Lipoprotein lipase-enhanced binding of human triglyceride-rich lipoproteins to heparan sulfate: modulation by apolipoprotein E and apolipoprotein C

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Abstract The objective of this study was to investigate whether compositional variation in apolipoprotein (apo) content of triglyceride-rich lipoproteins (TRLP) modulates binding of heparan sulfate proteoglycans (HSPG). Human TRLP was enriched with apoE and apoCs and the ability to bind biotin-conjugated heparan sulfate (b-HS) was studied in the presence or absence of heat-inactivated lipoprotein lipase (LPL). TRLP, associated with LPL, showed an increased capacity to bind b-HS compared with TRLP alone. Low density lipoproteins (LDL) bound both b-HS and LPL with a higher affinity than TRLP. ApoE enrichment of TRLP resulted in an enhanced binding of b-HS. Increased binding of b-HS to TRLP by the combination of apoE enrichment and LPL addition was found to be complementary, not affecting their individual binding capacity. TRLP enrichment with apoC led to the formation of an apoC-rich, apoE-poor particle; this alteration by itself did not change the ability to bind b-HS. ApoC enrichment of TRLP resulted in a reduced capacity to bind LPL and therefore a subsequently reduced capacity to bind b-HS, compared with control TRLP associated with LPL. Competition studies revealed that b-HS binding to TRLP was fully displaceable by lactoferrin but barely by heparan sulfate, dermatan sulfate, or chondroitin-4-sulfate. Using TRLP coated to microtiter wells and associated with LPL, the b-HS displacement patterns were comparable to those obtained with coated LDL in the presence or absence of LPL. The cell-free system that was used enabled us to identify the functions of apoC and apoE in the binding of TRLP to LPL and HSPG. Both LPL and apoE increased the ability of TRLP to bind HSPG. The apoC content of TRLP regulated the docking of TRLP to LPL. ApoC enrichment reduced the affinity or capacity of TRLP to LPL binding, and this has relevance for the lipolytic cascade.—**van Barlingen, H. H. J. J., H. de Jong, D. W. Erkelens, and T. W. A. de Bruin.** 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.

Supplementary key words heparan sulfate proteoglycans • biotin-conjugated heparan sulfate • low density lipoproteins

Triglyceride-rich lipoproteins (TRLP) are produced in the intestine and liver and are defined as chylomicrons and very low density lipoproteins (VLDL), respectively. The first step in TRLP catabolism is lipolysis of the triglyceride (TG) component. Lipolysis requires TRLP binding to lipoprotein lipase (LPL) which is anchored to the endothelial cell by heparan sulfate proteoglycans (HSPG). Apolipoprotein (apo) C-II is the cofactor for LPL (1) and apoC-III is considered a potential inhibitor of LPL activity (2). After TG hydrolysis, TRLP are released from the HSPG-LPL complex as remnants and rapidly cleared from the circulation (3). The clearance of TRLP-remnants from the plasma is mediated by a complex and coordinated action of apolipoproteins and receptors. Uptake of lipoproteins by the liver is mediated by the low density lipoprotein (LDL) receptor (LDL-r) and a remnant receptor, for which the LDL-receptor-related protein (LRP) is a candidate (4). Experimental data have shown that binding to the LDL-r is not the major pathway for remnant removal (5, 6).

ApoE is the high-affinity ligand for receptor-mediated removal of lipoproteins (7–9). ApoE also modulates the capacity of lipoproteins to bind to HSPG and thereby regulates their uptake (10). It is postulated that the uptake of remnants in the hepatic sinusoidal space involves apoE enrichment and interaction with HSPG followed by the transfer of the remnants to LRP for

Abbreviations: apo, apolipoprotein; b-HS, biotin-conjugated heparan sulfate; BSA, bovine serum albumin; FHTG, familial hypertriglyceridemia; HS, heparan sulfate; HRP, horseradish peroxidase; HSPG, heparan sulfate proteoglycans; GAG, glycosaminoglycans; LDL, low density lipoproteins: LPL, lipoprotein lipase; LRP, LDL-receptor-related protein; O.D., optical density; r-apoE, recombinant apoE; TRLP, triglyceride-rich lipoproteins; VLDL, very low density lipoproteins.

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internalization. This mechanism is known as the secretion-capture process of apoE (9, 11, 12). ApoCs diminish the capacity of lipoproteins to bind to receptors (13–15). This inhibitory effect of apoCs involves the displacement, masking, or alteration of apoE (4, 16). The role of apoCs was clearly shown in studies with human apoC-III and apoC-II transgenic mice. These mice developed hypertriglyceridemia, due to remnant accumulation, whereas no defect in lipolysis was found. Displacement of apoE from the remnants, which results in a lower binding affinity for heparan sulfate, may explain the accumulation of remnants (17–19). Subsequent overexpression of apoE corrected the remnant accumulation (20).

HSPG can be postulated to contribute to the catabolism of TRLP in two ways, separated by anatomical localization. First, binding of TRLP to LPL, anchored to HSPG on the endothelium, is an essential step for hydrolysis, and rate-limiting for triglyceride removal. In addition, TRLP-remnant binding to HSPG in the liver seems obligatory for (non-) receptor-mediated removal (21, 22). LPL in addition to apoE also enhances the binding of apoB-containing lipoproteins to HSPG and LRP, an action mainly independent of the LPL enzyme function (23–26).

We recently described that VLDL isolated from patients with hypertriglyceridemia were characterized by a high apoC content (27). The objective of the present study was to characterize the effect of this altered apolipoprotein composition of TRLP on their proteoglycan binding. We therefore studied the in vitro binding of HSPG to human TRLP, which had been enriched with human apoCs, apoE, or recombinant apoE3, in the presence or absence of LPL.

