

Expression of Human Hepatic Lipase in the Rabbit Model Preferentially Enhances the Clearance of Triglyceride-Enriched Versus Native High-Density Lipoprotein Apolipoprotein A-I

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Background—We have shown previously that triglyceride (TG) enrichment of HDL, as occurs in hypertriglyceridemic states, contributes to HDL lowering in humans by enhancing the clearance of HDL apolipoprotein (apo) A-I from the circulation. In the New Zealand White rabbit, an animal naturally deficient in hepatic lipase (HL), we demonstrated that TG enrichment of HDL per se is not sufficient to enhance HDL clearance in the absence of ex vivo lipolysis by HL. Here, we examined in the rabbit the interaction between in vivo HL lipolytic action and HDL TG enrichment on the subsequent metabolic clearance of HDL apoA-I.

Methods and Results—The clearance of HDL, TG-enriched with human VLDL (12% mass TG), was compared with a simultaneously injected native rabbit HDL tracer (8% TG) 5 to 7 days after injection of recombinant (r) adenovirus expressing either the human HL or lacZ transgene (n=6 animals each). In rHL-Adv rabbits, HL activity levels were 2- to 7-fold higher (versus rlacZ-Adv controls; $P<0.01$), and there were significant ($P<0.05$) reductions in HDL TG (−18%), cholesterol (−21%), cholesteryl ester (−24%), and phospholipid (−14%). Moreover, the clearance of TG-enriched versus native HDL was significantly greater (by 50%; 0.122 ± 0.022 versus 0.081 ± 0.015 pools/h; $P<0.01$) in rHL-Adv rabbits but not in controls.

Conclusions—These studies have shown that TG enrichment of HDL in the presence but not in the absence of in vivo expression of moderate levels of lipolytically active HL results in enhanced HDL clearance, demonstrating the important interaction between TG enrichment and HL action in the pathogenesis of HDL lowering in hypertriglyceridemic states. (*Circulation*. 2003;107:3066-3072.)

Key Words: lipoproteins ■ apolipoproteins ■ metabolism ■ cholesterol ■ hyperlipidemia

The strong inverse relationship between plasma HDL cholesterol (HDL-C) concentrations and the risk of developing atherosclerotic cardiovascular disease is well recognized.¹ As demonstrated in numerous studies, HDL-C levels are also inversely related to plasma triglyceride (TG) levels.² Therefore, one of the most frequent dyslipidemias observed in association with low HDL-C is hypertriglyceridemia.² The mechanisms responsible for the reduction of HDL levels in hypertriglyceridemic states, however, have not been fully elucidated. The interaction that occurs between HDL and TG-rich lipoproteins (VLDL and chylomicrons) in the circulation is enhanced in hypertriglyceridemic states.³ This is attributed to increased cholesteryl ester transfer protein-mediated mass transfer of TG from the expanded plasma pool of TG-rich lipoproteins to HDL and concomitant transfer of cholesteryl ester (CE) from HDL to TG-rich

lipoproteins.³ This process results in TG enrichment and relative depletion of the CE content of HDL in hypertriglyceridemic states.⁴

The TG content of HDL has been shown to have a major impact on the metabolism of HDL particles. We have shown previously in healthy humans that TG enrichment of HDL significantly enhances the fractional catabolic rate (FCR) of HDL apolipoprotein A-I (apoA-I), the major protein moiety of HDL.⁵ The apoA-I FCR is a surrogate marker of HDL holoparticle clearance and correlates closely with plasma HDL-C levels,⁶ although apoA-I can also dissociate from HDL and be cleared from the circulation independently. Therefore, the enhanced catabolism of apoA-I from TG-enriched HDL may underlie the reduction in HDL-C levels in hypertriglyceridemic states.² The precise mechanisms underlying the enhanced catabolism of TG-rich HDL apoA-I in

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vivo, however, are not fully known. Studies in vivo and in cell culture have shown that TG enrichment of HDL affects HDL particle metabolism by several different proteins, including phospholipid transfer protein,⁷ the scavenger receptor class B type I,⁸ and the intravascular lipolytic enzymes (lipoprotein lipase [LPL],⁹ hepatic lipase [HL]¹⁰), and endothelial lipase.¹¹

