HDL regulates the displacement of hepatic lipase from cell surface proteoglycans and the hydrolysis of VLDL triacylglycerol

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Abstract **We have previously shown that hepatic lipase (HL) is inactive when bound to purified heparan sulfate proteoglycans and can be liberated by HDL and apolipoprotein A-I (apoA-I), but not by LDL or VLDL. In this study, we show that HDL is also able to displace HL directly from the surface of the hepatoma cell line, HepG2, and Chinese hamster ovary cells stably overexpressing human HL. ApoA-I is more efficient at displacing cell surface HL than is HDL, and different HDL classes vary in their ability to displace HL from the cell surface. HDL_2s have a greater capacity to remove HL from the cell surface and intracellular compartments, as compared with the smaller HDL particles. The different HDL subclasses also uniquely affect the activity of the enzyme. HDL_2 stimulates HL-mediated hydrolysis of VLDL-triacylglycerol, while HDL_3 is inhibitory. Inhibition of VLDL hydrolysis appears to result from a decreased interlipoprotein shuttling of HL between VLDL and the smaller, more dense HDL particles. This study suggests that high HDL_2 levels are positively related to efficient triacylglycerol hydrolysis by their ability to enhance the liberation of HL into the plasma compartment and by a direct stimulation of VLDL-triacylglycerol hydrolysis.**—Ramsamy, T. A., J. Boucher, R. J. Brown, Z. Yao, and D. L. Sparks. **HDL regulates the displacement of hepatic lipase from cell surface proteoglycans and the hydrolysis of VLDL triacylglycerol. *J. Lipid Res.* 2003.** 44: **733–741.**

Supplementary key words heparan sulfate proteoglycans • apolipoprotein A-I • lipolysis

Human hepatic lipase (HL) is a 64 kDa glycoprotein anchored by heparan sulfate proteoglycans (HSPGs) to the surface of endothelial cells and hepatocytes (1). HL functions both as a cell surface ligand for lipoprotein uptake and as a lipolytic enzyme that mediates the clearance of triacylglycerol from the blood stream and the conversion of VLDL to LDL (2–9). It has been known for over five decades that displacement of lipolytic enzymes from cell surface binding sites with heparin results in rapid hydrolysis of triacylglycerol-rich lipoproteins in lipemic serum (10, 11). However, it is still commonly believed that both HL and lipoprotein lipase (LPL) are catalytically active when bound to cell surface proteoglycans and that this association may indeed enhance the lipolysis of triacylglycerol [as reviewed in ref. (12)].

This common view is in fact counterintuitive to the interfacial catalytic models proposed for lipases, which have shown a clear requirement for enzyme hopping or shuttling between substrates for optimal hydrolysis (13, 14). In agreement with this view, we showed that HL is active only when it is free in solution, and indeed is completely inactive when bound to pure HSPG (15). In addition, we showed that HL could be displaced from a pure HSPG matrix by HDL, and specifically by apolipoprotein A-I (apoA-I), but not by LDL or VLDL. In the present study, we have reevaluated this displacement phenomenon in HepG2 cells and in a Chinese hamster ovary cell line stably overexpressing human HL (CHO-hHL). As with our pure HSPG studies, we show that only apoA-I and HDL are able to displace cell surface HL. We further show that different HDL classes vary in their ability to displace HL, and demonstrate that the lowest density fractions (HDL_2) have the greatest capacity to remove HL from the cell surface and intracellular compartments.

In addition to their ability to displace cell surface HL, we have previously reported that apoA-I and HDL directly affect HL-mediated triacylglycerol hydrolysis, and showed that the rate of triacylglycerol hydrolysis is regulated by the amount of HDL in plasma (15). This observation sug-

Abbreviations: CHO-hHL, Chinese hamster ovary cell line stably overexpressing human hepatic lipase; ECM, extracellular matrix; EMEM, Eagle's minimal essential medium; FAF-BSA, essentially fatty acid-free BSA; HL, hepatic lipase; HRP, horseradish peroxidase; HSPG, heparan sulfate proteoglycan; [^{3}H]TG, [^{3}H]triolein; MAb, monoclonal antibody; pen/strep, penicillin/streptomycin; POPC, palmitoyl-2-oleoyl-phosphatidylcholine; rHDL, reconstituted HDL.

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gests that pre- and postheparin HL activities are a function of a direct effect of apoA-I and HDL on the enzyme activity, in addition to the amount of circulating enzyme. We have extended this previous work and now show that HL-mediated triacylglycerol hydrolysis is also a function of the kind of HDL in plasma. While the total HDL fraction is inhibitory to HL, the component HDL fractions affect HL quite differently. We find that the smaller HDL_3s are inhibitory to VLDL hydrolysis, while the larger and more buoyant HDL_2s are, conversely, quite stimulatory. Modulation of HL activity is therefore related to the lipidation and interfacial properties of the particles. In addition, we show that, in contrast to our earlier hypothesis, inhibition of VLDL hydrolysis is not caused by apoA-I transfer to the lipoprotein surface, but appears to result from alterations in the interlipoprotein shuttling of HL between VLDL and the different HDL particles.

In this study, we demonstrate that HDL regulates both the displacement of HL from cell surface HSPG and also the hydrolysis of VLDL-triacylglycerol. HDL speciation appears to play an important role in both the clearance of plasma triacylglycerol and the catabolism of VLDL.