Binding of LDL to vascular subendothelial matrix proteoglycans is considered a proatherogenic event (28, 29) and TRLP-remnants are potentially atherogenic (30). Therefore, the binding characteristics of glycosaminoglycans and lactoferrin, a known inhibitor of remnant clearance (31, 32), to TRLP and TRLP associated with LPL, were compared with LDL.

MATERIALS AND METHODS

Lipoprotein preparation

TRLP and LDL were isolated from the plasma of healthy nonfasting normolipidemic subjects. Phenylmethylsulfonyl fluoride, aprotinin (Bayer AG, Bayer, Germany), and NaN_3 were added to plasma at final concentrations of 1 mmol/l, 100 IU/ml, and 0.01%, respectively. TRLP was isolated from plasma as the density (d) fraction <1.006 g/ml, after centrifugation at 50,000 rpm for 22 h at 4°C (Ti-60 rotor, Beckman L8-80 centrifuge). LDL was isolated as the density band 1.019–1.063 g/ml using a discontinuous gradient (33) after ultracentrifugation at 36,000 rpm for 22 h at 4°C (SW40.1 rotor). TRLP and LDL were dialyzed extensively against 10 mmol/l phosphate-buffered saline (PBS: 1.5 mmol/l KH_2PO_4, 8.1 mmol/l Na_2HPO_4, 140 mmol/l NaCl, 2.7 mmol/l KCl, pH 7.4), stored at 4°C, and used within 5 days.

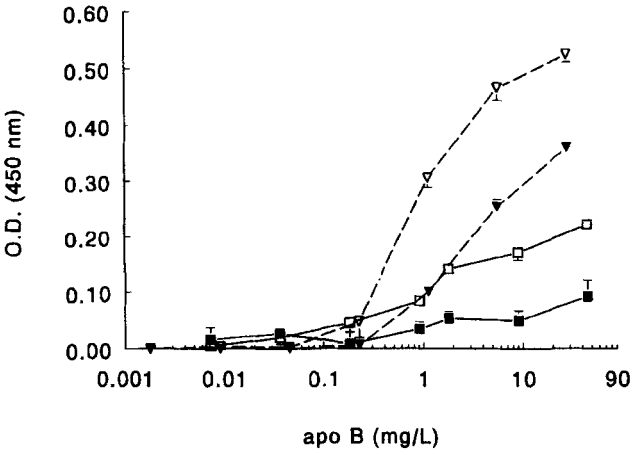

Fig. 1. Binding of b-HS to TRLP or LDL in the presence or absence of LPL. Wells were coated with TRLP at an apoB concentration ranging from 0.003 to 46 mg/l (■) or with LDL at an apoB concentration ranging from 0.002 to 28 mg/l (▼), and incubated for 1.5 h at 37°C. Plates were washed six times with PBS to remove unbound lipoproteins, and incubated with 5% BSA in PBS for 1 h at 37°C to block nonspecific binding sites. After washing with PBS, the plates were incubated with interaction buffer in the absence (closed symbols) or presence (open symbols) of 2.5 μg/ml LPL, for 1 h at 37°C. Plates were washed and incubated with biotin-conjugated heparan sulfate (b-HS, 10 μg/ml) for 1 h at 37°C. After washing and streptavidin peroxidase addition, the color reaction was performed for 6 min. Results represent the mean ± SD for wells measured in triplicate. The apoB concentration is on a log scale.

Apolipoprotein composition

To identify apolipoproteins, 50 μl TRLP was delipidated with 1.17 ml chloroform–methanol 1:1 and 1 ml diethylether. The pellets were resuspended in sample buffer containing 1.25% SDS, 10% glycerol, 30 mmol/l Tris, pH 6.8, 0.003% bromophenol blue, and 2.5% β-mercaptoethanol. Apolipoproteins were separated on precast 20% and 4–15% SDS-PAGE gels (Phastsystem, Pharmacia, Uppsala, Sweden). The gels were stained with silver according to the manufacturer's manual and analyzed for integrated absorbance with an image analysis scanning system (IBAS, Zeiss/Kontron, Eching, Germany) (34). The intra-assay variation (precipitation, silver staining, and scanning) was less than 10%.

Isolation of apolipoproteins

All isolation steps were performed at 4°C. TRLP were delipidated with 20 vol ethanol–ether 3:1. The pellet was

