Triglyceride enrichment of HDL does not alter HDL-selective cholesteryl ester clearance in rabbits

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Abstract Triglyceride (TG) enrichment of high density lipoprotein (HDL), which occurs in hypertriglyceridemic states, significantly enhances the rate at which HDL apolipoprotein (apo)A-I is cleared from the circulation of healthy humans. In the New Zealand White (NZW) rabbit, a species naturally deficient in hepatic lipase (HL), TG enrichment of HDL requires prior lipolytic modification to enhance apoA-I clearance. However, the effect of TG enrichment of HDL on the subsequent clearance of HDL cholesteryl ester (CE) has not previously been examined in vivo. Therefore, we investigated, in the NZW rabbit, the effect of ex vivo TG enrichment of rabbit HDL (by incubation with human very low density lipoprotein) on the clearance of HDL CE and apoA-I radiolabeled with ^{3}H-cholesteryl oleyl ether and with ^{131}I, respectively. In nine experiments, TG enrichment of rabbit HDL resulted in an 87% average increase in HDL TG and a corresponding 31% reduction in HDL CE content. The calculated apoA-I and CE fractional catabolic rates associated with TG-rich versus fasting HDL tracers were not significantly different (apoA-I: 0.119 ± 0.017 vs. 0.107 ± 0.024 pools per h, P = 0.68; CE: 0.147 ± 0.014 vs. 0.114 ± 0.019 pools per h, P = 0.20). In an animal model deficient in HL, TG enrichment of HDL did not alter the rates of HDL apoA-I or selective CE clearance. Further studies are needed to determine whether, in the presence of HL, TG enrichment of HDL alters selective HDL CE clearance.— Rashid, S., K. D. Uffelman, P. H. R. Barrett, P. Vicini, K. Adeli, and G. F. Lewis. **Triglyceride enrichment of HDL does not alter HDL-selective cholesteryl ester clearance in rabbits. *J. Lipid Res.* 2001.** 42: **265–271.**

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There is a strong inverse correlation between plasma levels of high density lipoprotein cholesterol (HDL-C) and the development of atherosclerotic cardiovascular disease (1, 2). Hypertriglyceridemia is commonly associated with low plasma levels of HDL-C (3–5). Although the molecular mechanism accounting for this association is not entirely clear, we and other researchers have postulated that changes in HDL lipid composition secondary to hypertriglyceridemia are an important determinant of plasma HDL-C levels (6). Indeed, we have previously demonstrated in humans that triglyceride (TG) enrichment of HDL markedly enhances the clearance of HDL-associated apolipoprotein (apo)A-I from the circulation (7).

Most in vivo studies to date that have examined the effect of hypertriglyceridemia on HDL metabolism have focused on the kinetics of HDL-associated apoA-l, the major protein component of the HDL particle (6). However, up to 65% of the cholesteryl ester (CE) component of HDL is currently believed to be selectively removed from HDL particles by tissues such as liver and adrenal, a process that is independent of HDL whole-particle uptake (8). The scavenger receptor class B, type 1 (SR-B1) was recently discovered to play a key role in the process of selective CE uptake from HDL (9, 10). The hypertriglyceridemia-related determinants of HDL CE metabolism may therefore not be the same as those for apoA-I, making it imperative that the effects of hypertriglyceridemia on HDL CE metabolism be studied directly.

Recent in vitro studies investigating the effect of TG enrichment of HDL, as occurs in hypertriglyceridemic states, on the process of selective CE uptake have shown opposing results. In one study, TG enrichment of human HDL was found to impair selective uptake of HDL CE in HepG2 and adrenal YI cells (11). In another study, TG-enriched

Abbreviations: apo, apolipoprotein; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FCR, fractional catabolic rate; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; HL, hepatic lipase; PAGGE, polyacrylamide gradient gel electrophoresis; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SR-B1, scavenger receptor type B, class 1; TCA, trichloroacetic acid; TG, triglyceride; TLC, thin-layer chromatography; NZW, New Zealand White; VLDL, very low density lipoprotein.

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HDL isolated from CE transfer protein (CETP)/apoA-I transgenic mice showed a significant increase in SR-B1-mediated HDL CE uptake in Chinese hamster ovary cells compared with control HDL isolated from apoA-I transgenic mice (12). The authors of the second study attributed the increase in HDL CE uptake to remodeling of HDL by CETP-mediated CE-TG interchange.