EXPERIMENTAL PROCEDURES

Materials

Free fatty acid (FFA) diagnostic kits were purchased from Roche Diagnostics (Laval, Canada). Novex polyacrylamide gels, Ham's F12 medium, Geneticin® Selective Antibiotic (G418 sulfate), Eagle's minimal essential medium (EMEM), L-glutamine, and penicillin/streptomycin (pen/strep) were purchased from Invitrogen (Burlington, Canada). The anti-mouse IgG horseradish peroxidase (HRP)-linked whole antibody (isolated from sheep), the broad range molecular weight markers, and the ^{125}I were obtained from Amersham Pharmacia Biotech (Baie d'Urfé, Canada). The SuperSignal West Pico and West Dura chemiluminescent substrates were purchased from Pierce Chemical Co. (Rockford, IL). Cytochalasin B, FBS, triolein, heparin, and essentially fatty acid-free (FAF)-BSA were obtained from Sigma (St. Louis, MO). [^{3}H]triolein ([^{3}H]TG) was purchased from NEN, PerkinElmer Canada, Inc. (Woodbridge, Canada). 1-Palmitoyl-2-oleoyl-phosphatidylcholine (POPC) was purchased from Avanti Polar Lipids (Alabaster, AL). CHO and HepG2 cell lines were obtained from ATCC (Manassas, VA). The anti-HL monoclonal antibody (MAb) XHL3-6, anti-apoA-I MAbs 4H1 and 5F6, and anti-apoB 100 MAbs 1D1 and 4G3 were obtained from Drs. Bensadoun, Marcel, and Milne, respectively. All other reagents were of analytical grade.

HL purification

HL was purified from postheparin human plasma by heparin affinity chromatography as described by Ehnholm et al. (16). Briefly, postheparin human plasma was collected from normolipidemic subjects, and a 20% triacylglycerol emulsion (Intralipid 20%, Baxter Corp., Toronto, ON) was added to the plasma. Lipid cakes were harvested centrifugally and delipidated. The aqueous solution of crude HL was then loaded onto a Heparin Sepharose CL-6B column and eluted with a salt gradient of 0.15 M to 1.50 M NaCl in 5 mM sodium barbital (pH 7.4), 20% glycerol. The HL fractions were pooled and stored at −80°C until required.

HL activity was characterized using a [^{3}H]TG emulsion and quantified into units of enzyme activity (where 1 unit = 1 μmol fatty acid hydrolyzed/h). The protein concentration of the purified HL was 0.114 mg/ml, and the specific activity of the isolated HL was determined to be 19,455 U/mg protein. The isolated HL was further characterized by SDS-PAGE (12%), and immunoblotting executed using the anti-HL MAb. A single band with an apparent molecular mass of 66 kDa was evident.

Isolation of lipoproteins

Plasma from fasting, normolipidemic subjects was collected. VLDL and HDL were isolated by sequential ultracentrifugation (17) within the density ranges $\rho < 1.006$ and $\rho = 1.063–1.250$ g/ml, respectively. The protein content was determined by the Lowry method as modified by Markwell et al. (18), and triacylglycerol content was determined enzymatically using diagnostic kits (Roche Diagnostics).

Isolation of HDL subfractions by density gradient ultracentrifugation

Plasma from fasting, normolipidemic subjects was collected, and HDL subfractions were isolated by KBr discontinuous gradient ultracentrifugation following an initial HDL ($\rho = 1.063–1.25$) isolation from plasma by sequential ultracentrifugation. Briefly, 50 mg of sucrose was added to the HDL isolated by KBr discontinuous gradient ultracentrifugation. Using the underlay method, 12.33 ml of 1 mM EDTA containing 0.02% NaN_3 was added to an open-top centrifugation tube followed by 10 ml of 1.080 g/ml density solution, then 6.67 ml of a 1.210 g/ml density solution, and finally 10 ml of HDL-KBr-sucrose mix. The HDL was centrifuged at 27,500 rpm for 22 h at 8°C in the SW28 rotor in a Beckman ultracentrifuge. Following centrifugation, the upper 14 ml were discarded and 12 2 ml fractions ranging in densities from 1.063 to 1.27 were obtained and dialyzed against PBS (pH 7.2) without EDTA or NaN_3. The densities of the HDL fractions were determined by refractometry.

Quantification of HL in CHO-hHL and HepG2 cells

CHO-hHL cells were plated in Ham's F12 medium containing 10% FBS and 500 μg/ml G418 (plating medium) and, following attachment, the medium was changed to Ham's F12 medium containing 1% FBS and 500 μg/ml G418 (complete medium). HepG2 cells were plated and grown to confluence in EMEM containing 10% FBS, 2% L-glutamine, and 1% pen/strep (complete medium). After an overnight incubation with serum-free media, the cells were washed and incubated with serum-free medium ± apoA-I or HDL at 37°C for various times. Following removal of the medium, the cells were washed with PBS and incubated with 60 μl of SDS sample buffer [62.5 mM Tris-HCl (pH 6.8), 20% glycerol, 2% SDS, 0.5% (w/v) bromophenol blue] overnight at room temperature. The solubilized extract was subjected to electrophoresis on an 8% polyacrylamide gel under denaturing conditions, transferred to a nitrocellulose membrane, and blocked overnight at 4°C in blocking solution (PBS containing 1% BSA and 0.2% Tween-20). The membrane was incubated for 2 h at room temperature with the anti-HL MAb in blocking solution containing 0.02% NaN_3, and following washes in PBS containing 0.2% Tween-20 (PBS-Tween), a sheep anti-mouse IgG HRP-linked whole antibody was used as the secondary antibody. After a 1 h incubation, the membranes were washed in PBS-Tween and visualized by chemiluminescence following a 10 min incubation with the Pierce Super Signal West Pico substrate or a 5 min incubation with a 1:5 (v/v) dilution of the Pierce Super Signal West Dura chemiluminescent substrate. The membranes were exposed to film for various times, and the amount of HL that was associated with the cell extract under the various conditions was

determined by densitometry using the BioRad Quantity One® (version 4.1) software.