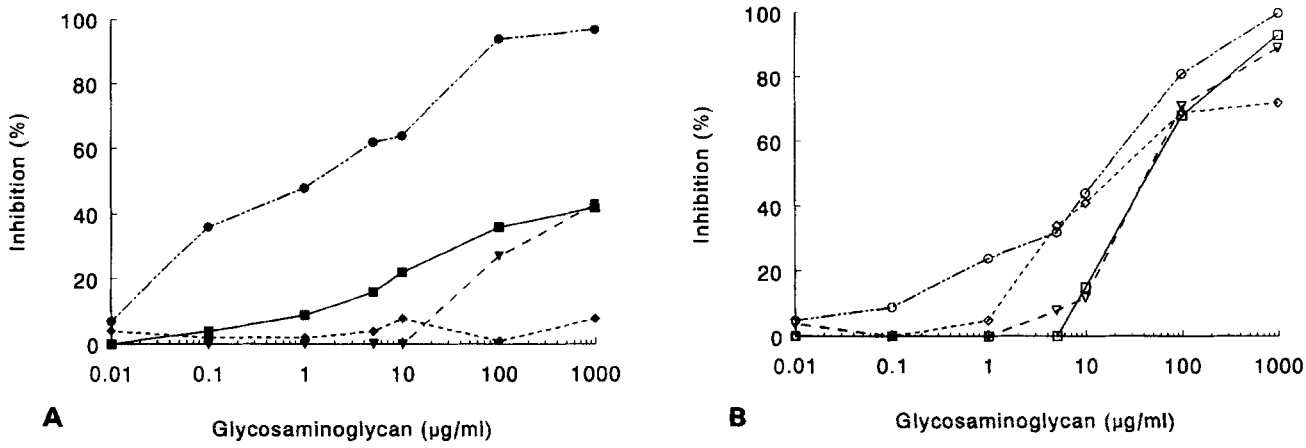

Fig. 2. Competition of b-HS binding to TRLP by glycosaminoglycans. Wells were coated with TRLP at a protein concentration of 100 mg/l. The competition experiments were performed in the absence of LPL (panel A, closed symbols) and presence of 2.5 μg/ml LPL (panel B, open symbols) in interaction buffer. b-HS (10 μg/ml) binding to TRLP was inhibited with heparan sulfate (■), dermatan sulfate (◆), chondroitin-4-sulfate (▼), or lactoferrin (●) in concentrations ranging from 0.01 to 1000 μg dry weight/ml. Measured absorbance in absence of competitors was fixed as zero percent inhibition, and effectiveness of competition was calculated as percentage of decrease in absorbance. Results represent the mean ± SD for wells measured in triplicate. The glycosaminoglycan concentration is on a log scale.

washed three times with ether, dried, and dissolved in running buffer A, (2 mmol/l NaH_2PO_4, 5 M urea, 0.5 mmol/l EDTA, 0.01% NaN_3, pH 8.5). ApoE was isolated by affinity chromatography on a heparin-Sepharose CL-6B column (Pharmacia, Uppsala, Sweden). ApoE was eluted with a gradient of 0–500 mmol/l NaCl in running buffer A. ApoE-containing fractions were pooled and dialyzed extensively against PBS. The void volume, which contained apoAs and apoCs, was dialyzed against PBS, and finally H_2O, lyophilized, and dissolved in running buffer B (200 mmol/l Tris, 6 M urea, 1 mmol/l EDTA, 0.01% NaN_3, pH 8.3). The apolipoproteins were separated by gel filtration on a Sephadex G-75 column (Pharmacia, Uppsala, Sweden).

The purity of apoE and apoCs was determined on SDS-PAGE gels. Purity of apolipoproteins was confirmed by immunoblotting, after transfer of the proteins to nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany) using the Phastsystem blotting system, incubated for 1 h in PBS containing 5% bovine serum albumin (BSA, Sigma, St. Louis, MO) at 20°C, washed six times with PBS, and incubated overnight at 20°C with monoclonal antibodies against apoE (1D7), apoA-I (5F8), (kind gifts from Dr. Y. L. Marcel, Ottawa, Canada), and with affinity-purified polyclonal antibodies against apoC-III and apoC-II (Daiichi Pure Chemicals, Tokyo, Japan). Membranes were washed six times with PBS and incubated with horseradish peroxidase (HRP)-conjugated anti-IgG antibodies (DAKO, Glostrup, Denmark) followed by development with tetramethylbenzidin (peroxidase substrate). Isoelectric focussing revealed that the apoC-II/apoC-III ratio (0.45) and the apoC-III isoform distribution (C-III-0, 25%; C-III-1, 42%; C-III-2, 32% of total apoC-III) of the apoC preparation was identical to that in VLDL of normolipidemic controls (27). Isolated apolipoproteins were dialyzed extensively against PBS and lyophilized for storage.

Modification of TRLP

TRLP was extensively dialyzed against 200 mmol/l Na-phosphate buffer, pH 6.5 at 4°C, and incubated at a TRLP:apolipoprotein ratio of 1:0.5 on protein basis, with isolated apoCs, apoE, or recombinant apoE (r-apoE, isoform E-3; kindly provided by Dr. T. Vogel, Bio-Technology General, Rehovot, Israel), respectively, for 1 h at 37°C. After addition of KBr to a density of 1.063 g/ml, the samples were kept on ice. Samples (2.5 ml) were underlayered with KBr solution (d 1.063 g/ml) and centrifuged for 9.87 ω^2t 24,500 rpm at 4°C (SW 50.1 rotor). After ultracentrifugation, modified TRLP was recovered in the top fraction by tube slicing at 1.25 cm from the top; the lower fraction contained unbound apolipoproteins. Modified TRLP was dialyzed against PBS.

Apolipoprotein B quantification

The concentration of apoB in TRLP and LDL fractions was measured by sandwich ELISA, as previously described (35), using a polyclonal sheep anti-human-apoB antiserum (Boehringer Mannheim GmbH, #13129021-08, Mannheim, Germany) as capture antibody, and the same antibody, affinity-purified on an LDL-Sepharose column and coupled with HRP for detection. Precinorm L (Boehringer Mannheim GmbH, Mannheim, Germany) was used as standard.