We and others have proposed that the enhanced lipolytic modification of TG-enriched HDL by HL, in particular, enhances the FCR of HDL in hypertriglyceridemic states.² Indeed, several studies have reported that hypertriglyceridemic humans with reduced concentrations of HDL have significant elevations in HL activity, and HL activity, moreover, has been found to be negatively correlated with concentrations of the TG-rich HDL₂ fraction in humans.^{12–14} Furthermore, in the New Zealand White (NZW) rabbit, an animal model deficient in HL but possessing normal human levels of LPL, we found that TG enrichment of rabbit HDL per se, in the absence of ex vivo lipolytic modification of the HDL by HL, did not enhance the FCR of HDL apoA-I or HDL CE.^{15,16}

In the present study, we examined in the HL-deficient NZW (ie, wild-type) rabbit the interaction between HL, using recombinant adenovirus expressing human HL, and HDL TG enrichment on the subsequent rate of clearance of HDL apoA-I. Our specific aim was to determine whether TG enrichment of HDL, similar to that observed in hypertriglyceridemic states, followed by in vivo interaction with catalytically active HL, would enhance the clearance of HDL apoA-I to a greater extent than native rabbit HDL.

Methods

Recombinant Adenovirus

Recombinant adenoviruses were constructed essentially as described previously.¹¹ Briefly, lacZ and human HL cDNAs were each subcloned at the E1 region of the pACCMV shuttle vector containing the cytomegalovirus (CMV) promoter and enhancer elements and the simian virus 40 polyadenylation signal. Cotransfection of pACCMV with PJM17 (Ad5 genome) in human embryonic kidney 293 cells generated the recombinant adenoviruses. The adenoviruses were propagated and amplified in 293 cells, purified by cesium chloride density ultracentrifugation, titered, and injected into rabbits. Chow-fed NZW male rabbits (Charles River, Quebec, Canada) 4.2±0.1 kg in weight received 4×10¹² to 5×10¹² plaque-forming units of recombinant adenovirus injected into the left marginal ear vein. All procedures followed were in accordance with institutional guidelines.

Isolation of HDL, Incubation With Human VLDL, and Radiolabeling

Blood was obtained via cardiac puncture from overnight-fasted male donor NZW rabbits sedated with ketamine and xylazine. To prepare the TG-rich tracers, 15 mL of rabbit serum was incubated ex vivo with postprandial human VLDL for 6 hours, essentially as described previously.^{15,16} *m*-Parachloromercuriphenyl sulfonate (0.002 mol/L) was included in the incubation mixture to inhibit lecithin-cholesterol acyltransferase activity.¹⁷ Rabbit HDL derived from an overnight-fasted rabbit (henceforth referred to as native HDL) and TG-rich rabbit HDL (1.063<*d*<1.21 g/ml) were isolated by sequential ultracentrifugation at 4°C of whole rabbit serum¹⁵ and then dialyzed in Tris-EDTA buffer.

The native and TG-rich HDL preparations (2.4±0.1 mg) were alternately iodinated with 500 μCi of either ¹³¹I or ¹²⁵I (NEN)

according to a modification of the iodine monochloride method of McFarlane.¹⁵ Cold HDL carrier protein in the amount of 2.3±0.2 mg was added to the iodinated HDL. The HDL tracers were then washed at *d*=1.21 g/ml at 4°C, followed by dialysis in Tris-EDTA buffer.

HDL Turnover Study

Five days after infusion of recombinant adenovirus, rabbits were injected with native and TG-rich HDL tracers simultaneously, containing 3.2±0.6 mg HDL protein and 4.2±0.5×10⁷ cpm of iodinated HDL, into the right marginal ear vein. Blood samples (2 mL) were obtained over the next 2 days from a vein in the opposite ear at the following time intervals: 10 minutes and 1, 2, 3, 4, 6, 24, 27, 30, and 48 hours. HDL (1.063<*d*<1.21) was isolated (from ≈1 mL serum) by sequential ultracentrifugation.¹⁵ The amount of radioactivity specifically associated with HDL apoA-I (isolated by use of 15% SDS-PAGE) was determined as described previously.¹⁶

Blood Sampling

At baseline and days 2, 4, and 6 after adenovirus injection, animals were fasted 4 to 6 hours before collection of blood into tubes containing EDTA. HDL lipids were determined after precipitation of apoB-containing lipoproteins with heparin and manganese chloride.