To differentiate intracellular from matrix-bound HL, the cells were washed and incubated with medium (in the absence of FBS) containing cytochalasin B at a final concentration of 10 μg/ml for 1 h at 37°C. Plates were then placed on ice, and cells were subjected to repeat aspiration of the medium to remove the cell monolayer. This process was repeated with three washes of PBS. The cells and wash buffer were centrifuged at 1,300 rpm for 10 min at 8°C in the Sorvall RT 6000D low-speed centrifuge. The supernatant was removed, and the pellet was solubilized in 60 μl of SDS sample buffer. The extracellular matrix (ECM), which remained on the plate, was also solubilized in 60 μl of SDS sample buffer. The solubilized pellet and the ECM were electrophoresed, transferred to a nitrocellulose membrane, and the HL content was analyzed by Western blot analysis as detailed above. The membranes were exposed to film for various times, and HL content was quantified by densitometry.

Determination of HL activity in CHO-hHL and HepG2 cell media

CHO-hHL and HepG2 cells were grown to confluence. After an overnight incubation with serum-free media, the cells were washed and incubated with serum-free medium ± HDL or heparin at 37°C for various times. One hundred fifty microliters of the medium was removed from the cells, and the HL activity was determined using a [^{3}H]TG emulsion, as previously described (16). Briefly, each test tube contained 200 μl substrate emulsion, 150 μl cell media, and 150 μl incubation buffer (0.33 M Tris-HCl, pH 8.3, 1% FAF-BSA, 3.33 M NaCl, and 5 mM $CaCl_2$). Incubations were carried out for 1 h for CHO-hHL cell medium and 3 h for HepG2 cell medium at 37°C. The reactions were terminated by the addition of 3 ml methanol-chloroform-heptane (1.41:1.25:1, v/v/v) and 750 μl of 0.14 M K_2CO_3-H_3BO_3 buffer (pH 10.5). The samples were vortexed and the phases separated by centrifugation at 2,200 rpm for 15 min in a Sorvall RT 6000D low-speed centrifuge. One milliliter of the aqueous phase was removed to scintillation vials, and the amount of [^{3}H]fatty acid released during the incubation was determined.

Determination of apoA-I association with VLDL

Removawells were coated with an anti-apoB MAb (1D1) (at a predetermined dilution) overnight at 4°C, washed with PBS, and saturated with 0.5% gelatin in PBS. Serial dilutions of VLDL in the presence or absence of HDL or apoA-I (in PBS with 0.5% gelatin) that had been incubated with or without hHL for 3 h at 37°C were added to 1D1-coated Removawells for 2 h at room temperature. After three washes (PBS with 0.05% Tween-20), the Removawells were incubated for 1 h with ^{125}I-labeled anti-apoA-I MAbs (4H1 and 5F6) in PBS with 0.5% gelatin (~200,000 cpm/well). Removawells were washed three times with PBS-Tween, and the amount of radioactivity was measured.

Purification of apoA-I and preparation of reconstituted HDL complexes

ApoA-I was isolated by size exclusion chromatography on a Sephacryl S-200 HR column (19). Prior to use, the apoA-I was resolubilized in 6 M guanidine HCl, 10 mM Tris (pH 7.2), and dialyzed extensively against PBS (pH 7.2). Reconstituted LpA-I complexes [reconstituted HDL (rHDL) containing one (Lp1A-I) or two (Lp2A-I) molecules of apoA-I] were prepared by cosonicating POPC and apoA-I (molar ratios indicated in Fig. 6) as previously described (20). Briefly, specific amounts of lipids in chloroform were dried under nitrogen in a 12 × 75 mm test tube. Eight hundred microliters of PBS was added, and the lipid-PBS mixture was successively sonicated under nitrogen for 1 min at constant output, incubated at 37°C for 30 min, and sonicated again for 5 min at 95% duty cycle. ApoA-I (2 mg of a 1.4 mg protein/ml PBS solution) was added to the lipid mixture and cosonicated for 4 × 1 min at 90% duty cycle with 1 min cooling periods between sonications.

Total HL activity

VLDL hydrolysis by HL was characterized in the presence or absence of HDL fractions or rHDL particles. Each test tube contained the lipoprotein substrate (350 μM VLDL), purified HL (26 units), 75 μl incubation buffer (0.33 M Tris-HCl, pH 8.3, 1% FAF-BSA, 5 mM $CaCl_2$), PBS (to a final volume of 250 μl), and increasing concentrations of HDL fractions or rHDL particles, as indicated in Figs. 4 and 6, respectively. Incubations were carried out for 30 min, and the reactions were terminated on ice. The total amount of fatty acid released during the incubation was determined using FFA diagnostic kits.

RESULTS

Characterization of HL in CHO-hHL and HepG2 cells

Previous reports from this laboratory showed that HL can be displaced from pure HSPG by HDL and apoA-I (15). In order to extend these studies to a cell culture system, CHO-hHL and HepG2 cells were characterized and then utilized to explore the ability of HL to be displaced from the cell surface. CHO-hHL and HepG2 cells were grown to confluence in complete medium, and the adherent cells were either extracted with SDS sample buffer to produce whole-cell lysates or treated with cytochalasin B to separate the intact cells from their ECMs (21) and allow differentiation of both intracellular and cell surface HL. Whole-cell lysates, ECMs, and cell extracts were individually treated with SDS sample buffer and then electrophoresed and probed for HL. Results showed that HL from whole-cell lysates of both cell lines exhibits two distinct bands, one at 66 kDa and the other at 52 kDa, which appear to represent two differently glycosylated forms of the enzyme. The HL associated with the cell surface ECM is similar in size to that of purified human HL obtained from postheparin human plasma and has an apparent molecular mass of 66 kDa. The HL present in the cell extract appears to be an intracellular form that is smaller and has an apparent molecular mass of 52 kDa (data not shown).

