained on laboratory chow. New Zealand White rabbits (1.5–2 kg) were maintained for 3–4 weeks on laboratory chow containing 1.5% cholesterol (w/w).

Preparation of lipoproteins

β-VLDL (d < 1.006 g/ml) were isolated from the blood of rabbits fed a diet containing 1.5% cholesterol (w/w) for 3–4 weeks (31). Small chylomicron remnants were produced by a standard procedure (8). Lymph was collected from rats with an intestinal lymph fistula while 50 ml of 0.15 M NaCl containing 5 g glucose was infused into the duodenum for 25 h (32). Chylomicrons were isolated by centrifugal flotation at 3×10^7 g_{av}-min (33). Chylomicron remnants were produced in vitro by incubation of lymphatic chylomicrons with the d > 1.019 g/ml plasma fraction obtained after injection of heparin into rats (8). The protein and lipid compositions of lymph chylomicrons and chylomicron remnants were similar to that reported previously (8). In indicated experiments, 3 mg (protein) β-VLDL was incubated with 12 μg LPL for 30 min on ice.

Preparation of macroglobulins

Human α_2M was prepared from pooled citrate plasma using Zn^{2+} chelate-affinity chromatography as described earlier (34). α_2M was complexed with trypsin (α_2M-trypsin) as described (27). The complexed proteins were purified either by Sephacryl S-300 gel filtration or Superose 12 fast protein liquid chromatography. All preparations of native and proteinase-complexed macroglobulins were monitored by reducing and nonreducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Radiolabeling of ligands

Chylomicron remnants were labeled with ^{125}I by the iodine monochloride method of McFarlane (35) to a specific activity of 200–300 cpm/ng protein, as described (36). α_2M-trypsin was iodinated as described previously (27).

In vivo metabolism studies

To study the plasma removal of lipoproteins and macroglobulins in vivo, rats were anesthetized with diethyl ether and the radiolabeled ligands were injected into a femoral vein; blood samples were taken from the tail vein. In competition experiments unlabeled ligands were injected into a femoral vein 5 min before the radiolabeled ligand was given. The injected dose of tracer per estimated plasma volume was taken as 100%. The calculation was based on a plasma volume of 4.5% of body weight. Thirty min after injection of the radiolabeled ligand livers were removed, weighed, and radioactivity was determined in aliquots.

Statistical analysis

For the levels of significance between experimental groups, a two group unpaired two-tailed *t*-test was performed.

RESULTS

In this study we investigated the plasma turnover and hepatic uptake of β-VLDL, isolated from cholesterol-fed rabbits, rat chylomicron remnants, and human α_2M-trypsin. The plasma turnover of β-VLDL is shown in **Fig. 1:** two min after intravenous injection of ^{125}I-labeled β-VLDL (20 μg protein) more than 70% of the tracer was cleared from the circulation; 30 min after injection only 2.8% of ^{125}I remained in the circulation. The removal mechanism of β-VLDL can be saturated by injection of high dosages of the ligand. After intrafemoral injection of saturating amounts of β-VLDL (2 mg protein equivalent to 23 mg cholesteryl esters) and 20 μg (protein) ^{125}I-labeled β-VLDL, less than 15% of the tracer was eliminated from the circulation 2 min after injection and less than 45% 30 min after injection (Fig. 1). Tracer amounts of ^{125}I-labeled α_2M-trypsin were eliminated from the circulation at comparable rates as low dosages of β-VLDL. Fifty-two percent of ^{125}I-labeled α_2M-trypsin could be recovered from the blood 2 min after injection and 3.6% 30 min after injection (**Fig. 2**). The removal of α_2M-trypsin could also be saturated by injection high doses of the ligand, as demonstrated previously (28, 29).