Preparation of biotin-conjugated heparan sulfate

Heparan sulfate (HS) from bovine kidney (Sigma, St. Louis, MO) was used. This well-characterized preparation has a sulfation level intermediate between rat liver and human endothelial cells (36). HS was conjugated with biotin by mixing HS with N-hydroxysuccinimide-biotinate (Bio-Rad, Richmond, CA) at a 1:10 molar basis (37). The mixture was incubated for 4 h at 4°C under continuous stirring, and dialyzed subsequently against 20 mmol/l Tris-buffer, pH 7.4, to block unbound N-hydroxysuccinimide-biotinate. Free biotin was separated from b-HS through a PD-10 column (Pharmacia, Uppsala, Sweden). The biotin-conjugated HS was stored at 4°C until used. The b-HS concentration was determined (38) with HS (dry wt/ml) as a standard. Bovine mucosa dermatan sulfate, bovine trachea chondroitin-4-sulfate, and lactoferrin were purchased from Sigma (St. Louis, MO).

LPL isolation

Bovine LPL was isolated from bovine milk according to the method of Posner, Wang, and McConathy (39), as previously described (27). LPL was eluted as one single fraction at 1.3 mol/L NaCl, dialyzed against interaction buffer containing 10 mmol/l Tris, 50 mmol/l NaCl, 5 mmol/l $CaCl_2$, pH 7.0, heat-inactivated by incubation at 50°C for 4 h (24), and stored at -70°C. In our lipolysis assay (27), heat-inactivated LPL was not able to generate free fatty acids from a TRLP suspension. Protein concentrations were determined by SDS-Lowry assay (40) with BSA as a standard. The heat inactivation procedure did not alter the detection of LPL protein by immunoblot using antibodies against bovine LPL (mouse monoclonal 5D2, a kind gift from Dr. J. D. Brunzell, Seattle, WA, or rabbit polyclonal 380, a kind gift from Dr. H. Jansen, Rotterdam, The Netherlands). No LPL degradation products were detectable but enzyme activity was completely lost.

Assay for the binding of b-HS to TRLP

A modification of the methods of Baker and Christner (37) and Edwards et al. (29) was used. TRLP and LDL (100 µl/well) in PBS were immobilized by passive adsorption to the wells of flat-bottom microtiter plates (Greiner GmbH, Frichenhausen, Germany) for 1.5 h at 37°C. The plates were washed with PBS, and nonspecific binding sites were blocked by incubation with 300 µl 5% BSA in PBS per well for 1 h at 37°C. The wells were then washed with PBS. Where indicated, 2.5 µg/ml LPL (100 µl/well) in interaction buffer containing 1% BSA was added. One hundred µl of interaction buffer containing 1% BSA was added to the control wells. After a 1-h incubation at 37°C, unbound LPL was removed by washing the wells with PBS and 100 µl/well b-HS (10 µg/ml) in interaction buffer containing 0.1% BSA was added. Where indicated, competing glycosaminoglycans diluted in 50 µl interaction buffer containing 0.1% BSA was added to the wells immediately after the addition of 50 µl 20 µg/ml b-HS in interaction buffer containing 0.1% BSA. Unbound b-HS was removed by washing the wells with PBS. Streptavidin peroxidase (Bio-Rad, Richmond, CA), 100 µl of a 1/5000 dilution in 50 mmol/l Tris, pH 7.0, containing 30 mmol/l $CaCl_2$ and 1% BSA, was added to the wells and the plates were incubated for 1 h at 37°C. The wells were washed with PBS and 100 µl of freshly prepared staining solution (0.1 g/l tetramethylbenzidin, 0.005% H_2O_2 in 100 mmol/l Na-acetate, pH 5.5) was added to the wells. The reaction was stopped after 15 min by the addition of 50 µl 2 mol/l H_2SO_4 per well. The plates were vortexed and absor-

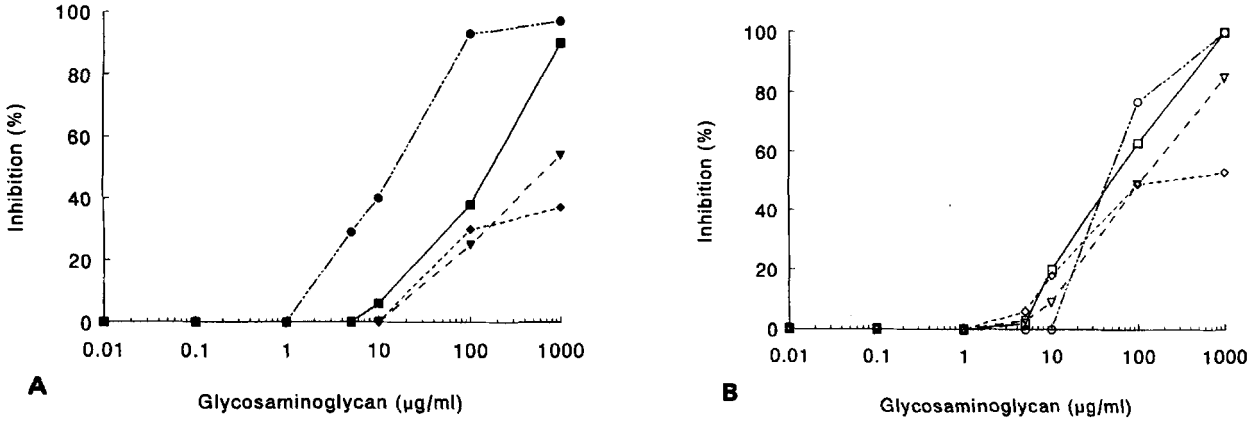

Fig. 3. Competition of b-HS binding to LDL by glycosaminoglycans. LDL was coated on microtiter wells at a protein concentration of 50 mg/l. Subsequently LDL was incubated in the absence of LPL (panel A, closed symbols) or presence of 1 µg/ml LPL (panel B, open symbols). b-HS (10 µg/ml) binding to LDL was inhibited with heparan sulfate (■), dermatan sulfate (◆), chondroitin-4-sulfate (▼), or lactoferrin (●) in concentrations ranging from 0.01 to 1000 µg dry weight/ml. Measured absorbance in the absence of competitors was fixed as zero precent inhibition; effectiveness of competition was calculated as the percentage of decrease in absorbance. Results represents the mean ± SD for wells measured in triplicate. The glycosaminoglycan concentration is on a log scale.