Displacement of HL from CHO-hHL and HepG2 cells by HDL and apoA-I

To determine if HDL and apoA-I could displace HL from the cell surface, CHO-hHL and HepG2 cells were treated with apoA-I or HDL (150 μg protein/ml of medium) for various times at 37°C. **Figure 1** shows the HL present in whole-cell lysates of CHO-hHL cells following treatment with either apoA-I (Fig. 1A) or HDL (Fig. 1B) for various times. Although both forms of HL decrease as a function of time when treated with either HDL or apoA-I, the 66 kDa cell surface-associated enzyme is depleted much faster than is the 52 kDa intracellular HL. Figure 1C is a composite graph of the Western blots shown in Fig. 1A and Fig. 1B after band quantification by densitometry. The displacement curves show HL to be more efficiently displaced from the cell surface by apoA-I than by HDL; HL displacement by apoA-I is complete after 1 h, while only 60% of HL is displaced by HDL by 2 h. Figure 1D

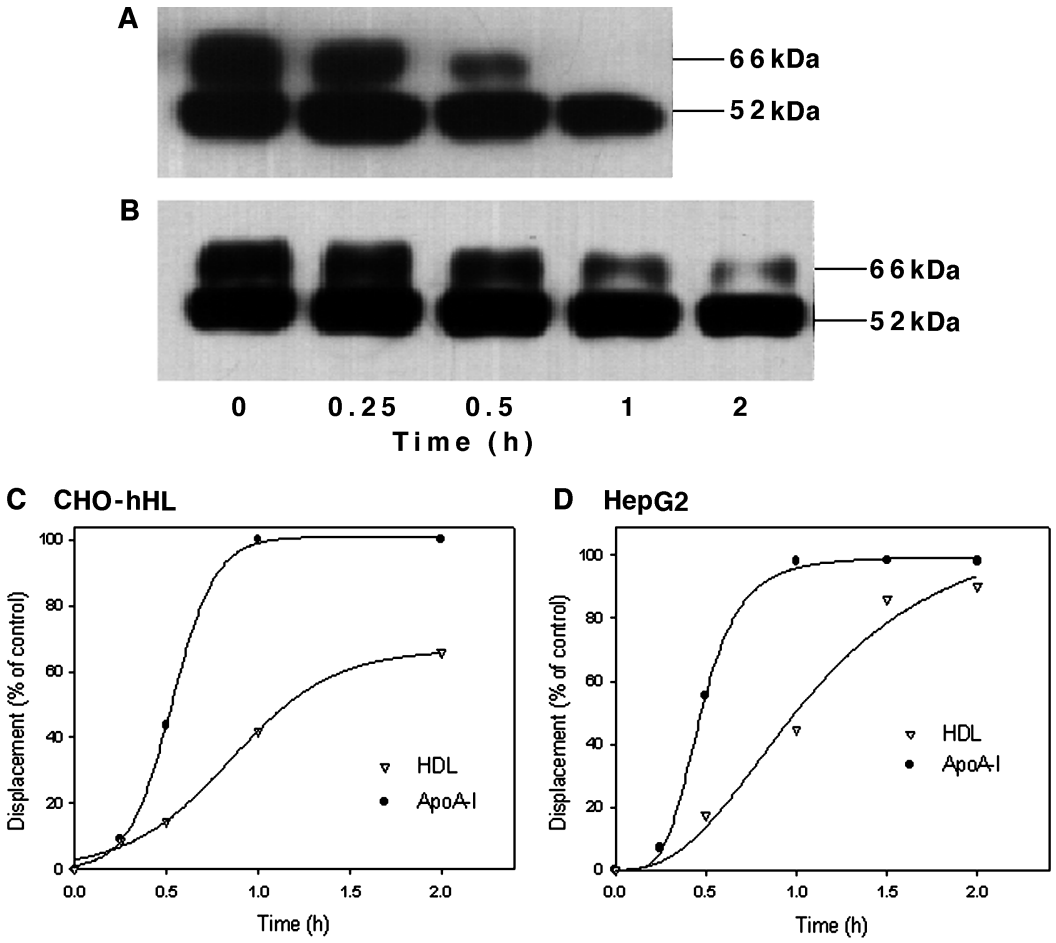

Fig. 1. Displacement of hepatic lipase (HL) from the cell surface of Chinese hamster ovary cell line stably overexpressing human hepatic lipase (CHO-hHL) and HepG2 cells by HDL and apolipoprotein A-I (apoA-I). CHO-hHL (A–C) and HepG2 (D) cells were grown to confluence in complete medium. Cells were washed and incubated with serum-free medium ± apoA-I or HDL at 37°C for the times indicated. Once the medium was removed, the cells were washed with PBS and incubated with SDS sample buffer overnight at 24°C. The cell lysates were electrophoresed and transferred to a nitrocellulose membrane. Samples were probed with an anti-HL monoclonal antibody (MAb) and with an anti-mouse IgG horseradish peroxidase (HRP)-linked secondary antibody. Western blots of apoA-I and HDL displacement of HL in CHO-hHL cells are shown in A and B, respectively. Apparent molecular weight determinations were derived from molecular weight markers. Relative HL displacement by apoA-I (circle) and HDL (triangle) was quantified by densitometry and is graphically shown in C for CHO-hHL cells and D for HepG2 cells. Data are representative of duplicate determinations from three different experiments for CHO-hHL cells and duplicate determinations from two different experiments for HepG2 cells.

shows that both HDL and apoA-I can also displace HL from the surface of HepG2 cells. Displacement curves appear similar to those observed with the CHO-hHL cells; apoA-I promotes complete displacement by 1 h, while HDL requires 2 h to completely liberate the cell surface HL.