Chylomicron remnants and α_2M-trypsin are thought to be taken up by the LRP/α_2M-receptor in vitro. The following experiments were performed to examine, whether receptor-active α_2M-trypsin and β-VLDL might compete

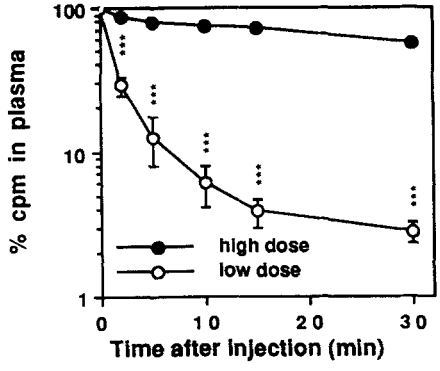

Fig. 1. Plasma elimination of ^{125}I-labeled β-VLDL in rats. Blood samples were drawn from the tail vein after intrafemoral injection of 20 μg protein (low dose) or 2 mg protein (high dose) ^{125}I-labeled β-VLDL. Values are given as percent of the injected ^{125}I remaining in blood plasma. Each point represents the mean of three experiments (low dose) and two experiments (high dose); bars indicate 1 SD. Level of significance: $^*P < 0.1$; $^{**}P < 0.01$; $^{***}P < 0.001$.

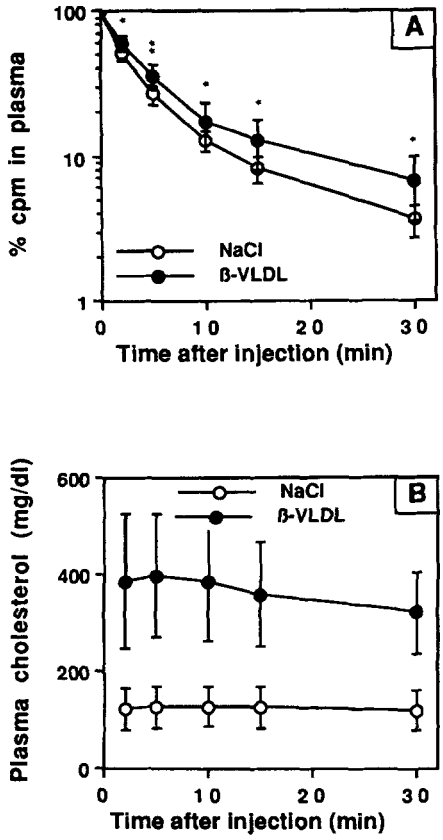

Fig. 2. In vivo competition of ^{125}I-labeled α_2M-trypsin and β-VLDL in rats. Two mg unlabeled β-VLDL (β-VLDL) was injected intrafemorally followed 5 min later by 2 μg ^{125}I-labeled α_2M-trypsin. In control rats (NaCl) a volume of 0.15 M NaCl equivalent to that of the unlabeled ligand was injected. Blood was drawn from the tail vein; values are given as percent of ^{125}I remaining in blood plasma (A). Cholesterol concentrations were measured from plasma samples of control rats and rats after injection of β-VLDL (B). Each point represents the mean of nine experiments, bars indicate 1 SD. Level of significance: $^{*}P < 0.1$; $^{**}P < 0.01$; $^{***}P < 0.001$.

in vivo. For these studies radiolabeled tracers of α_2M-trypsin were injected into a femoral vein 5 min after saturating concentrations of unlabeled lipoproteins were given. In control rats similar volumes of 0.15 M NaCl-solution were injected. Under these conditions the removal of ^{125}I-labeled α_2M-trypsin was almost unimpaired. Two, 10, 15, and 30 min after injection there was no significant difference ($P > 0.01$) in the plasma elimination of ^{125}I-labeled α_2M-trypsin of control rats and rats given a load of β-VLDL (Fig. 2A). Injection of saturating dosages of β-VLDL (2 mg protein equivalent to 30 mg cholesteryl esters) raised the plasma cholesterol concentration about threefold at all time intervals after injection. In these animals there was a reduction of the cholesterol concentration in plasma of only 17% within 30 min, indicating that saturating dosages of unlabeled lipoproteins were given (Fig. 2B).