An important question, which has yet to be answered, is whether TG enrichment of HDL also modulates the process of selective CE clearance in vivo. Therefore, in the present study, we examined the influence of ex vivo TG enrichment of rabbit HDL particles in determining the subsequent clearance of both HDL apoA-l and HDL CE from the circulation of normal (i.e., wild-type) rabbits.

We chose the rabbit as the model to study the metabolism of HDL in hypertriglyceridemic states because rabbits are deficient in the enzyme hepatic lipase (HL) (13). The lipolytic and nonlipolytic effects of HL on HDL metabolism have recently been extensively reviewed (14). In the present study, we wished to investigate the effect of TG enrichment of HDL per se on the clearance of HDL CE, independent of the effects of HL. Our specific aim was to determine whether TG enrichment of HDL, similar to that observed in hypertriglyceridemic states, without substantial in vivo hydrolysis and enhancement of uptake by HL, is sufficient to enhance the clearance of HDL CE, as compared with the clearance of apoA-I in the HDL particle.

MATERIALS AND METHODS

Animals

New Zealand White (NZW) male rabbits, 3.95 to 5.5 kg in weight, were used in a total of nine experiments. All rabbits received a standard chow-fed diet (17% protein, 2.1% fat, 18% fiber). The study protocol comprised two phases: In phase 1, HDL from fasting rabbit serum and from serum that had been enriched with TG by incubation with human very low density lipoproteins (VLDL; see below) was isolated and radiolabeled. In the second phase of the study, the two HDL tracers (fasting and TG-enriched) were injected into two weight-matched recipient rabbits and the clearance of apoA-I and CE associated with each tracer was determined.

Phase 1: Isolation and radiolabeling of fasting and TG-enriched rabbit HDL

Blood was obtained via cardiac puncture from male NZW rabbits following an overnight fast (16 h) and sedation with 0.2 ml/kg body weight of ketamine (Rogar/STB Pharmaceuticals, London, Ontario, Canada) and 0.2 ml/kg body weight of xylazine (Bayer, Etobicoke, Ontario, Canada). Individual donor rabbits were used for each experiment. Next, 13 ml of rabbit serum was incubated ex vivo with human VLDL for 4 h at 37°C to enrich the HDL particles with triglycerides. VLDL was obtained immediately prior to each experiment from a healthy normolipidemic donor human subject. The amount of VLDL included in the incubation mix was sufficient to contribute ~1 mM of triglycerides.

Fasting serum and serum incubated with VLDL were then incubated for 2 h at 37°C with ^{3}H-cholesteryl oleyl ether (2–5 μCi/ml of serum) to whole-label HDL CE. CETP within the rabbit serum mediated the exchange of ^{3}H-cholesteryl oleyl ether from VLDL to HDL CE. Fasting and TG-rich HDL tracers were isolated by sequential ultracentrifugation of whole serum at consecutive densities of 1.063 g/ml for 20 h at 40,000 rpm and 1.21 g/ml for 24 h at 55,000 rpm at 4°C in a Beckman 70.1 Ti rotor, and then dialyzed overnight in a buffer containing 0.15 M NaCl, 0.01 M Tris-base, and 5 mM ethylenediaminetetraacetic acid, pH 8.0 (hereafter referred to as Tris buffer). Approximately 1–3 mg of fasting protein and TG-rich HDL were iodinated by a modification of the iodine monochloride method of McFarlane (15) using 250 μCi of ^{131}I. Briefly, HDL was first added to a mixture of 20 μmol ICl/μmol HDL protein and 250 μCi of radioactive iodine. The mixture was then eluted through a 0.2 M Glycine-Sephadex G-50 column, pH 10 (Pharmacia Biotech, Uppsala, Sweden) to allow for the iodination of HDL and the separation of the HDL-bound radioactive iodine from the free iodine. The radiolabeled HDL was then eluted through a saline-Sephadex G-50 column to bring the pH of the solution containing the radiolabeled HDL back to physiological range (pH 7.4). An equal amount of unlabeled TG-rich and fasting HDL was added to the radioiodinated HDL fractions as a cold carrier (2.39 ± 0.25 mg HDL protein) and HDL tracers were washed at d = 1.21g/ml, 55,000 rpm, 4°C, in a 70.1 Ti rotor for 24 h, followed by dialysis in Tris buffer at 4°C. The composition of each radiolabeled tracer was measured using commercially available enzymatic assay kits as described below. The size and integrity of the fasting and TG-rich HDL tracers were also analyzed by 4–30% nondenaturing polyacrylamide gradient gel electrophoresis (PAGGE). HDL particle size was determined from the migration of standard molecular weight proteins of known diameter (HMW Calibration Kit, Pharmacia, Piscataway, NJ). Peak and mean radius of small and large HDL particles were determined by densitometric scanning (Image Master DTS densitometer with Imagemaster computer software, Pharmacia LKB, Uppsala, Sweden) based on relative migration distance (R_f) of standards and using two distinct approaches. First, the estimated radius of the major peak in each scan was identified as the HDL peak particle size. A mean (or weighted) HDL particle size was also calculated using a modification of the method described by Li et al. (16). The mean HDL particle size for each subject was obtained by multiplying the size of each band by its fractional area. This mean HDL particle radius (in nm) therefore combines the HDL size distribution as well as the relative concentration of each HDL band (16).