Laboratory Measurements

Lipids in plasma and HDL samples (collected at baseline and days 2, 4, and 6 after adenovirus injection and during the turnover study) and in HDL injectates were quantified by enzymatic assays with commercially available kits for TGs, total cholesterol, phospholipid (PL) (Boehringer Mannheim GmbH Diagnostica), and free fatty acids (Wako Chemicals). CE was measured with the cholesterol kit after free cholesterol in the samples had been degraded with a mixture of cholesterol oxidase, peroxidase, phenol, Triton-X, and phosphate buffer.¹⁵ Total HDL protein was measured by the technique described by Lowry et al.¹⁵ The injectates were also analyzed for apolipoprotein content by isoelectric focusing techniques¹⁸ and ELISA using anti-human polyclonal antibodies.

ApoB concentration in ultracentrifuged VLDL and LDL fractions was also measured during the turnover study. In brief, apoB in each fraction was quantified by subtracting the total protein content of the fraction (measured by the method of Lowry et al.¹⁵) by the protein content remaining in the fraction after precipitation of apoB-containing lipoproteins with manganese heparin.¹⁵ Liver enzymes were measured with kits for ALT and AST (Sigma Diagnostics).

The size of the HDL tracers and HDL samples from the turnover study were determined by 4% to 30% nondenaturing polyacrylamide gradient gel electrophoresis and analyzed as described previously.^{15,19} Briefly, the radius of the major peak in each scan was identified as the HDL peak particle size, and mean (or weighted) HDL particle size was calculated by multiplying the size of each band by its fractional area.¹⁹

HL and LPL Activities

At the end of the HDL turnover study, on day 7 after adenovirus injection, blood was collected from rabbits 10 minutes after injection of heparin (500 U/kg IV).²⁰ Postheparin HL and LPL activities were measured as described previously using ¹⁴C-triolein as substrate in the presence and absence, respectively, of 1 mol/L NaCl.¹⁶

Kinetic Analysis

The radioactivity die-away curves were analyzed with a 2-pool model using the SAAM II program (SAAM Institute). Iterated 2-stage population kinetic parameters were estimated from the individual parameter values, as described previously,¹⁵ using the SAAMII Population Kinetics program (SAAM Institute). The population parameters were then used to generate mean die-away curves for the fasting and TG-enriched apoA-I HDL tracers. The average coefficient of variation for apoA-I FCRs was 25±7% for fasting HDL and 12±2% for TG-rich HDL.

TABLE 1. Mean Radius and Lipid Composition (% of HDL Mass) of the HDL Tracers (n=6 for Each Unless Otherwise Noted)

	TG-Rich HDL	Native HDL
Radius, nm (mean)	5.18±0.14*	4.99±0.11
TGs, %	12.2±0.7‡	7.5±0.9
Cholesterol, %	8.8±0.9	10.8±1.0
CE, %	6.3±0.8*	9.7±0.9
PL, %	33.8±1.2†	31.3±1.6
Proteins, %	45.2±1.7‡	50.4±2.0
Total apolipoproteins, % (n=5 each)		
ApoA	79±1	78±2
ApoC	11±1	12±1
ApoE	10±1	10±1
Human apolipoproteins, mg/dL		
ApoC-I	0.37±0.08†	0
ApoC-III	3.16±0.64†	0.01±0.002
ApoE	0.26±0.08	0.15±0.03

Values are mean±SEM.

* $P<0.05$, † $P<0.01$, ‡ $P<0.0001$, significantly different from native HDL (paired t tests).