Effect of HDL on HL activity in CHO-hHL and HepG2 cell media

To determine whether HL displacement resulted in an increase in HL activity, the media of CHO-hHL and HepG2 cells preincubated with either HDL or heparin were tested for enzyme activity. The cells were washed and incubated in serum-free medium containing either HDL (150 μg protein/ml of medium) or heparin (200 U/ml of medium) for various times at 37°C. The medium was removed and HL activity was measured by tracking hydrolysis of a standard [^{3}H]TG emulsion. **Figure 2** shows that, in the absence of HDL or heparin, only a small amount of HL activity is observed in the medium of both the CHO-hHL or the HepG2 cells (inset of Fig. 2). Incubating CHO-hHL cells with heparin for times ranging from 0–2 h results in a progressive increase of HL activity into the medium. Similarly, treatment of the cells with HDL also results in an increase of HL activity in the medium; however, hydrolytic rates were less than that observed for heparin. Results obtained using HepG2 cells (inset) paralleled those obtained with CHO-hHL cells. These data show that HDL acts much like heparin and can release catalytically active HL from the surface of both CHO-hHL and HepG2 cells into the medium. The fact that more activity is detectable when the cells are incubated with heparin relative to HDL appears to reflect an inhibi-

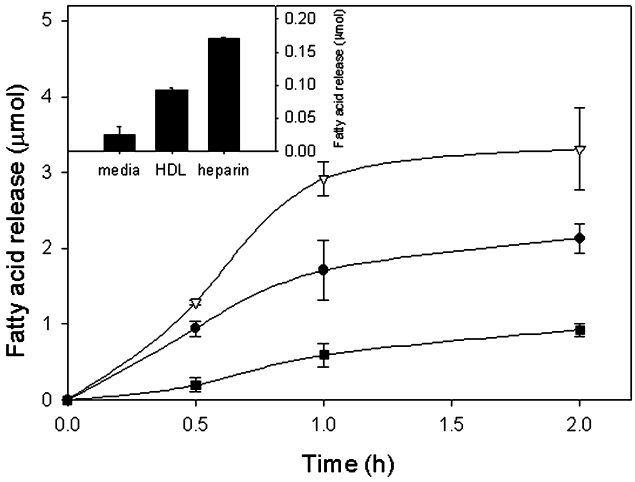

Fig. 2. Effect of HDL on HL activity in CHO-hHL and HepG2 cell media. CHO-hHL and HepG2 (inset) cells were grown to confluence in complete medium. Cells were washed and incubated with serum-free medium (square), +HDL (circle) or +heparin (triangle) at 37°C for various times. An aliquot of the medium was removed, and triacylglycerol hydrolytic rates were measured after incubation with a [^{3}H]triolein emulsion. The graph shows [^{3}H]fatty acid released during a 1 h hydrolytic assay using CHO-hHL cell medium that had been incubated with HDL or heparin. Inset shows [^{3}H]fatty acid released during a 3 h hydrolytic assay using HepG2 cell medium, which had been incubated with HDL or heparin for 4 h. Hydrolytic values are the means ± SD of triplicate determinations and are representative of two experiments for each cell line.

tory effect of HDL on HL, much as we had previously reported (15).

Effect of HDL subfractions on HL displacement from the cell surface

Experiments were also undertaken to determine whether various HDL density subclasses differ in their ability to displace HL. HDL was isolated from plasma by sequential ultracentrifugation in order to remove VLDL, LDL, and albumin fractions ($\rho < 1.063$ and >1.25 g/ml). The HDL was then separated into fractions of varying densities (ρ = 1.064–1.25 g/ml) by discontinuous density gradient ultracentrifugation, and the ability of the various HDLs to displace HL was investigated. CHO-hHL cells were grown to confluence in complete medium, and the cells were then incubated for 1 h with serum-free medium ± HDL (ρ = 1.1 to 1.22 g/ml) at 37°C. As detailed in **Fig. 3**, there is an inverse relationship between HDL density and HL displacement. While the smaller HDLs (ρ = 1.22) could only displace ~50% of the HL from the cell surface, the larger HDLs (ρ = 1.10) displaced >95% the enzyme. Furthermore, the more buoyant HDLs also caused a greater depletion in the intracellular stores of the enzyme. Taken together, these data indicate that HDL composition influences the displacement of HL from cell surface proteoglycans and mobilization of the enzyme from the cell.

Effect of HDL subfractions on VLDL hydrolysis

To determine whether the HDL fractions differed in their ability to affect the hydrolysis of VLDL by HL, coincubation hydrolytic assays were performed. The results in **Fig. 4** show the relative total lipid hydrolytic rates for VLDL ± HDL or apoA-I and are expressed as percent of hydrolysis of VLDL alone. The figure shows that HDL density has significant effects, both stimulatory and inhibitory, on HL-mediated VLDL hydrolysis. As the density of the HDL increases, progressively less stimulation and more inhibition of HL-mediated VLDL hydrolysis can be observed. While the most buoyant HDL fraction (ρ = 1.11) was found to stimulate HL activity by ~30%, the more dense HDL fractions (ρ = 1.19) inhibited the enzyme's activity by up to 50%. As previously shown, apoA-I inhibits HL-mediated VLDL hydrolysis by 80–90%. The data therefore suggest that HDL composition plays a role in determining whether HDL stimulates or inhibits HL-mediated VLDL hydrolysis.

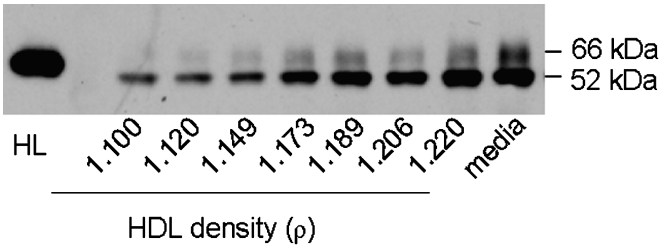

Fig. 3. Displacement of HL from the cell surface of CHO-hHL by HDL fractions. CHO-hHL cells were grown to confluence in complete medium. Cells were washed and incubated with serum-free medium ± HDL density fractions (150 μg protein/ml medium) at 37°C for 1 h. Cells were washed with PBS, incubated with SDS sample buffer overnight, and then the cell lysates were electrophoresed and transferred to a nitrocellulose membrane. Samples were probed with an anti-HL MAb and with an anti-mouse IgG HRP-linked secondary antibody. Images are representative of duplicate determinations from three different experiments.