The binding affinity of chylomicron remnants and β-VLDL to the LRP/α_2M-receptor can be substantially stimulated in vitro by the addition of LPL to the remnant particle (23). Therefore, the plasma turnover of ^{125}I-labeled α_2M-trypsin was investigated in the presence of β-VLDL, previously loaded with LPL. Plasma cholesterol concentrations were raised about threefold at all time intervals after injection of unlabeled LPL-enriched β-VLDL, as demonstrated for native β-VLDL. However, no inhibition of the plasma elimination of ^{125}I-labeled α_2M-trypsin could be observed in these rats (**Fig. 3A**). To study the effect of β-VLDL, enriched with endogenous lipoprotein lipase, saturating doses of β-VLDL together with 1.000 IE heparin were injected 5 min before ^{125}I-labeled α_2M-trypsin was given. Again, no retardation of the plasma turnover of ^{125}I-labeled α_2M-trypsin could be detected under these conditions (Fig. 3B).

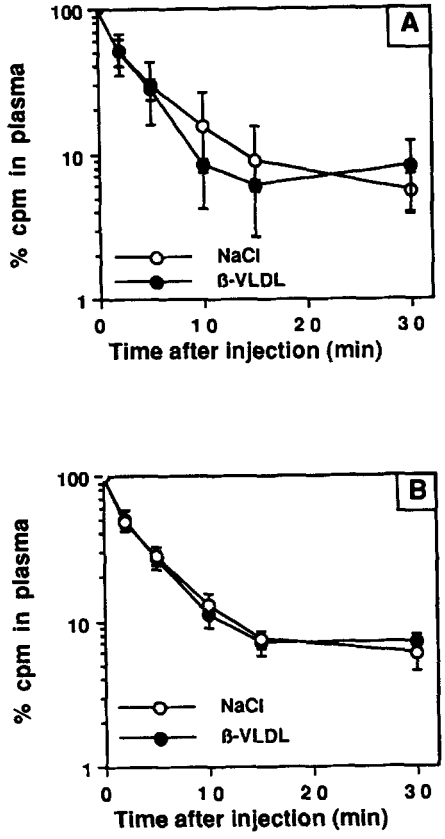

Fig. 3. In vivo competition of ^{125}I-labeled α_2M-trypsin and LPL-enriched β-VLDL in rats. (A) Three mg β-VLDL was incubated with 12 μg LPL for 30 min on ice and injected into a femoral vein followed 5 min later by 2 μg ^{125}I-labeled α_2M-trypsin. (B) Two mg β-VLDL and 1.000 IE heparin were injected simultaneously followed 5 min later by 4 μg ^{125}I-labeled α_2M-trypsin. In control rats (NaCl) a volume of 0.15 M NaCl equivalent to that of the unlabeled ligand was injected. Blood was drawn from the tail vein; values are given as percent of ^{125}I remaining in blood plasma. Each point represents the mean of three experiments; bars indicate 1 SD. Level of significance: $^{*}P < 0.1$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Similar experiments were performed using radiolabeled chylomicron remnants and, as a competitor, saturating amounts (10 mg) of unlabeled α_2M-trypsin. Plasma elimination of ^{125}I-labeled chylomicron remnants remained unchanged after previous injection of α_2M-trypsin (**Fig. 4**). Small chylomicron remnants were removed from the circulation at a slower rate than β-VLDL but much faster than LDL, as demonstrated previously (13).

α_2M-trypsin was almost completely taken up by the liver. The liver uptake of ^{125}I-labeled α_2M-trypsin remained unchanged after injection of saturating dosages of β-VLDL. There was also no significant difference ($P > 0.1$, n = 6) for the uptake in spleen, lungs, heart, kidneys, and adrenal glands (**Fig. 5**). The liver uptake was also unchanged after injection of lipase-enriched β-VLDL and after injection of heparin together with β-VLDL (**Table 1, A**). Vice versa, the rate of hepatic removal of ^{125}I-labeled chylomicron remnants remained unaltered after injection of a load of α_2M-trypsin (Table 1, B).