Phase 2: HDL turnover study

An aliquot of each tracer containing 1.38 ± 0.25 mg HDL protein, 6.5 ± 0.9 ($\times 10^7$) cpm of ^{131}I-HDL, and 1.1 ± 0.5 ($\times 10^6$) dpm of ^{3}H-cholesteryl ether was injected simultaneously into the right marginal ear vein, the TG-rich tracer into one male NZW rabbit, and the fasting HDL tracer into another. Blood samples (2 ml) were obtained over the next 3 days from a vein in the opposite ear at the following time intervals: 10 min, and 1, 2, 3, 4, 6, 24, 27, 30, 48, 51, and 54 h.

Isolation of HDL and apoA-I for radioactivity counting

Serum was immediately separated from red cells by centrifuging at 3,000 rpm for 20 min at 4°C. HDL was isolated (from ~1) ml serum) by sequential ultracentrifugation as follows: *1*) the serum was first adjusted to a density of 1.063 g/ml and centrifuged for 5 h at 100,000 rpm at 4°C in an Optima TLX ultracentrifuge (Beckman Instruments, Palo Alto, CA) using a TLA 100.3 rotor, *2*) the bottom two-thirds fraction from this spin was adjusted to a density of 1.21 g/ml and centrifuged for 5 h at 100,000 rpm, and *3*) the top one-third fraction from the centrifugation was washed for a further 5 h at 100,000 rpm. Aliquots of total serum and of the d < 1.063 g/ml HDL and d >1.21 g/ml

serum fractions were taken to measure the proportion of ^{131}I protein and ^{3}H-cholesteryl ether radioactivity found in each of these fractions. To determine ^{3}H-cholesteryl oleyl ether radioactivity, each sample was counted in Ready Solve liquid scintillation cocktail (Beckman, Fullerton, CA) using a Beckman 6500 liquid scintillation counter. All ^{3}H-cholesteryl oleyl ether radioactivity counts were adjusted back to total serum radioactivity in dpm/ml. In a preliminary experiment, delipidated HDL tracers were analyzed using thin-layer chromatography (TLC). It was found that greater than 97% of both fasting and TG-rich HDL lipid tracer radioactivity co-migrated with cholesteryl ether on TLC.

To measure the ^{131}I radioactivity specifically associated with apoA-I, 500-μl aliquots of the dialyzed HDL were delipidated as previously described (17) and dissolved in a 0.5 M phosphate buffer with 1% SDS, 1% mercaptoethanol, pH 7.2, overnight. The samples were then run on 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to isolate apoA-I. The apolipoproteins were stained overnight with R250 Comassie blue colloidal stain and destained for 2 h in water, and the A-I band was sliced from the gel. Radioactivity in each serum fraction and in the apoA-I band on the gel was counted in a Beckman 5500 gamma counter. The apoA-I radioactivity in the gel was taken as a percentage of the radioactivity found in that gel and adjusted back to total serum radioactivity in cpm/ml. All radioactivity counts were corrected for the half-life of the isotope and adjusted back to the time of injection. In addition to the use of 15% SDS-PAGE, the injectates were also analyzed for the percentage of ^{131}I counts in the various apolipoproteins using isopropranol precipitation and isoelectric focusing techniques. The percentage of radioactivity of the injectates present in a bound versus free form was also analyzed using trichloroacetic acid (TCA) precipitation.