Statistics

Results are presented as mean±SEM. Paired t tests were performed to compare FCR values between native HDL and TG-rich HDL tracers and to test differences in tracer composition and size. Analyses of various parameters in rabbits before and after injection of recombinant adenovirus were also performed with paired t tests. Between-group comparisons were performed with unpaired t tests.

Results

Table 1 presents the compositions of the HDL tracers. TG enrichment of rabbit HDL was performed with VLDL isolated postprandially from 12 healthy human subjects (age, 30±3 years; body mass index, 22±1; postprandial plasma TG, 1.3±0.2 mmol/L; postprandial plasma cholesterol, 4.1±0.2 mmol/L). TG enrichment of rabbit HDL resulted in a highly significant 63% increase in HDL TG content ($P<0.0001$) in the TG-rich versus the native HDL tracer. Compared with native HDL, the TG-rich HDL injectate also contained a greater % mass of PL (8% greater; $P<0.01$) and markedly less % mass CE and protein (−36% and −10%, respectively; $P<0.05$ and $P<0.0001$, respectively), which is a natural consequence of the lipid-exchange process of cholesteryl ester transfer protein that we used to enrich the HDL tracers with TG.⁹ Isoelectric focusing analysis demonstrated that the percentages of apoA, apoC, and apoE overall were similar in the 2 groups of tracers (n=5 in each group, $P=NS$). Analysis by ELISA showed that incubation of native rabbit HDL with human VLDL resulted in significant enrichment of the TG-rich HDL injectates (n=7) with human apoC-I and apoC-III (0.37±0.08 and 3.16±0.64 mg/dL, respectively). The human apolipoprotein antibodies showed a small amount of cross-reactivity with the respective rabbit apolipoproteins.

It is important to note that there was no significant difference in the lipid, total protein, or apolipoprotein compositions of the fasting and TG-rich HDL tracers used in the

TABLE 2. Lipid Profile of rIacZ-Adv (n=6) and rHL-Adv (n=6) Rabbits at Baseline and 6 Days After Adenovirus Injection

	TG	Cholesterol	CE	PL
rIacZ-Adv				
Plasma				
Baseline	47.8±5.5	33.4±5.1	23.0±2.3	125.0±13.5
After injection	70.7±9.0	34.2±4.6	24.0±2.6	115.4±16.6
% Change	51*	3	5	−8
HDL				
Baseline	27.2±2.9	17.3±0.9	10.4±2.0	45.7±2.8
After injection	25.0±2.6	15.1±1.4	9.9±1.9	46.2±2.8
% Change	−7	−7	−6	2
rHL-Adv				
Plasma				
Baseline	44.9±3.6	31.8±3.2	20.2±1.7	113.9±11.1
After injection	51.4±4.9	29.6±3.1	17.3±2.0	90.9±7.1
% Change	14	−7	−13	−19*
HDL				
Baseline	25.3±2.6	17.9±2.5	7.9±0.6	56.6±11.0
After injection	20.3±1.8	13.1±2.5	5.9±0.4	50.2±11.7
% Change	−18*	−21*	−24*	−14*

Values are mg/dL, mean±SEM. Postinjection lipids are values from day 6 after adenovirus administration. % Change indicates the relative change in lipid concentrations in the rHL-Adv and rIacZ-Adv animals above or below their respective baseline values.

* $P<0.05$, significant difference between baseline and posttreatment values (paired t tests).

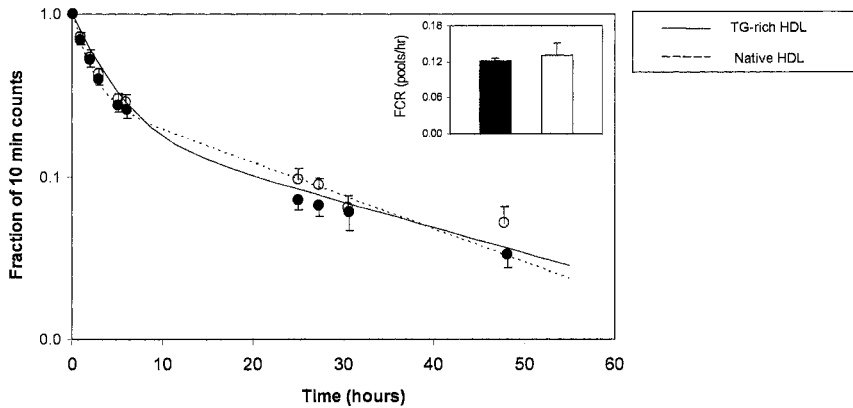
rHL-Adv versus the rIacZ-Adv animals. The iodinated HDL tracers had similar % counts in a protein-bound form (90±1% and 90±2% for rHL-Adv and rIacZ-Adv, respectively, $P=NS$) as determined by trichloroacetic acid precipitation. Analysis of HDL size showed that the TG-rich HDL tracer was larger than fasting HDL (mean radius, 5.18±0.14 and 4.99±0.11 nm, respectively, $P<0.05$).