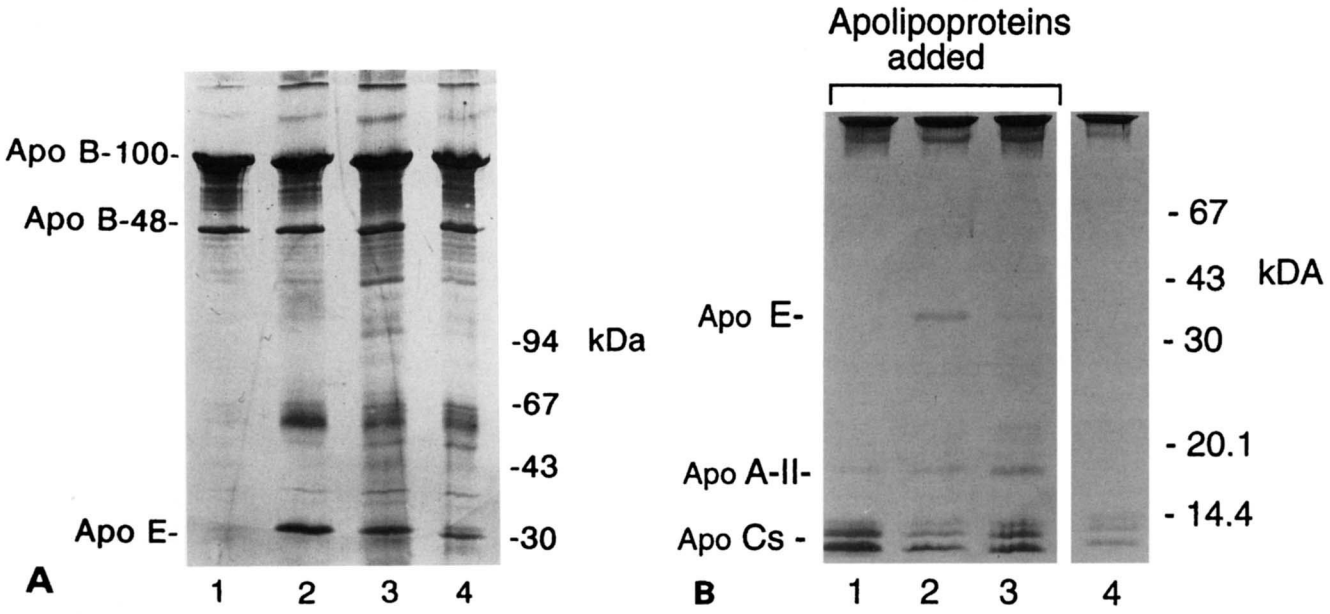

Fig. 4. A: Analysis of control and apolipoprotein-enriched TRLP. TRLP protein (0.50 μg) was separated on 4–15% SDS-PAGE gradient gels. The proteins were stained with silver. Lane 1 contained TRLP incubated with apoC; lane 2, TRLP incubated with r-apoE; lane 3, TRLP incubated with apoE; and lane 4 shows unmodified TRLP (control). The position of low molecular weight SDS-PAGE standards (Pharmacia, Uppsala, Sweden) in kDa is indicated on the right side. The positions of apoB-100, apoB-48, and apoE are indicated on the left side. The amounts of apoB-100 and apoB-48 loaded were identical for all lanes, as assessed by integrated absorbance measurements. B: Analysis of apolipoprotein-enriched TRLP. TRLP protein (0.50 μg) was separated on 20% SDS-PAGE gel and stained with silver. Lane 1 contained TRLP incubated with apoC; lane 2, TRLP incubated with r-apoE; lane 3, TRLP incubated with apoE; and lane 4 shows unmodified TRLP (control). The position of low molecular weight SDS-PAGE standards is indicated on the right side. The positions of apoE, apoA-II, and apoC are indicated on the left side.

bance at 450 nm was measured on a 340 ATTC microtiter plate reader (SLT Labinstruments, Salzburg, Austria). Nonspecific binding measured in BSA-coated wells, incubated with LPL and/or b-HS and/or avidin-HRP, was always less than 10% of total binding. In all experiments the measured absorbance was corrected for background. In lipoprotein-coated wells incubated with avidin-HRP, a similar background was observed as compared with BSA-coated wells. All experiments were performed at least in duplicate with freshly isolated and modified TRLP.

To determine binding of TRLP or LDL to microtiter plates, the affinity-purified HRP-coupled polyclonal sheep anti-human-apo-B antiserum was used. After TRLP or LDL coating and blocking with BSA, wells were washed with PBS and incubated with anti-apoB antibody for 1 h at room temperature. Wells were washed and the color reaction was performed as described.

Statistical analysis

Correlations and comparisons of binding curves were made by Multivariant ANOVA (MANOVA) with contrast polynomial, using the SPSS/PC+ program (41).

RESULTS

Enhancement of b-HS binding to TRLP or LDL in the presence or absence of LPL

The initial studies demonstrated that incubation of coated TRLP in the presence of LPL increased the binding capacity of b-HS in a gradual fashion, depending on LPL concentration, with near saturation at 2.5 μg LPL/ml.