Laboratory measurements

Cholesterol was measured using the CHOD-PAP enzymatic colorimetric kit (Boehringer Mannheim GmbH Diagnostica, Montreal, Canada). CE was measured using the cholesterol kit after degrading free cholesterol in the samples with a mixture of cholesterol oxidase, peroxidase, phenol, triton-X, and phosphate buffer (18). Protein was measured by the technique described by Lowry et al. (19). Triglycerides were measured as esterified glycerol using an enzymatic colorimetric kit (Boehringer Mannheim GmbH Diagnostica). Free glycerol was eliminated from the sample in a preliminary reaction followed by enzymatic hydrolysis of triglyceride with subsequent determination of the liberated glycerol by colorimetry. Phospholipids were measured using a kit (Test-Combination Phospholipids; Boehringer Mannheim GmbH Diagnostica).

Kinetic analysis

The radioactivity die-away curves were analyzed using a two-pool model as previously described (17, 18). This model assumes the existence of an intravascular pool in equilibrium with a nonvascular pool, and all losses from the system are assumed to occur from the intravascular pool. For each radiolabeled tracer, "population" kinetic parameters were computed by population kinetic analysis using an iterative two-stage method (20) based on using the population mean at each iteration as a priori for improving the individual parameter estimates. Population kinetic analysis takes into consideration intersubject variability to estimate kinetic parameters for a group or population of individuals (17). The population kinetic parameters calculated as above were then used to generate mean die-away curves for the fasting and TG-enriched apoA-I and CE HDL tracers. As previously described (17), computing population kinetics is the process of fitting a model to an individual's data while using statistical information from all subjects to help generate the parameters of the population. Population kinetic parameters are similar to mean kinetic parameters obtained from individual fitting of subjects in a group (for data-dense situations) (17), but unlike mean kinetic parameters, they are obtained by taking into account the errors associated with the individual parameter estimates, thus reducing the variability of the estimates (17). The estimates of parameters obtained by the population analysis were compared to the individual fit [simulation, analysis, and modeling II (SAAMII)] and determined to be similar, but were associated with lower coefficients of variation.

The two-pool model provided good fits to HDL apoA-I and CE tracer data to yield estimates of the fractional catabolic rate (FCR). Estimates of the precision of the individual FCRs, coefficients of variation (CV), are determined during the fitting process. These CV are functions of the noise in the experimental data and the structure of the model. The average CV for apoA-I FCRs were 11 ± 1% for TG-rich HDL and 20 ± 4% for fasting HDL. The average CV for CE FCRs were 15 ± 2% for TG-rich HDL and 27 ± 4% for fasting HDL. The proportion of CE catabolized via selective clearance from the HDL fraction was calculated as the difference between the FCR of HDL CE and the FCR of ^{131}I apoA-I (21).

Statistics

Results are presented as mean ± SEM. Unpaired *t*-tests were performed to compare population FCR values between fasting and TG-rich HDL tracers. Paired *t*-tests were used to test differences in tracer composition and size.

RESULTS

In a total of nine HDL turnover studies, the physiological variation in HDL TG, cholesterol, CE, phospholipid, and protein averaged 9%, 7%, 11%, 9%, and 6%, respectively, in the animals receiving fasting tracer; and 8%, 11%, 17%, 8%, and 4%, respectively, in the animals receiving TG-rich tracer.

Table 1 presents the mean lipid and protein composition of the fasting and TG-rich rabbit HDL tracers. TG enrichment of rabbit HDL with human VLDL resulted in a large (87%) increase in HDL TG content ($P < 0.0001$) in the TG-rich HDL tracer compared with the fasting HDL tracer. TG enrichment also produced reciprocal reductions

TABLE 1. Mean lipid and protein characterization of the TG-rich HDL and fasting HDL tracers

	TG-Rich HDL	Fasting HDL	% Difference[a]
	% of HDL weight		
Triglycerides	13.4 ± 1.2	7.2 ± 1.2	87.1[b]
Cholesterol	9.6 ± 1.2	14.3 ± 1.2	−32.9[b]
Esters	6.9 ± 0.9	10.1 ± 0.6	−31.3[c]
Free	3.0 ± 0.5	4.5 ± 1.1	−32.8
Phospholipids	24.6 ± 0.9	25.7 ± 2.8	−4.2
Proteins	51.0 ± 2.8	51.0 ± 2.1	0.2

Values are mean ± SEM.