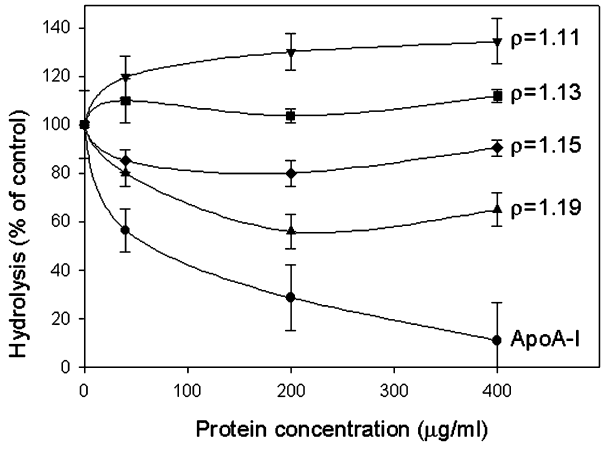

Fig. 4. Effect of HDL fractions on the hydrolysis of VLDL by HL. VLDL (350 μM triacylglycerol) was incubated with HL (26 units) and increasing amounts of different HDL density fractions [ρ = 1.11 (inverted triangle), ρ = 1.13 (square), ρ = 1.15 (diamond), ρ = 1.19 (triangle), or apoA-I (circle)] for 30 min at 37°C. An aliquot was removed, and fatty acid release was measured. Hydrolytic values are the means ± SD of triplicate determinations and are representative of two experiments.

HDL subfractions and apoA-I transfer to VLDL

To determine whether the ability of HDL to either stimulate or inhibit VLDL lipolysis is related to binding of apoA-I to VLDL, solid-phase radioimmunometric assays were performed. VLDLs that had been previously incubated for 3 h with HL ± HDL or apoA-I were added to Removawells coated with a MAb to apoB (1D1). ApoA-I association with the VLDL was then measured with a pooled mixture of two ^{125}I-labeled MAbs to apoA-I (4H1 and 5H6). As can be seen in **Fig. 5**, significant amounts of apoA-I could be found associated with the VLDL that had been incubated with either apoA-I or the different HDL fractions, but not with the native VLDL. The inset shows that an inverse relationship exists between HDL density and apoA-I transfer to VLDL. As the HDL density increased (toward the small VHDL), there was progressively less apoA-I transferred to the VLDL. It is also of note that similar amounts of apoA-I were transferred to VLDL in incubations with pure apoA-I, or the ρ = 1.100 g/ml HDL density fraction (HDL_2), and yet apoA-I is inhibitory to HL and the less dense HDLs are stimulatory.

Effect of apoA-I lipidation on VLDL hydrolysis

In order to determine whether the stimulatory-inhibitory effects seen with the HDL fractions were related to the particle physical properties, hydrolytic assays were performed to evaluate the effect of apoA-I lipidation on VLDL hydrolysis. **Figure 6** shows that the amount of phospholipid associated with apoA-I directly affects its ability to both stimulate and inhibit HL. Addition of a few molecules of phospholipid to apoA-I (POPC-apoA-I, mol/mol, 5:1) converted the apolipoprotein from an inhibitor of HL to a stimulator. However, while the poorly lipidated Lp1A-I complex profoundly stimulated HL-mediated VLDL hydrolysis, additional lipidation of apoA-I reduced this stimulatory effect. The 35:1 Lp2A-I complex stimulated HL to a much lesser extent than the 5:1 particle, and the 60:1 Lp2A-I complex actually inhibited the enzyme activity by almost 50%. This therefore suggests that the lipidation-dependent physical properties of HDL may affect its ability to regulate HL.

DISCUSSION

HL is anchored by HSPG to the surfaces of liver sinusoidal endothelial cells and hepatocytes (1). Early studies (22–24) identified HL as a triacylglycerol lipase activity that was not detectable in regular plasma samples, but was present in postheparin plasma and in heparin perfusates of the liver. It is now well established that when HL and LPL are released from the cell surface HSPG by heparin