Increasing amounts of TRLP or LDL were coated to the wells and incubated with 2.5 μg/ml LPL, after removal of unbound TRLP or LDL. As shown in **Fig. 1,** with both LDL and TRLP the binding of b-HS increased with increasing apoB concentrations. The binding of b-HS to either TRLP or LDL was higher in the presence of LPL. b-HS and LPL had a higher capacity for binding to LDL than TRLP, based on the concentration of apoB plated. In order to quantify the LPL binding to the different lipoprotein particles, similar amounts of LDL or TRLP were immobilized to the microtiter plate. This was confirmed by measuring the amount of apoB immobilized, by the detecting antibody used in the apoB ELISA, (measured absorbance: LDL, 0.717 ± 0.011; TRLP, 0.692 ± 0.022). LPL associated with either LDL or TRLP was measured using the anti-LPL antibody 5D2: LDL associated with LPL, 0.445 ± 0.011; TRLP associated with LPL, 0.244 ± 0.040; and using the anti-LPL polyclonal 380 antibody: LDL associated with LPL, 0.447 ± 0.005; and TRLP associated with LPL, 0.342 ± 0.016. To accommodate for this difference we used an LPL concentration of 1 μg/ml LPL in all our experi-

ments with coated LDL binding as this LPL concentration elicited an increase in b-HS binding for LDL similar to 2.5 μg/ml LPL for TRLP.

Displacement of b-HS from lipoproteins, in the presence or absence of LPL, by glycosaminoglycans

The binding characteristics of b-HS to coated TRLP or TRLP associated with LPL were investigated by addition of increasing concentrations of GAGs or lactoferrin. In the absence of LPL, only lactoferrin could fully displace b-HS from TRLP. Heparan sulfate and chondroitin-4-sulfate hardly competed with b-HS for binding to TRLP, and dermatan sulfate was ineffective (**Fig. 2A**). In contrast, increasing concentrations of lactoferrin, heparan sulfate, chondroitin-4-sulfate, and dermatan sulfate displaced b-HS from TRLP associated with LPL (Fig. 2B). To exclude the possibility that non-displaceable binding of b-HS to TRLP was caused by the binding of biotin to TRLP, free biotin (concentration ranging from 0.01 to 100 μg dry weight/ml) was used instead of b-HS in the binding assay. Biotin did not bind to TRLP (data not shown) and, moreover, addition of the competitor concomitantly with b-HS, or changing the order of addition to the well, did not alter the pattern observed with either TRLP or TRLP associated with LPL.

To understand the discrepancy between the characteristics of b-HS binding to TRLP and TRLP associated with LPL, we performed identical experiments with LDL and LDL associated with LPL. LDL was coated in a concentration of 50 mg apoB/l and incubated in the presence or absence of 1 μg/ml LPL. The best competitors (**Fig. 3**) were lactoferrin and heparan sulfate and less effective were chondroitin-4-sulfate and dermatan sulfate, although this was only obvious at the highest concentration used. The effective competition of lactoferrin and glycosaminoglycans with b-HS for binding to LDL or LDL associated with LPL (Fig. 3B) showed characteristics comparable to those observed with b-HS binding to TRLP associated with LPL (Fig. 2B). These characteristics include a consistent but moderate displacement by chondroitin-4-sulfate and dermatan sulfate and full displacement by heparan sulfate. TRLP had a different binding strength to b-HS compared with TRLP associated with LPL, LDL, and LDL associated with LPL, because TRLP showed tight binding to b-HS that was only 40% displaceable by GAGs. As the non-displaceable binding was not observed with TRLP associated with LPL, we postulate that LPL could reduce or block the tight binding of b-HS to TRLP. To analyze this further, we studied the accessibility of antibodies against apoB and apoE on TRLP, after LPL incubation. Equal amounts of coated TRLP and LDL, based on apoB concentrations, were used. After washing, the anti-apoB antibody was applied. TRLP associated with LPL showed a strong reduction in detectable apoB (absorbance 0.222 ± 0.043) compared with TRLP alone (absorbance 0.692 ± 0.022); this was not seen for LDL. This finding indicated that binding of LPL to TRLP covered epitopes for the used anti-apoB antibody; these epitopes can be involved in the non-displacable b-HS binding. On the other hand, incubation with LPL did not reduce the binding of the anti-apoE antibody (1D7) to coated TRLP, indicating that LPL binding to TRLP did not hinder the accessibility of apoE.

Effect of modified TRLP

Incubation of TRLP with apoE or r-apoE resulted in a more than twofold increase in the apoE/apoB ratio,