Table 2 presents the plasma and HDL lipid concentrations at baseline and 6 days after injection of adenovirus. In rIacZ-Adv rabbits, plasma TG levels increased significantly, by 51% ($P<0.05$), whereas the levels of other plasma lipids did not change significantly. In rHL-Adv animals, there was a significant reduction in plasma PL (−19%, $P<0.05$) but no significant change in other plasma lipids. It should be noted that the low levels of lipids inherently present in the rabbit model make effects on plasma lipid levels by HL expression difficult to detect.

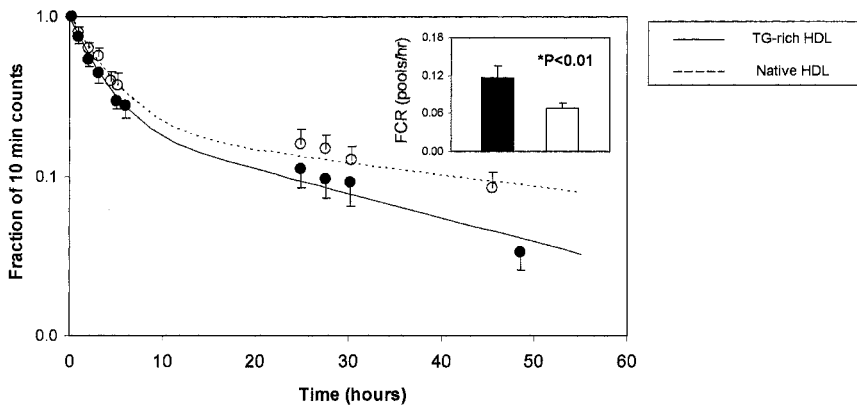
In rIacZ-Adv control rabbits, there was no significant change in HDL TG, CE, or PL, and the decline in HDL cholesterol was of borderline significance (−7%, $P=0.05$) (Table 2). In contrast, in animals receiving rHL-Adv recombinant adenovirus, there was a significant decline in HDL TGs, cholesterol, CE, and PL (−18%, −21%, −24%, and −14%, respectively, $P<0.05$ for all) after adenovirus injection.

The mean concentration of VLDL apoB, measured over the course of the turnover experiments (ie, days 5 to 7 after adenovirus injection), was 30% lower in the rHL-Adv group

A Mean Decay of HDL Apolipoprotein A-I Radioactivity rIacZ-Adv Expressors (n=6)



B Mean Decay of HDL Apolipoprotein A-I Radioactivity rHL-Adv Expressors (n=6)



Simulated curves of HDL apoA-I radioactivity. Die-away curves for TG-enriched HDL tracer (●) and native HDL tracer (○) were generated from estimated population analysis parameters. Insets show FCR associated with TG-rich (black bar) and native (white bar) HDL. A, Decay curves for animals receiving rIacZ-Adv recombinant adenovirus. B, Results for rabbits receiving rHL-Adv recombinant adenovirus. Radioactivity data for each tracer were normalized as a function of radioactivity measured at first time interval (10 minutes). Statistics are by *t* test of FCR values for each experiment (paired *t* test for intragroup comparisons and unpaired *t* test for intergroup comparisons).