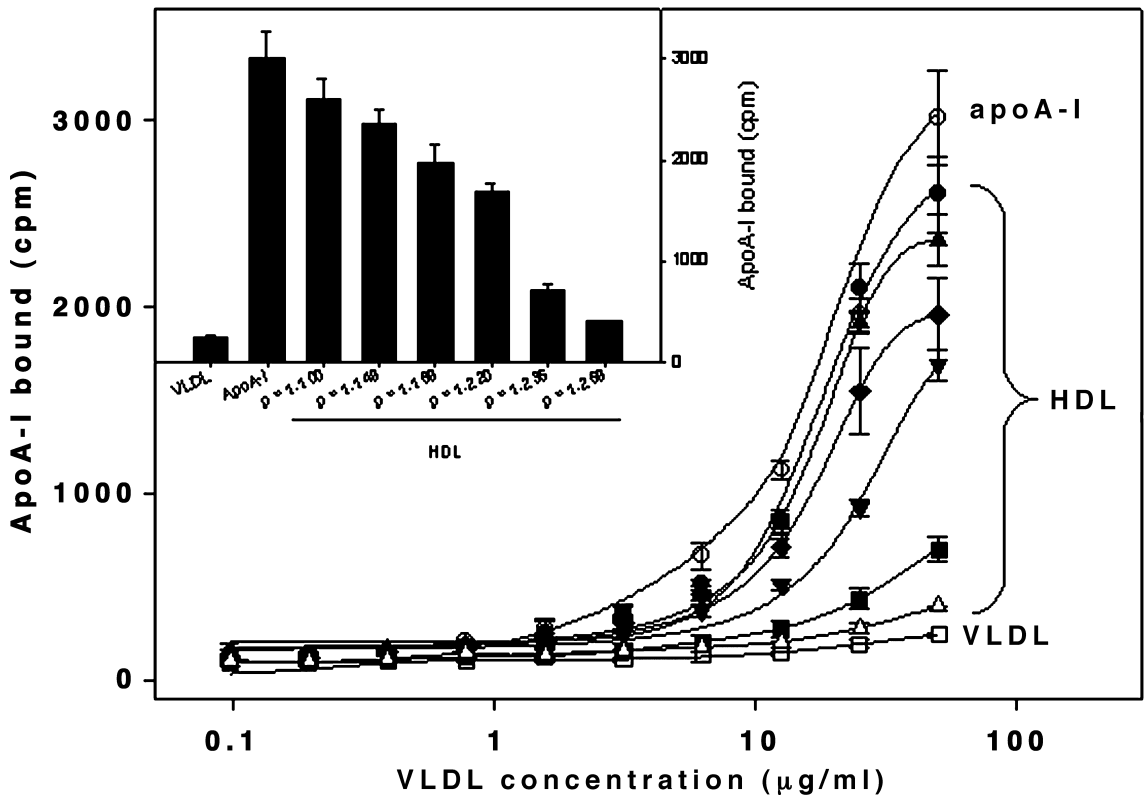

Fig. 5. VLDL-apoA-I association by a solid-phase radioimmunometric assay. Removawells coated with a MAb to apoB (1D1) were incubated with VLDL (open square) that had been preincubated (3 h) with HL ± apoA-I (open circle) or HDL density fractions [ρ = 1.100 (closed circle), ρ = 1.149 (closed triangle), ρ = 1.189 (closed diamond), ρ = 1.220 (closed inverted triangle), ρ = 1.236 (closed square), ρ = 1.269 (open triangle)]. ApoA-I association with the VLDL was then measured by the addition of two ^{125}I-labeled MAbs to apoA-I (4H1 and 5F6), followed by washing and radioactivity measurement. Values were corrected for background association and are the means ± SD of triplicate determinations, and are representative of two different experiments.

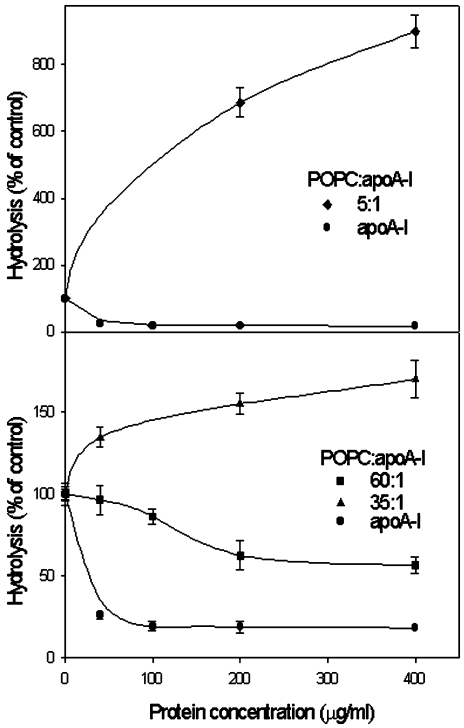

Fig. 6. Effect of reconstituted HDL (rHDL) on the hydrolysis of VLDL by HL. VLDL (350 μM triacylglycerol) was incubated with HL (26 units) and increasing amounts of rHDL particles [palmitoyl-2-oleoyl-phosphatidylcholine-apoA-I; 5:1 (diamond), 35:1 (triangle), 60:1 (square), or apoA-I (circle)] for 30 min at 37°C. An aliquot was removed, and fatty acid release was measured. Hydrolytic values are the means ± SD of triplicate determinations and are representative of two experiments.

administration, triacylglycerol hydrolysis and clearance from the blood stream is increased (11, 25). This suggests that these enzymes are less active when bound to the cell surface and is consistent with our previous work showing that VLDL hydrolysis by HL is significantly inhibited when HL is bound to pure HSPG (15). The data also suggest that efficient lipolysis requires displacement of HL from the cell surface matrix, and the factors that regulate displacement may therefore be critical to achieving rapid triacylglycerol hydrolysis.

We have further evaluated the factors that regulate HL displacement in two different cell lines; HepG2 cells that endogenously express HL and a CHO-hHL. We chose to study displacement in a CHO cell culture model in which human HL was under the control of a foreign gene promoter (cytomegalovirus) to circumvent any possible gene regulatory affects of lipoproteins or apoA-I and consequential changes in the production and secretion of the expressed protein. Western blots showed that the CHO-hHL extracts exhibit two immunoreactive HL proteins, one that represents the full-sized HL molecule and is associated with the cell surface matrix and a second, lower-molecular-mass intracellular species. This smaller intracellular HL protein appears to be a less-glycosylated form of the enzyme similar to that observed by others (26). We showed that both apoA-I and HDL are able to readily displace cell surface HL and that, as with the pure HSPG displacement studies, apoA-I is >2-fold more able to displace surface HL than is HDL. Experiments in HepG2 cells had almost identical results, which suggests that HDL predominantly acts by a displacement of the surface enzyme and may have less effect on the regulation of the HL gene. Displacement of cell surface HL was further shown to be concomitant with the liberation of an active enzyme and increased lipid hydrolysis. HL displacement was shown to also parallel decreases in intracellular HL levels. This suggests that apoA-I-mediated HL displacement from the cell surface is more rapid than its replenishment by the newly synthesized and secreted HL. It also suggests that the displaced HL remains tightly bound to the (lipoprotein) components in the medium and does not reassociate with the cell surface.