[a] % Difference indicates the relative change in the TG-rich HDL above or below the fasting HDL values.

[b] $P < 0.0001$ compared with fasting HDL.

[c] $P = 0.05$.

in HDL total cholesterol and CE content [33% ($P < 0.0001$) and 31% ($P < 0.05$), respectively] of the TG-rich HDL. Finally, HDL phospholipid and protein concentrations were not modified to a significant extent by incubation with human VLDL.

Analysis of the HDL tracers pre- and postiodination showed that the iodination procedure had no significant effect on the composition of the particles (results not shown) or on the integrity of the radiolabeled tracers when monitored on 4–30% PAGGE. There was no significant difference in either the weighted mean or peak sizes of the fasting and TG-rich HDL tracers (weighted size: 5.44 ± 0.11 vs. 5.61 ± 0.10, respectively; peak size: 5.47 ± 0.10 vs. 5.63 ± 0.09 nM, respectively). Both tracers were found to have more than 92% of their ^{131}I radioactivity in a protein-bound form, as assessed with TCA precipitation. To determine percentage of ^{131}I counts in the various apolipoproteins, the injectates were also analyzed using 15% SDS-PAGE, isopropanol precipitation, and isoelectric focusing techniques. Thirty-seven percent of this radioactivity was bound to apoA-I [n = 9 experiments; P = not significant (NS) between TG-rich and fasting HDL tracers], with the remainder of the counts distributed in apoB, apoE, and apoC (n = 3 experiments; P = NS between TG-rich and fasting HDL tracers).

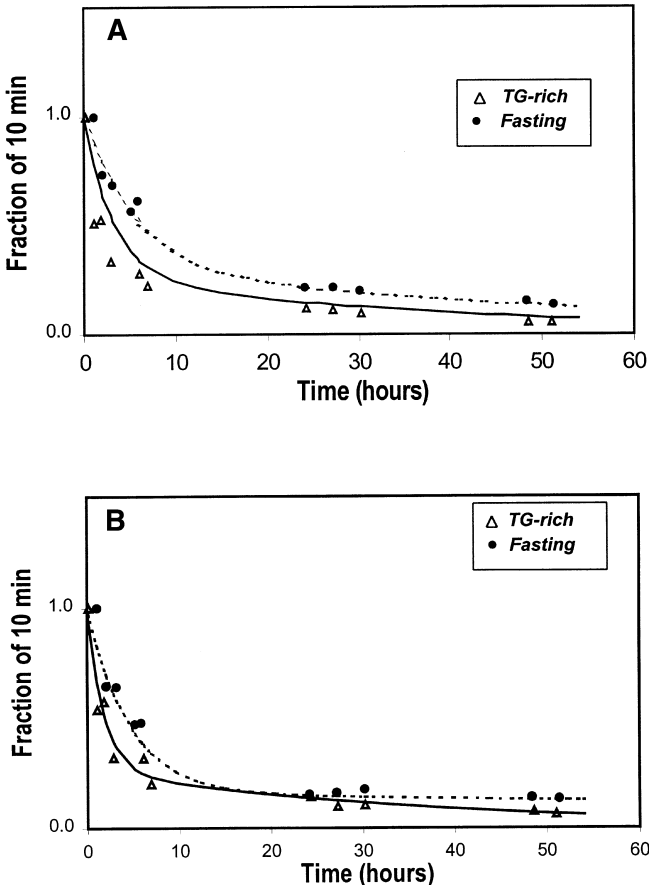

Fig. 1. Die-away curves of HDL apoA-I (A) and CE (B) radioactivity from one representative experiment. Results for the TG-enriched HDL tracer (observed data: open triangles; modeled data: solid line) and fasting HDL tracer (observed data: closed circles; modeled data: dashed line) are shown. The data presented on the y axis of graph A represent the radioactivity on HDL apoA-I isolated by gel electrophoresis of the delipidated HDL fraction (see Materials and Methods). The data presented on the y axis of graph B represent the CE radioactivity in the total HDL fraction. The radioactivity data for each tracer were normalized as a function of the radioactivity measured at the first time interval (10 min).