than in the rIacZ-Adv control group (107.7 ± 7.4 versus $154.3 \pm 15.2 \mu\text{g/mL}$, $P=0.02$), whereas LDL apoB tended to be lower by 20% in rHL-Adv animals (255.5 ± 57.8 versus $310.6 \pm 46.3 \mu\text{g/mL}$, $P=NS$), consistent with the results of previous studies.²¹

Postheparin plasma lipase activities were measured on day 7 after adenovirus administration. Postheparin LPL activities were similar in the rIacZ-Adv and rHL-Adv groups (12.5 ± 0.4 and $12.6 \pm 0.6 \mu\text{mol free fatty acid/h}$, $P=NS$) and were in the range of LPL values reported previously in the rabbit.²² Postheparin HL activities were markedly greater (4-fold higher, $P<0.01$) than baseline values in rHL-Adv rabbits (6.3 ± 1.11 [$n=6$] versus 1.6 ± 1.2 [$n=3$] $\mu\text{mol free fatty acid/h}$, respectively) but not in rIacZ-Adv controls ($n=6$, $P=NS$). In accordance with the effect of HL on HDL particle size, the size of the HDL samples collected during the HDL turnover experiments (measured by polyacrylamide gradient gel electrophoresis) was significantly smaller in the rHL-Adv animals versus controls. Mean HDL peak radius on days 1 to 3 of the turnover studies was 4.86 ± 0.03 nm in rHL-Adv animals and 5.03 ± 0.02 nm in rIacZ-Adv animals ($P<0.0001$).

Plasma ALT and AST levels, measured on days 2 to 6 after adenovirus injection, were similar in the 2 recombinant

adenovirus groups (rIacZ-Adv, 31.4 ± 1.4 and 15.3 ± 0.4 Sigma-Frankel units, respectively [$\text{mean} \pm \text{SEM}$]; rHL-Adv, 33.9 ± 1.8 and 17.2 ± 0.9 Sigma-Frankel units, respectively) and did not increase significantly from baseline values.

The Figure shows the die-away curves of radiolabeled HDL apoA-I from TG-enriched and native HDL in the rIacZ-Adv and rHL-Adv groups (A and B, respectively). As described in Methods, the isotope clearance curves were constructed using the counts in the isolated apoA-I band ($42 \pm 1\%$ of total HDL counts, $n=24$; 2 tracers \times 12 animals in all). The proportion of injected radioactivity that was recovered during the turnover studies within the HDL fraction was $48 \pm 1\%$. Results from the kinetic experiments (Table 3) in the rIacZ-Adv animals showed no systematic difference ($-6 \pm 12\%$) in the rate of clearance of TG-rich compared with fasting HDL apoA-I (0.121 ± 0.007 versus 0.129 ± 0.023 pools/h, $P=0.70$). In contrast, there was a more rapid clearance of apoA-I associated with TG-rich HDL compared with native HDL apoA-I in all 6 rHL-Adv experiments, with a $50 \pm 19\%$ greater mean apoA-I FCR of TG-rich versus fasting HDL (0.122 ± 0.022 and 0.081 ± 0.015 pools/h, respectively, $P<0.01$). There were no significant differences in FCR between native HDL in rHL-Adv versus rIacZ-Adv ($P=0.11$).

TABLE 3. Kinetic Parameters of the HDL ApoA-I Tracers in rIacZ-Adv (n=6) and rHL-Adv (n=6) Transfected Rabbits

	FCR, hr ⁻¹ (CV, %)*	
	TG-Rich HDL	Native HDL
rIacZ-Adv	0.121 (6.9)	0.152 (14.2)
	0.134 (19.5)	0.228 (7.6)
	0.147 (21.4)	0.127 (13.8)
	0.106 (13.5)	0.072 (17.3)
	0.107 (6.5)	0.091 (10.9)
	0.113 (15.6)	0.105 (13.5)
Mean±SEM	0.121±0.007	0.129±0.023
rHL-Adv	0.094 (11.5)	0.038 (15.0)
	0.125 (7.2)	0.079 (14.2)
	0.207 (9.1)	0.137 (75.9)
	0.160 (12.7)	0.112 (11.6)
	0.075 (6.2)	0.065 (4.3)
	0.070 (9.9)	0.056 (6.8)
	0.122±0.022†	0.081±0.015

*CV%: coefficient of variation is the SD divided by the estimated FCR, expressed as percent. The CV is an estimate of the degree of precision of the FCR.