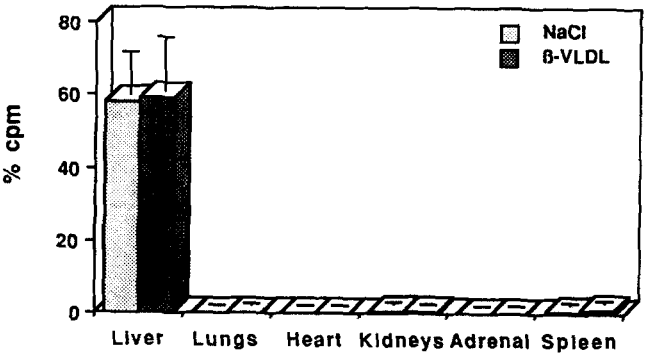

Fig. 5. Uptake of ^{125}I-labeled α_2M-trypsin after injection of β-VLDL into rats. Organs were removed 35 min after injection of 2 mg β-VLDL (β-VLDL) and 30 min after injection of 2–4 μg ^{125}I-labeled α_2M-trypsin. In control rats (NaCl) a volume of 0.15 M NaCl equivalent to that of β-VLDL was injected. Values are given as percent of the injected ^{125}I recovered in the organs. Each column represents the mean of six experiments; bars indicate 1 SD. Level of significance: $^*P < 0.1$; $^{**}P < 0.01$; $^{***}P < 0.001$.

DISCUSSION

We have demonstrated that in the rat there is no cross-competition of small rat chylomicron remnants and rabbit β-VLDL with human α_2M-trypsin. Plasma elimination and liver uptake of radiolabeled chylomicron remnants and α_2M-trypsin remained almost unchanged after injection of saturating doses of unlabeled α_2M-trypsin and β-VLDL, respectively. Hussain et al. (30), however, demonstrated some cross-competition of chylomicron remnants, β-VLDL, and activated α_2M in mice. Yet, the concentrations of competing ligands were much higher than would be expected from the equilibrium constants and only partial competition could be obtained in plasma (30). In the experiments of Hussain et al. (30), [^{3}H]retinol-labeled chylomicrons were isolated from the thoracic duct of dogs and chylomicron remnants were prepared by injecting chylomicrons into hepatectomized rabbits, while in our experiments chylomicron remnants were produced in vitro by incubation of lymphatic rat chylomicrons with the d > 1.019 g/ml plasma fraction obtained after injection of heparin into rats. In the experiments of Hussain et al. (30), β-VLDL were obtained from rabbits fed a 0.5% cholesterol diet for 4 days; in our study β-VLDL were isolated from the plasma of rabbits fed a 1.5% cholesterol diet for 3–4 weeks. In the experiments of Hussain et al. (30) the liver uptake of native and apolipoprotein E-enriched chylomicron remnants was decreased to about 70% by saturating dosages of activated α_2M. The hepatic removal of activated α_2M was decreased to about 70% by saturating amounts of apolipoprotein E-enriched chylomicron remnants, indicating only incomplete cross-competition of these ligands (30). Yet, in our studies there was no significant cross-competition of triglyceride-rich lipoproteins and α_2-macroglobulin for the detectable liver uptake.