The proportion of injected ^{131}I radioactivity that was recovered during the turnover studies within the HDL fraction was similar whether derived from the fasting (50 ± 8%) or the TG-rich HDL (43 ± 5%) tracers. The proportion of serum radioactivity in the d < 1.063 g/ml and the d >1.21 g/ml fractions were also similar (P = 0.41 for both) for the fasting and the TG-enriched HDL turnover studies (d < 1.063 g/ml: 13 ± 4% and 12 ± 3%; d >1.21 g/ml: 37 ± 5% and 46 ± 3%, respectively). The percentage of ^{3}H-cholesteryl ether radioactivity recovered in the different lipoprotein fractions was also calculated during the turnover studies. For both fasting HDL and TG-rich HDL, the majority of ^{3}H-cholesteryl ether radioactivity was recovered in HDL (69 ± 6% and 71 ± 6%, respectively) and the recoveries were similar for both tracers in the HDL (P = 0.79) and the d < 1.063 g/ml fractions (31 ± 6% and 29 ± 6%, P = 0.79).

Effect of TG enrichment of HDL on the FCR of HDL apoA-I

Fig. 1A shows the die-away clearance curves of radiolabeled HDL apoA-I from fasting HDL and from the TG-enriched HDL for one representative experiment. As described in Materials and Methods, the isotope clearance curves were constructed using the counts in the isolated apoA-I band. The kinetic parameters (FCR) derived from the clearance curves are presented in **Table 2**. Results from nine experiments showed no systematic difference in the rate of clearance of fasting compared with TG-rich HDL apoA-I (0.107 ± 0.024 vs. 0.119 ± 0.017 pools per h), with an 11.6% greater mean FCR of TG-rich versus fasting HDL CE, P = 0.68). **Fig. 2A** illustrates the die-away curves for radiolabeled fasting and TG-rich HDL apoA-I generated from the estimate of the mean population parameters.

TABLE 2. Kinetic parameters of apoA-I from TG-rich and fasting HDL in rabbits[a]

Experiment	TG-Rich HDL	Fasting HDL
	FCR h^{-1} (CV, %)[b]	
1	0.104 (8.3)	0.069 (11.2)
2	0.107 (8.0)	0.068 (17.9)
3	0.082 (10.7)	0.037 (35.0)
4	0.177 (15.5)	0.249 (11.6)
5	0.092 (19.4)	0.062 (37.1)
6	0.226 (10.3)	0.150 (23.3)
7	0.077 (8.0)	0.046 (9.9)
8	0.098 (11.1)	0.100 (7.6)
9	0.109 (7.8)	0.181 (30.6)
Mean ± SEM	0.119 ± 0.017	0.107 ± 0.024

[a] No significant difference between fasting and TG-rich HDL, P = 0.68.

[b] CV, %: coefficient of variation is the standard deviation divided by the estimated FCR, expressed as percent. The CV is an estimate of the degree of precision of the FCR.