† $P < 0.01$, significant difference between TG-rich and native HDL apoA-I. There were no significant differences in FCR between native HDL in rHL-Adv vs rIacZ-Adv ($P = 0.11$).

Discussion

We have shown in these experiments that in vivo expression of a moderate level of HL enzyme activity in NZW rabbits, a species naturally deficient in HL, enhances the subsequent metabolic clearance of TG-enriched HDL apoA-I (by 50%) in comparison with native HDL. In HL-expressing animals, there was also a significant reduction in all HDL lipids measured (TG, cholesterol, CE, and PL). In contrast, in control rabbits expressing β -galactosidase (rAdv-lacZ), TG-enrichment of HDL did not increase HDL apoA-I clearance or significantly alter HDL lipids. Power calculations indicate that in the control HL-deficient (rAdv-lacZ) group, the experimental sample size would need to be increased to >199 to result in a statistically significant difference in apoA-I clearance between TG-enriched and native HDL. Overall, our results demonstrate that the process of TG enrichment of HDL in the presence of lipolytically active HL in vivo can explain, at least in part, the enhanced catabolism and deleterious reduction of HDL observed in hypertriglyceridemic states.

We showed previously in the NZW rabbit that TG-rich HDL that had been lipolytically modified ex vivo by incubation with HL was cleared faster than lipolytically modified native HDL.¹⁶ The present study extends our previous observation by comparing the clearance of TG-rich versus native HDL in both the presence and the absence of HL action. It also demonstrates the in vivo relevance of HDL TG and HL interactions in altering HDL apoA-I catabolism. Furthermore, the present study can explain our previous opposing findings

in humans and the rabbit model as follows. Whereas TG-rich HDL was cleared significantly faster than native HDL apoA-I in healthy human subjects,⁵ in HL-deficient NZW rabbits, we observed no difference in the clearance of the 2 tracers in the absence of ex vivo incubation of the particles with catalytically active HL.¹⁵ We speculated that a deficiency of HL in rabbits explained the contrasting effects of TG enrichment on HDL metabolism in the 2 species, an explanation that is supported by the present study. It has been shown in vitro that TG enrichment of reconstituted HDL particles alters the surface charge and structural conformation of apoA-I within the particles, making apoA-I more likely to dissociate from the particle.²³ In an in vivo setting, both the alterations in surface charge and structure of HDL as a result of TG enrichment and decreased stability of HDL apoA-I because of lipolysis by HL may be necessary to enhance HDL apoA-I clearance.

Fan et al²⁰ previously investigated the effect of HL expression on HDL metabolism in transgenic rabbits. In their studies, overexpression of HL (up to 80-fold above baseline) produced a significant reduction in HDL-C, with a preferential reduction of the large TG-rich HDL₁ and HDL₂ subfractions and a lesser decrease of the denser, relatively lipid-poor HDL₃ subfraction.²⁰ HDL turnover studies were not conducted in this previous study²⁰ or, to the best of our knowledge, in any other previous study investigating HL expression in the rabbit; however, these results are consistent with our key observation that TG-rich HDL particles are cleared from the circulation faster than native rabbit HDL.

Although our experiments were not designed to determine whether HL expression enhances the clearance of native rabbit HDL, previous studies indicate that although supra-physiological levels of HL activity (induced in several animal models) can have a major impact on native HDL levels and catabolism,^{20,24} smaller physiological differences in HL activity (in humans and animals) do not seem to alter HDL apoA-I metabolism in the absence of hypertriglyceridemia or HDL TG enrichment.^{25,26} For example, in normolipidemic humans grouped according to HL promoter genotype, de Oliveira e Silva et al²⁵ found that although HL activity was significantly different among the 3 genotypes present (≈ 3 -fold difference between the genotypes with highest and lowest mean HL activities), there was no significance difference in either the FCR of HDL apoA-I or apoA-I levels among the 3 groups. Furthermore, the fact that the native HDL in the present study was not cleared more rapidly in rHL-Adv versus rIacZ-Adv rabbits provides the most powerful evidence to date, consistent with our previous observations,¹⁶ that HDL that is not TG-enriched is not cleared more rapidly in the presence of moderate expression of HL. These data are consistent with the concept that moderate changes in HL activity influence plasma HDL-C and apoA-I concentrations primarily when HDL particles are enriched with TG.