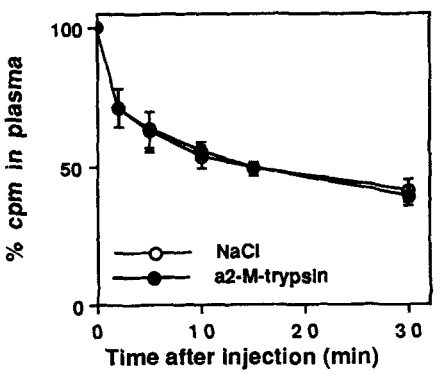

Fig. 4. In vivo competition of ^{125}I-labeled chylomicron remnants and α_2M-trypsin in rats. Ten mg unlabeled α_2M-trypsin (α_2M-trypsin) was injected intrafemorally followed 5 min later by 10 μg ^{125}I-labeled chylomicron remnants. In control rats (NaCl) a volume of 0.15 M NaCl equivalent to that of the unlabeled ligand was injected. Blood was drawn from the tail vein; values are given as percent of the injected ^{125}I remaining in blood plasma. Each point represents the mean of three experiments; bars indicate 1 SD. Level of significance: $^*P < 0.1$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Hussain et al. (30) suggested that α_2M and remnants, which do not share sequence homology in their binding domains (37, 38) bind to different binding sites of the LRP molecule. The incomplete competition of remnants and α_2M-trypsin found in the studies of Hussain et al. (30) could be explained by steric hindrance of the used ligands. The differences in results may be due to different methods for the preparation of chylomicron remnants and β-VLDL and different experimental designs of the in vivo studies. Yet, in both studies there was no complete cross-competition of remnants and α_2M in vivo, indicating that different binding domains of LRP are involved. This view

TABLE 1. Liver uptake of ^{125}I-labeled α_2M-trypsin and ^{125}I-labeled chylomicron remnants in rats

	A. Uptake of ^{125}I-Labeled α_2M		B. Uptake of ^{125}I-Labeled Chylomicron Remnants	
Treatment	Control	β-VLDL	Control	α_2M-Trypsin
β-VLDL[a]	58.26 ± 11.30	58.94 ± 14.64		
LPL-β-VLDL[b]	59.75 ± 2.53	44.99 ± 4.24*		
Heparin-β-VLDL[c]	46.82 ± 8.78	52.36 ± 2.51		
α_2M-trypsin[d]			37.15 ± 5.30	36.67 ± 3.71

Organs were removed 35 min after intrafemoral injection of unlabeled ligands (β-VLDL or α_2M-trypsin) and 30 min after injection of 2–4 μg ^{125}I-labeled α_2M-trypsin (A) or 10 μg ^{125}I-labeled chylomicron remnants (B). In control rats, a volume of 0.15 M NaCl equivalent to that of unlabeled β-VLDL and α_2M-trypsin was injected. Values are given as percent of the injected ^{125}I recovered in the liver. Each value represents the mean of three experiments ± 1 SD. Level of significance: *, $P < 0.1$.

[a]Two mg (protein) β-VLDL.

[b]Three mg (protein) β-VLDL after addition of 12 μg LPL.

[c]Three mg (protein) β-VLDL and 1.000 IE heparin.

[d]Ten mg α_2M-trypsin.

is strongly supported by findings of Moestrup and Gliemann (39) in vitro, who demonstrated no cross-competition of β-VLDL and α_2M for the binding to the purified LRP (A. Nykjaer, G. Bengttson-Olivecrona, S. Moestrup, W. Weber, U. Beisiegel, and J. Gliemann, unpublished results). A 39–40 kD-protein associated to purified LRP inhibits binding of both α_2M-trypsin (39) and β-VLDL (40) probably by binding to both binding sites.

Van Dijk et al. (41) investigated the competition of lactoferrin with chylomicron remnants, β-VLDL and methylamine-activated α_2M in the rat. Large chylomicron remnants were isolated from rat lymph after infusion of lipid emulsions into the duodenum; β-VLDL was obtained from rats, fed a diet containing cholesterol, olive oil, and cholic acid for 16 days. Lactoferrin blocked the liver uptake of chylomicron remnants and β-VLDL leaving the removal of activated α_2M unaffected (41). These findings could also be explained by binding of lipoprotein remnants and α_2M to different binding sites of LRP, if the positively charged lactoferrin binds specifically to the "lipoprotein-binding site" but not to the "α_2M-binding site" of LRP.