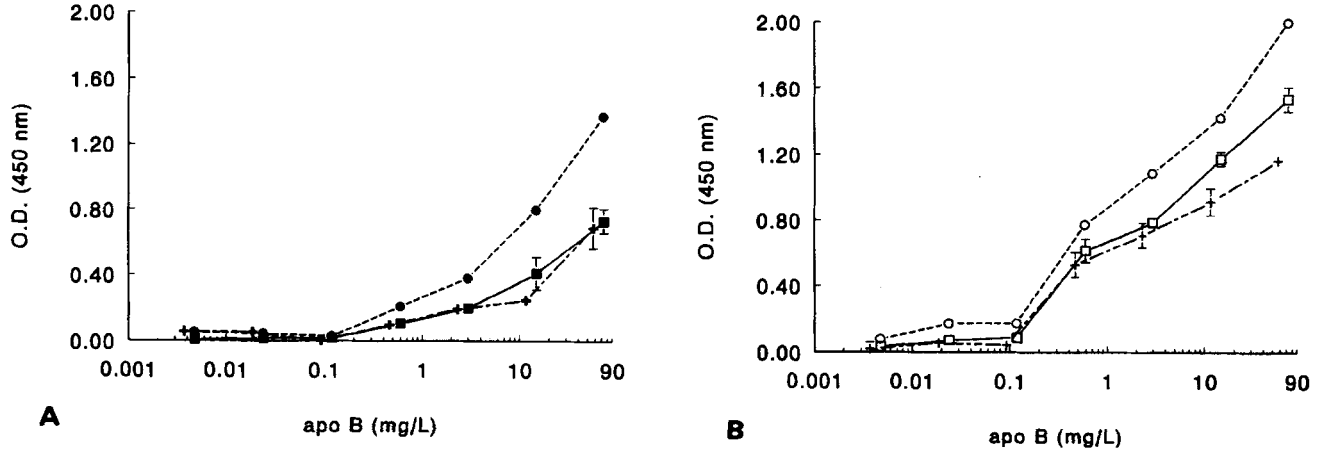

Fig. 5. b-HS binding to modified and control TRLP. Wells were coated with control TRLP (■), TRLP enriched with apoE (●) or apoC (✚), each preparation at an apoB concentration ranging from 0.005 to 90 mg/l. Unbound TRLP was removed by washing. Experiments were done in the absence of LPL (panel A, closed symbols) or presence of 2.5 μg/ml LPL (panel B, open symbols). Finally 10 μg/ml b-HS was added. Results represent the mean ± SD for wells measured in triplicate.

as measured by the ratio of the absorbance of the apoE and apoB bands on 4–15% SDS-PAGE (**Fig. 4A**), compared with control TRLP on the same gel. Addition of apoE resulted in a twofold reduction of the apoCs/apoE ratio (Fig. 4B). However, addition of apoCs resulted in a fourfold increase in the apoCs/apoE ratio, due to apoC enrichment and displacement of apoE from the TRLP. This was also confirmed by the concomitant reduction in the apoE/apoB ratio to approximately 25% of the control TRLP on the 4–15% SDS-PAGE gel. After TRLP enrichment with apoC, no change in the individual apoC contribution was found between control and apoC-enriched TRLP: apoC-II/apoC-III ratio (0.45 versus 0.38), and apoC-III isoform distribution (C-III-0, 22%; C-III-1, 47%; C-III-2, 32% of total apoC-III for apoC-enriched TRLP, versus 22%, 48%, and 30% for control-TRLP, respectively), as measured by isoelectric focussing (27).

To characterize the effect of TRLP concentration and TRLP modification on binding to microtiter wells, increasing amounts of modified TRLP and control TRLP were added to the wells and incubated with anti-apoB antibody. The measured absorbance was the same for the tested samples based on the concentration of apoB added to the well (results not shown), indicating that modified TRLP bound in a similar, quantitative manner as native control TRLP.

Binding experiments with modified TRLP (**Fig. 5**) showed that addition of apoE or r-apoE (identical results, not shown) increased the capacity of TRLP to bind b-HS, irrespective of the presence or absence of LPL ($P < 0.001$ vs. control TRLP curve). ApoC enrichment of TRLP and consequently apoE depletion did not affect the binding of b-HS to the particle. In contrast, when apoC-enriched TRLP was associated with LPL, the increase in binding capacity for b-HS due to LPL was significantly lower ($P < 0.001$), as compared to control TRLP associated with LPL, although the binding was still higher than in the absence of LPL.

DISCUSSION

Interaction of human TRLP with proteoglycans and LPL is functionally regulated by apoC and apoE. Our results stress the importance of the apolipoprotein composition of TRLP for their binding to HSPG or LPL-HSPG complexes. The presence of apoC, although known to be essential for triglyceride hydrolysis, inhibits the tight binding of TRLP to LPL-HSPG when present in excess. This may be functional at the level of the endothelial cell to regulate the lipolytic cascade. The loss of apoC from the TRLP remnant and subsequent apoE enrichment may promote the clearance of the TRLP remnant within the liver by an HSPG and/or receptor-mediated pathway.

The level of sulfation and thereby negative charge of the HS molecule is a major determinant of its binding properties. As we were interested in the effect of TRLP composition on HS binding, we chose an HS-preparation with a sulfation level intermediate to that in rat liver and human endothelial cells and comparable to human skin fibroblasts, the most frequently used cell system in lipoprotein binding studies (36). The possibility remains that the cell-specific structure of HS modulates TRLP binding, and this is currently under investigation in our laboratory.

In our cell-free assay, both immobilized human TRLP and LDL bound b-HS. Interaction of the immobilized lipoproteins with LPL increased b-HS binding, because the association of LPL with TRLP or LDL induced extra binding sites for b-HS. The binding capacity of LDL for b-HS was higher than that of TRLP, irrespective of the presence or absence of LPL. In addition to its higher b-HS binding capacity, LDL also bound more LPL.

The competition experiments revealed that the ability of glycosaminoglycans to compete with b-HS for binding to TRLP associated with LPL closely resembles that observed for LDL, or LDL associated with LPL. On the other hand, in the absence of LPL, heparan sulfate and chondroitin-4-sulfate hardly affected the binding of b-HS to TRLP, and dermatan sulfate did not compete at all with b-HS. At present we cannot explain why there was little competition for binding to TRLP. One possibility is the expression of specific apoB epitopes on TRLP. Using antibodies directed against apoB we found evidence that LPL is capable of masking epitopes on apoB, extending previous results obtained by studies using monoclonal antibodies (42). ApoB epitopes on TRLP, covered by LPL, and not present or of minor importance for b-HS binding to LDL, can be responsible for the different binding characteristics. We have no evidence indicating that the differences in binding characteristics could be ascribed to apoB-48 present in our TRLP preparations. We used preparations with various amounts of apoB-48, ranging from 1 to 18% of total apoB; however, in all experiments results were similar. Therefore we have no data to suggest that apoB-48-containing TRLP interact differently with heparan sulfate than apoB-100-containing TRLP.