HDL samples collected during the HDL turnover experiments were analyzed for size and were found to be significantly smaller in HL-expressing animals than in control animals, consistent with the results of previous studies in HL-transgenic rabbits.²⁰ In accordance with these findings, it has been suggested that a precursor-product relationship

exists in the circulation of hypertriglyceridemic individuals between large TG-enriched HDL, which serves as a substrate for HL, and the resulting lipolyzed HDL.⁵ O'Meara et al²⁷ first showed that in vivo lipolysis (stimulated via intravenous injection of heparin) of HDL in hypertriglyceridemic humans induces the formation of distinct HDL particles reduced in size and density. In vivo studies further indicated that TG enrichment of HDL was a necessary prerequisite for the formation of such "remnant HDL" products from HL hydrolytic action.¹⁰ Remnant human HDL, moreover, has been shown to undergo greater catabolism in perfused rabbit liver than unmodified, relatively TG-poor human HDL²⁸ and also demonstrated high-affinity binding and internalization in human hepatoma (HepG2) cells.²⁹ These findings suggested that remnant HDL undergoes greater holoparticle uptake at the liver. The present study, showing significantly greater clearance of apoA-I associated with TG-rich versus native HDL in animals expressing catalytically active HL and an absence of a difference in control animals, could provide in vivo support for this concept, although loss of apoA-I from HDL particles followed by rapid clearance of lipid-poor HDL from the circulation is an equally plausible explanation.

Although there was no difference between the clearance of TG-rich versus native HDL apoA-I in the control rIacZ-Adv animals, the absolute FCRs of these tracers were not different from the those of the TG-rich HDL tracer in HL-expressing animals. A "null" virus is an appropriate control in the present study to differentiate the nonspecific viral effects from the effects of the gene of interest. Nevertheless, some inaccuracies could arise with intergroup (ie, comparing the absolute FCR of native or TG-rich HDL in rIacZ-Adv rabbits versus the absolute FCR of native or TG-rich HDL in rHL-Adv rabbits) rather than intragroup comparisons (ie, comparing the FCR of native versus TG-rich HDL within either the rIacZ-Adv or rHL-Adv rabbits). These inaccuracies arise because the experiments in the rHL-Adv and rIacZ-Adv animals were not paired. That is, the tracer preparations for the 2 groups and the in vivo kinetic experiments for the groups were done separately, at different times, and using different donor rabbit HDL. In addition, HDL catabolism is very sensitive to inflammatory stimuli, thought to be mediated by serum amyloid A and/or secretory phospholipase A₂.³⁰ It is possible that the nonspecific effects of the rIacZ-Adv on HDL clearance may have been greater than those of the rHL-Adv. We believe, therefore, that the valid comparison in the present studies is the intragroup paired comparisons of the TG-rich versus native HDL apoA-I FCR in the rIacZ-Adv and rHL-Adv groups (as opposed to comparisons across the 2 groups), as per the experimental design of the studies.

In conclusion, both HDL TG enrichment and sufficient HL activity are necessary to enhance the clearance of HDL-associated apoA-I. Because the FCR of HDL apoA-I is a close correlate of HDL-C levels,⁶ the processing of TG-rich HDL by HL can partially explain the deleterious reduction in HDL-C levels in hypertriglyceridemia. Our data indicate that HL enzyme action results in a decline in the concentration of all major HDL lipids and a reduction in HDL size. Future studies are necessary to elucidate more precisely how alter-

ations in HDL composition combined with HL processing alter HDL interaction with lipoprotein receptors to enhance HDL clearance in hypertriglyceridemic states.

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