Alternatively, in the rat, chylomicron remnants and α_2M-trypsin might bind at least partially to different receptors in vivo. The LDL receptor seems to participate in the clearance of chylomicron remnants from plasma. Chylomicron remnants and β-VLDL bind in vitro to the hepatocytic LDL receptor (5–9), their uptake is mediated by the LDL receptor in different cell lines, and their removal is altered by interventions that are known to alter the number of LDL receptors (10–13). The removal of chylomicron remnants can be up- and down-regulated in vivo by interventions that regulate the LDL receptor but not LRP (13).

It has been demonstrated previously that binding of chylomicron remnants and β-VLDL to HepG2 cells and fibroblasts could be increased about 30- to 40-fold by addition of LPL (23). With cross-linking experiments, it was demonstrated that LRP is the protein responsible for the LPL-enhanced binding of chylomicrons (23). Therefore, cross-competition experiments were performed with ^{125}I-labeled α_2M-trypsin and a load of LPL-enriched β-VLDL to study the interactions of both ligands in vivo. Alternatively, unlabeled β-VLDL were injected simultaneously with heparin, to mobilize endogenous lipase from the endothelium and to enrich β-VLDL with endogenous lipoprotein lipase. Again, no significant difference of the plasma elimination and hepatic uptake of ^{125}I-labeled α_2M-trypsin was observed under these conditions. The lack of cross-competition indicates that LPL, like apoE, does not bind to the same site as the α_2M-trypsin. However, apoE and LPL are positively charged heparin-binding proteins and it is very likely that they are recognized by the same binding site. In further studies, we (C. Huber, A. Mann, and U. Beisiegel) demonstrate whether the effect of LPL on the lipoprotein uptake observed in vitro can be confirmed in turnover studies in vivo.

We conclude that triglyceride-rich lipoproteins and α_2M-trypsin bind in vivo to different binding domains of LRP or to different receptors. The contribution of LRP for the hepatic removal of chylomicron remnants and β-VLDL in vivo still has to be elucidated.