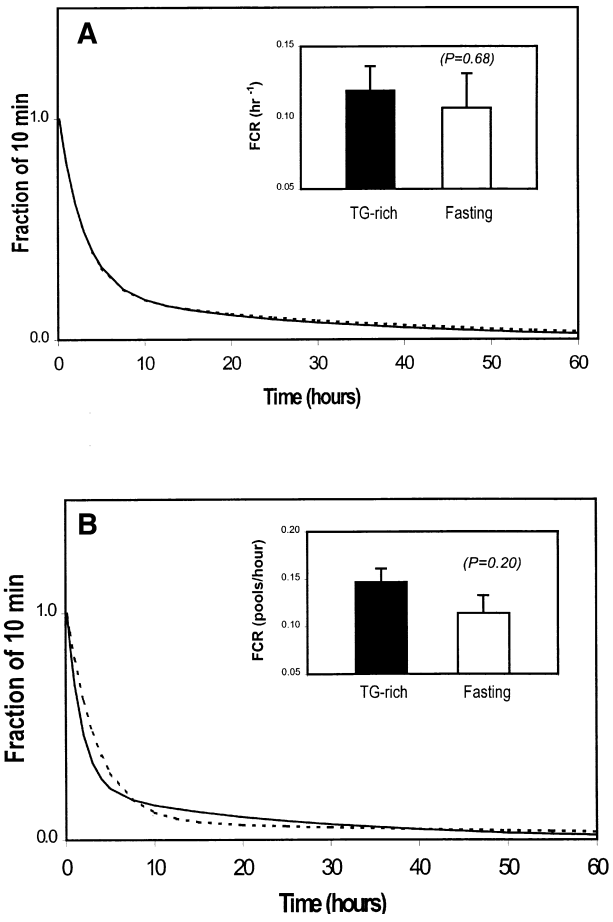

Fig. 2. Simulated die-away curves of HDL apoA-I (A) and CE (B) radioactivity. The die-away curves for the TG-enriched HDL tracer (solid line) and fasting HDL tracer (dashed line) were generated from the estimated population analysis parameters. The radioactivity data for each tracer were normalized as a function of the radioactivity measured at the first time interval (10 min). Insets show the FCR associated with TG-rich and fasting HDL.

Effect of TG enrichment of HDL on the FCR of HDL cholesteryl ether and on selective CE clearance

The individual kinetic data for HDL cholesteryl ether derived from each tracer are presented in **Table 3**. The die-away curves of HDL cholesteryl ether from fasting and TG-enriched HDL are shown in Fig. 1B (one representative experiment) and Fig. 2B (simulated curve derived by using mean population parameters). The curves were constructed from the total counts in the HDL pool. In nine experiments, the mean percentage difference in clearance of apoA-I from TG-rich HDL versus fasting HDL was 27.8%. This trend toward a greater HDL apoA-I FCR resulting from TG enrichment of HDL was not significant (0.146 ± 0.014 vs. 0.114 ± 0.019 pools per h, $P = 0.20$) compared with fasting HDL.

The proportion of CE catabolized via selective clearance from the HDL fraction was calculated as the difference between the FCR of HDL cholesteryl ether and the FCR of ^{131}I apoA-I (21) (**Table 4**). The rates of selective CE clearance were not significantly different between fasting and TG-rich HDL ($P = 0.52$).

TABLE 3. Kinetic parameters of CE from TG-rich and fasting HDL in rabbits[a]

Experiment	TG-Rich HDL	Fasting HDL
	FCR h^{-1} (CV, %)[b]	
1	0.161 (22.3)	0.117 (26.9)
2	0.132 (12.1)	0.091 (27.4)
3	0.158 (22.9)	0.099 (32.9)
4	0.225 (13.3)	0.062 (52.4)
5	0.156 (22.2)	0.102 (25.5)
6	0.162 (11.5)	0.173 (25.6)
7	0.092 (10.4)	0.024 (19.4)
8	0.131 (13.5)	0.137 (11.2)
9	0.094 (10.8)	0.221 (21.4)
Mean ± SEM	0.147 ± 0.014	0.114 ± 0.019

[a] No significant difference between fasting and TG-rich HDL, $P = 0.20$.

[b] CV, %: coefficient of variation is the standard deviation divided by the estimated FCR, expressed as percent. The CV is an estimate of the degree of precision of the FCR.

DISCUSSION

The present study shows that TG enrichment of HDL, a common metabolic consequence of hypertriglyceridemia, does not significantly enhance the metabolic clearance rates of HDL apoA-I and CE in rabbits, a species naturally deficient in HL (13). Similarly, selective clearance of HDL CE was not altered by TG enrichment of HDL. Indeed, an 87% average increase in the TG content of HDL did not significantly alter HDL apoA-I and CE clearance rates or selective CE clearance from the HDL fraction. There was a trend toward greater apoA-I and CE FCR with TG-rich compared with fasting rabbit HDL particles (11.6% and 27.7%, respectively), but this trend did not reach statistical significance. Power calculations indicate that the experimental sample size would need to be increased to more than 170 for HDL apoA-I and to 26 for HDL CE to result in a statistically significant greater clearance in the TG-rich compared with fasting HDL. This is in contrast to the highly significant increase in apoA-I FCR in TG-enriched HDL, which we recently observed in a study of six human subjects (7). Whether TG enrichment of HDL

TABLE 4. Kinetic parameters of selective CE clearance[a] from TG-rich and fasting HDL in rabbits[b]

Experiment	TG-Rich HDL	Fasting HDL
	FCR h^{-1}	
1	0.057	0.048
2	0.025	0.024
3	0.076	0.063
4	0.048	−0.187
5	0.065	0.040
6	−0.064	0.023
7	0.015	−0.022
8	0.033	0.037
9	−0.015	0.040
Mean ± SEM	0.027 ± 0.015	0.007 ± 0.026

[a] Selective CE clearance = FCR cholesteryl ether − FCR apoA-I.