We showed that the lowest density HDL fractions have the greatest capacity to remove HL from both the cell surface and the intracellular compartments. It is striking that almost the entire cell surface and a significant amount of the intracellular HL are removed from the CHO-hHL cell line after 1 h incubation with an HDL_2 fraction. The depletion appeared to be an acute phenomenon, as both intracellular and cell surface HL were replenished after extended incubations (data not shown). In contrast to HDL_2, the smaller high-density HDL fractions were much less able to displace HL. Since HL is readily displaced by lipid-free apoA-I, the ability of HDL to displace HL from the cell surface may be a function of the amount of loosely bound or exchangeable apoA-I on the surface of

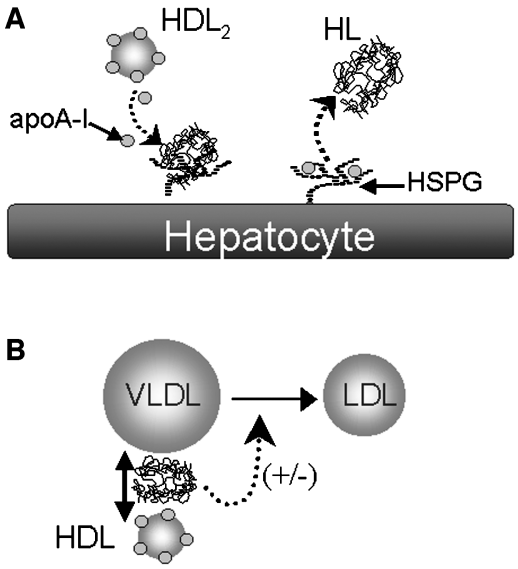

Fig. 7. Effect of HDL on the displacement and catalytic activity of HL. ApoA-I molecules on the more buoyant HDL are less tightly bound and can become dissociated from the lipoprotein particle. These dissociated apoA-I molecules bind to cell surface heparan sulfate proteoglycan and cause the displacement of cell surface-bound HL (A). The activity of HL is then dependent on the kinds of HDL in the plasma. The smaller HDL_3 and lipid-free apoA-I are inhibitory to HL, while the more buoyant HDL_2 stimulates VLDL-triacylglycerol hydrolysis (B).

the larger HDL_2 particles. It has been previously reported that HDL_2 may allow for a greater dissociation of lipid-poor apoA-I than the smaller HDL particles (27, 28), and this is consistent with our VLDL-apoA-I association studies. Figure 5 shows that more apoA-I can be transferred to the VLDL surface from large and less dense HDL fractions than from the smaller particles.

These data suggest that higher levels of HDL_2 may promote a greater displacement of cell surface HL and enhanced triacylglycerol hydrolysis by HL (**Fig. 7**). This appears to be consistent with a number of studies that have shown that there is an inverse relationship between the magnitude of postprandial lipemia and plasma levels of HDL_2 (29, 30). However, the data does not appear to be consistent with reports that have identified an inverse relationship between plasma HDL_2 levels and postheparin HL activities (31, 32). However, with this new view that HDL acts like heparin and displaces cell surface HL, it follows that if HDL_2 levels are high and HL is readily displaced from cell surface HSPG, lower amounts of HL may be liberated by heparin and this may result in reduced postheparin activities.

Consistent with this view, larger HDL fractions also appear to directly stimulate VLDL-triacylglycerol hydrolysis by HL (Fig. 7B). Therefore, the well-described inverse relationship between HDL_2 and postprandial lipemia may be due to both an enhanced liberation of HL into the plasma compartment and a direct stimulation in triacylglycerol hydrolysis. Conversely, the smaller HDL fractions are much less able to liberate HL and actually inhibit HL activity. While it is known that the apoCs readily transfer between HDL and VLDL during lipolysis (33, 34), differences do not appear to be due to unique apolipoprotein effects, as both stimulation and inhibition of HL could be demonstrated with different kinds of rHDL containing only apoA-I. Of note, the lipid-poor rHDLs were much more stimulatory of HL than the phospholipid-enriched particles (Fig. 6). The most stimulatory of the recombinant particles was the 5:1 PC-apoA-I particle, a complex that we have previously shown to also be highly reactive with LCAT and similar in structure and charge to the preβ HDL subfractions described by others (35). Previous studies by Barrans and Guendouzi have shown that HL has the ability to generate preβ HDL from triacylglycerol-enriched HDL_2 (27, 28). HDL_2 may therefore stimulate HL by generating a novel substrate that catalyzes triacylglycerol hydrolysis.

It is less clear how the smaller HDL particles may be acting to inhibit HL-mediated hydrolysis of VLDL. We had proposed that this inhibition may be due to the transfer of apoA-I from the HDL fraction to VLDL (15); however, this does not appear to be the case, as HDL_2 has a greater propensity to transfer apoA-I to VLDL than do the smaller HDL classes (Fig. 5). Since inhibition of VLDL hydrolysis by HL does not appear to be a function of apoA-I association, it appears that the different classes of HDL may be affecting VLDL hydrolysis through a regulation of interfacial association. Our data suggest that HL preferentially binds to HDL relative to VLDL, and that inhibition may be associated with a very high affinity binding and impaired interlipoprotein shuttling of HL. Both crosslinking and apoA-I immunoprecipitation studies show that HL associates with HDL particles and that this association is unaffected by the presence or absence of VLDL (Ramsamy et al., unpublished observations). It therefore appears that with the smaller HDL particles, high affinity HL association may affect interlipoprotein movement of the enzyme and thereby inhibit VLDL hydrolysis.

It is now well established that efficient triacylglycerol hydrolysis and clearance from the blood stream is directly related to the amount and kinds of HDL in the blood stream. This study may explain some of these poorly understood relationships between HDL and triacylglycerol metabolism. The well-described inverse relationship between HDL_2 and postprandial lipemia may in fact be a direct relationship and due to both an enhanced liberation of HL into the plasma compartment by this lipoprotein (Fig. 7A) and a direct stimulation of VLDL-triacylglycerol hydrolysis (Fig. 7B). Conversely, low HDL_2 and high levels of small HDL particles in the blood would be expected to reduce the displacement of cell surface HL and to directly inhibit the enzyme. This would link the low HDL_2 levels with high postheparin HL activity measurements, and impaired VLDL hydrolysis would be consistent with observations in numerous laboratories (29, 31, 32).

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