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REFERENCES

1. Havel, R. J., and R. L. Hamilton. 1988. Hepatocytic lipoprotein receptors and intracellular lipoprotein catabolism. *Hepatology.* **8:** 1689–1704.
2. Hussain, M. M., R. W. Mahley, J. K. Boyles, M. Fainaru, W. J. Brecht, and P. A. Linquist. 1989. Chylomicron-chylomicron remnant clearance by liver and bone marrow in rabbits: factors that modify tissue-specific uptake. *J. Biol. Chem.* **264:** 9571–9582.
3. Borensztain, J., G. S. Getz, R. J. Padley, and T. J. Kotlar. 1982. The apoprotein B-independent uptake of chylomicron remnants. *Biochem. J.* **204:** 609–612.
4. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
5. Kita, T., J. L. Goldstein, M. S. Brown, Y. Watanabe, C. Hornick, and R. J. Havel. 1982. Hepatic uptake of chylomicron remnants in WHHL rabbits: a mechanism genetically distinct from the low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* **79:** 3623–3627.
6. Wade, D. P., B. L. Knight, and A. K. Soutar. 1986. Binding of low-density lipoprotein and chylomicron remnants to the hepatic low-density lipoprotein receptor of dogs, rats and rabbits demonstrated by ligand blotting: failure to detect a distinct chylomicron-remnant-binding protein by ligand blotting. *Eur. J. Biochem.* **159:** 333–340.
7. Jensen, E., C-H. Florén, and Å. Nilsson. 1987. Cell-density-dependent uptake of chylomicron remnants in rat hepatocyte monolayers. Effects of compactin and mevalonic acid. *Biochim. Biophys. Acta.* **917:** 74–80.
8. Windler, E., J. Greeve, W. H. Daerr, and H. Greten. 1988. Binding of rat chylomicrons and their remnants to the hepatic low-density-lipoprotein receptor and its role in remnant removal. *Biochem. J.* **252:** 553–561.
9. Jäckle, S., S. E. Brady, and R. J. Havel. 1989. Membrane binding sites for plasma lipoproteins on endosomes from rat liver. *Proc. Natl. Acad. Sci. USA.* **86:** 1880–1884.
10. Nagata, Y., J. Chen, and A. D. Cooper. 1988. Role of low density lipoprotein receptor-dependent and -independent sites in binding and uptake of chylomicron remnants in rat liver. *J. Biol. Chem.* **263:** 15151–15158.
11. Bowler, A., T. G. Redgrave, and J. C. L. Mamo. 1991. Chylomicron-remnant clearance in homozygote and heterozygote Watanabe-heritable-hyperlipidaemic rabbits is defective. *Biochem. J.* **276:** 381–386.
12. Jäckle, S., E. Runquist, S. Brady, R. L. Hamilton, and R. J. Havel. 1991. Isolation and characterization of three endosomal fractions from the liver of normal rats after lipoprotein loading. *J. Lipid Res.* **32:** 485–498.
13. Jäckle, S., F. Rinninger, J. Greeve, H. Greten, and E. Windler. 1992. Regulation of the hepatic removal of chylomicron remnants and β-very low density lipoproteins in the rat. *J. Lipid Res.* **33:** 419–429.
14. Cooper, A. D., and P. Y. S. Yu. 1978. Rates of removal and degradation of chylomicron remnants by isolated perfused rat liver. *J. Lipid Res.* **19:** 635–643.
15. Mahley, R. W., D. Y. Hui, T. L. Innerarity, and K. H. Weisgraber. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man; apoB,E and apo-E receptors. *J. Clin. Invest.* **68:** 1197–1206.
16. Arbeeny, C. M., and V. A. Rifici. 1984. The uptake of chylomicron remnants and very low density lipoprotein remnants by the perfused rat liver. *J. Biol. Chem.* **259:** 9662–9666.
17. Rubinsztein, D. C., J. C. Cohen, G. M. Berger, D. R. Van der Westhuyzen, and G. A. Coetzee. 1990. Chylomicron remnant clearance from the plasma is normal in familial hypercholesterolemic homozygotes with defined receptor defects. *J. Clin. Invest.* **86:** 1306–1312.
18. Herz, J., U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl, and K. K. Stanley. 1988. Surface location and high affinity for calcium of a 500 kD liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J.* **7:** 4119–4127.
19. Beisiegel, U., W. Weber, G. Ihrke, J. Herz, and K. K. Stanley. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature.* **341:** 162–164.
20. Kowal, R. C., J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. 1989. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* **86:** 5810–5814.
21. Lund, H., K. Takahashi, R. L. Hamilton, and R. J. Havel. 1989. Lipoprotein binding and endosomal itinerary of the low density lipoprotein receptor-related protein in rat liver. *Proc. Natl. Acad. Sci. USA.* **86:** 9318–9322.
22. Kowal, R. C., J. Herz, K. H. Weisgraber, R. W. Mahley, M. S. Brown, and J. L. Goldstein. 1990. Opposing effects of apolipoproteins E and C on lipoprotein binding to low density lipoprotein receptor-related protein. *J. Biol. Chem.* **265:** 10771–10779.
23. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
24. Strickland, D. K., J. D. Ashcom, S. Williams, W. H. Burgess, M. Migliorini, and W. S. Argraves. 1990. Sequence identity between the α_2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. *J. Biol. Chem.* **265:** 17401–17404.
25. Kristensen, T., S. K. Moestrup, J. Gliemann, L. Bendtsen, O. Sand, and L. Sottrup-Jensen. 1990. Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the α_2-macroglobulin receptor. *FEBS Lett.* **276:** 151–155.
26. L. Sottrup-Jensen. 1989. α_2-Macroglobulins: structure, shape, and mechanism of proteinase complex formation. *J. Biol. Chem.* **264:** 11539–11542.
27. Gliemann, J., and O. Davidsen. 1986. Characterization of receptors for α_2-macroglobulin-trypsin complex in rat hepatocytes. *Biochim. Biophys. Acta.* **885:** 49–57.
28. Imber, M. J., and S. V. Pizzo. 1981. Clearance and binding of two electrophoretic "fast" forms of human α_2-macroglobulin. *J. Biol. Chem.* **256:** 8134–8139.
29. Davidsen, O., E. I. Christensen, and J. Gliemann. 1985. The plasma clearance of human α_2-macroglobulin-trypsin complex in the rat is mainly accounted for by uptake into hepatocytes. *Biochim. Biophys. Acta.* **846:** 85–92.
30. Hussain, M. M., F. Maxfield, J. Mas-Oliva, I. Tabas, Z-S. Ji, T. L. Innerarity, and R. W. Mahley. 1991. Clearance of chylomicron remnants by the low density lipoprotein