In vivo, the firm binding of human TRLP to HSPG in the vessel wall may lead to local lipoprotein and lipid deposition. This phenomenon can contribute to the tendency for premature atherosclerosis in patients with hypertriglyceridemia and delayed remnant clearance, for instance familial combined hyperlipidemia and dysbetalipoproteinemia, where there is excess of TRLP in the circulation (34, 43).

Lactoferrin was the best competitor of b-HS binding to TRLP or LDL, in the presence or absence of LPL. The inhibition profile of lactoferrin differed from that of the glycosaminoglycans. This is consistent with the conclusion that lactoferrin binds to b-HS directly, inhibiting b-HS from binding to TRLP, confirming the results by Ji and Mahley (44). The ability of lactoferrin to bind heparan (45) is probably based on its structural homology with the heparan binding site of apoE and on its overall positive charge (46). This leads to the conclusion that lactoferrin is able to block the apoE interaction with the remnant receptor (31) and the HSPG-mediated catabolism of TRLP. Both mechanisms may occur simultaneously.

Increasing the apoE/apoB ratio by saturating TRLP with isolated human apoE or recombinant apoE3 resulted in a significantly increased binding of b-HS. Incubation of immobilized apoE-enriched TRLP with LPL further increased the binding of b-HS. Our data confirm and extend the results obtained by Ji et al. (12) who showed that saturation of remnant lipoproteins with apoE increased their binding to HSPG. This, in turn, may lead to a faster removal of lipoproteins via receptor-dependent and -independent mechanisms. The magnitude of increased binding of b-HS elicited by LPL was identical for apoE-enriched TRLP to that observed with non-modified TRLP. Thus, it can be concluded that apoE saturation increases the capacity of TRLP to bind HSPG without affecting LPL-mediated HSPG binding. Furthermore, using antibodies directed against apoE or LPL, we showed that apoE and LPL did not bind each other or compete for binding to TRLP (42, 47). Because LPL binding to HSPG and TRLP involved different sites of the molecule (48), the present results indicate that the increased binding of b-HS to TRLP enriched with apoE is independent of the increased binding of b-HS to TRLP associated with LPL. These independent mechanisms are cooperative in b-HS binding to TRLP, and the results are in agreement with the effects of apoE and LPL on the cellular binding of LDL (49).

In the absence of LPL, no significant effect of apoC saturation was observed on b-HS binding to TRLP. This indicated that displacement of apoE by apoC did not directly affect the binding of TRLP to HSPG. The reduction in the number of apoE molecules, and thereby the binding capacity of the lipoprotein, may have been compensated for by the increased total apolipoprotein content of the TRLP. In contrast, in the presence of LPL, apoC-enriched TRLP showed a lower binding of b-HS than control TRLP associated with LPL. The present data show that massive apoC enrichment hampered the binding of LPL to TRLP. However, a high apoC content in TRLP does not affect their substrate-affinity for LPL in solution (27) and therefore does not reduce the hydrolysis of the triglyceride component. We postulate that apoCs prevent TRLP from being fully hydrolyzed, when bound at one position on endothelial HSPG. This intermittent regulation (by binding, release, and binding) will enable the widespread distribution of TRLP and their hydrolysis products, free fatty acids, in the plasma compartment. Furthermore, the relatively lower binding affinity of apoC-enriched TRLP for LPL can diminish the potential uptake of TRLP by the endothelium through an LPL-HSPG pathway (50).

The transfer of apoC from TRLP to HDL is probably essential for the clearance of TRLP remnants, as apoC inhibits receptor binding (13–15). TRLP hydrolysis by LPL leads to a reduction in size and the subsequent transfer of apolipoprotein C to HDL (51), leading to the formation of a TRLP remnant particle. This TRLP remnant eventually reaches the space of Disse and becomes enriched with apoE, binds to HSPG, probably in cooperation with hepatic lipase and resident LPL, and is subsequently internalized after interacting with LRP or LDL-r (9, 11, 12). The present results indicate that apoC displacement by apoE is less efficient than vice versa (Fig. 4B), illustrating the importance of apoC transfer to HDL during remnant formation.

An increased apoC content was found in VLDL isolated from patients with familial hypertriglyceridemia (FHTG) (27). Our results predict that in FHTG subjects, there will be a reduced binding of TRLP to LPL, anchored to HSPG, and therefore a delayed or reduced hydrolysis of triglycerides. The high apoC content of the lipoproteins in FHTG may delay their apoE enrichment and therefore impair remnant catabolism.

Our results provide new information on the role of apoC in the catabolism of TRLP. Experiments with hypertriglyceridemic apoC-III and apoC-II transgenic mice have shown that lipolysis was not delayed in vitro. Nevertheless, the present results predict that in vivo delayed lipolysis occurs at the level of the vascular wall when there is reduced binding of apoC-rich TRLP to LPL, anchored by HSPG on endothelial cells. In addition, a high apoC content will delay apoE enrichment and therefore decrease remnant uptake in the liver, in agreement with recent observations (17–19).

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