receptor-related protein α_2-macroglobulin receptor. *J. Biol. Chem.* **266:** 13936–13940.
31. Kovanen, P. T., M. S. Brown, S. K. Basu, D. W. Bilheimer, and J. L. Goldstein. 1981. Saturation and suppression of hepatic lipoprotein receptors: a mechanism for the hypercholesterolemia of cholesterol-fed rabbits. *Proc. Natl. Acad. Sci. USA.* **78:** 1396–1400.
32. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J. Lipid Res.* **19:** 712–722.
33. Windler, E., Y-S. Chao, and R. J. Havel. 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. *J. Biol. Chem.* **255:** 5475–5480.
34. Sottrup-Jensen, L., T. E. Petersen, and S. Magnusson. 1980. A thiol-ester in α_2-macroglobulin claved during proteinase complex formation. *FEBS Lett.* **121:** 275–279.
35. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53–57.
36. Sigurdsson, G., S-P. Noel, and R. J. Havel. 1978. Catabolism of the apoprotein of low density lipoproteins by the isolated perfused rat liver. *J. Lipid Res.* **19:** 628–634.
37. Roche, P. A., D. K. Strickland, J. J. Enghild, and S. V. Pizzo. 1988. Evidence that the platinum-reactive methionyl residue of the α_2-macroglobulin receptor-recognition site is not in the carboxyl-terminal receptor binding domain. *J. Biol. Chem.* **263:** 6715–6721.
38. Mahley, R. W., T. L. Innerarity, S. C. Rall, Jr., K. H. Weisgraber, and J. M. Taylor. 1990. Apolipoprotein E: genetic variants provide insights into its structure and function. *Curr. Opin. Lipidol.* **1:** 87–95.
39. Moestrup, S., and J. Gliemann. 1991. Analysis of ligand recognition by the purified α_2-macroglobulin receptor (low density lipoprotein receptor-related protein). Evidence that high affinity of α_2-macroglobulin-proteinase complex is achieved by binding to adjacent receptors. *J. Biol. Chem.* **266:** 14011–14017.
40. Herz, J., L. J. Goldstein, D. K. Strickland, Y. K. Ho, and M. S. Brown. 1991. 39 kDa protein modulates binding of ligands to low density lipoprotein receptor-related protein/α_2-macroglobulin receptor. *J. Biol. Chem.* **266:** 21232–21238.
41. Van Dijk, M. C. M., G. J. Ziere, W. Boers, C. Linthorst, M. K. Bijsterbosch, and T. J. C. Van Berkel. 1991. Recognition of chylomicron remnants and β-migrating very-low-density lipoproteins by the remnant receptor of parenchymal liver cells is distinct from the liver α_2-macroglobulin-recognition site. *Biochem. J.* **279:** 863–870.