[b] No significant difference between fasting and TG-rich HDL, $P = 0.52$.

enhances selective CE clearance from HDL in humans is currently not known. The finding from the present study indicates that the increase in HDL TG content alone does not fully explain the increase in HDL apoA-I FCR and associated reduction in apoA-I and HDL-C levels observed in hypertriglyceridemic states. There are many differences in lipoprotein metabolism between rabbits and humans, but given the important function of HL in the metabolism of HDL (14, 22), we speculate that HL deficiency in the rabbit may have accounted for a lack of observed difference in the clearance of apoA-I and CE from TG-rich versus fasting HDL. This hypothesis will need to be tested in future experiments.

Further indirect evidence that the absence of HL in the rabbit may have accounted for the lack of effect of HDL TG enrichment in enhancing HDL apoA-I and CE clearance comes from a recent study by Lambert et al. (23). These authors showed that HL promotes the selective uptake of HDL CE via SR-B1. Furthermore, the small calculated difference we observed between HDL apoA-I FCR and HDL CE FCR (selective CE clearance) in the present study for both TG-rich and fasting HDL tracers may have been due to the natural deficiency of HL in the rabbit. In a previous study, Goldberg, Beltz, and Pittman (21) estimated that only 20% of HDL CE is cleared by selective uptake in the rabbit, a far smaller percentage than has been observed in other species. In addition, the authors of that study, similar to the present study, observed a wide variability in the contribution of the selective uptake pathway to cellular uptake of HDL CE (from 0% to 47% of the total). They attributed the variability in selective uptake to widely varying CETP activities and TG levels in rabbits, both of which correlated positively with selective HDL CE uptake (21).

In addition to the marked increase in TG content of TG-rich compared to fasting HDL tracers used in the rabbit kinetic studies, there were significant reciprocal reductions in total cholesterol, CE, and free cholesterol mass in the TG-rich HDL. CETP in serum mediates the bidirectional exchange of TG and CE between HDL and the more buoyant lipoproteins (VLDL, LDL, and CM) (24), a process that is enhanced in hypertriglyceridemia (25). Overall, this study was designed to test the effect of the TG content of HDL particles on HDL metabolism. Besides the normal physiological depletion of HDL CE associated with TG enrichment of HDL, other measured components of HDL composition remained unaltered. The fasting and TG-rich HDL tracers were similar in size (both mean peak and weighted mean sizes) and contained similar percentage mass of total protein and phospholipid. Use of intralipid, a synthetic TG emulsion, to enrich HDL particles with TG in our previous kinetic studies in humans and rabbits lead to alterations in the phospholipid content of HDL (7). Hence, to avoid this change in phospholipid content associated with intralipid, we used VLDL from normotriglyceridemic human subjects to enrich HDL with TG in the present study. Thus, the physiological relevance of the tracers was an important consideration in our present investigation.

A number of mechanisms have been proposed to explain the reduction in serum HDL cholesterol levels and serum apoA-I in hypertriglyceridemic states (6). These mechanisms include thermodynamic instability of TG enriched and CE-depleted HDL particles, irreversible loss of apoA-I from HDL during lipolysis of the TG-rich HDL particle by HL, and rapid clearance from the circulation of the lipolytic end-product smaller HDL particle, to mention a few (6). Nonetheless, as the present study demonstrates, TG enrichment of HDL particles, by incubating rabbit serum with human VLDL, was not sufficient to enhance serum HDL apoA-l clearance in this model. Our previous work in rabbits (17, 18) as well as that of others (26) has indicated that TG enrichment, without substantial lipolysis of the particle, is insufficient to enhance the clearance of HDL-associated apoA-l. Our previous studies were confounded, however, by the fact that TG-enriched HDL was derived from hypertriglyceridemic human subjects, and their catabolism subsequently traced in a rabbit model. The present study avoids that confounding factor, and yet it confirms our previous findings that TG enrichment per se, without substantial subsequent lipolytic modification of the HDL particle, does not enhance HDL-associated apoA-I clearance.

Moreover, the effect of TG enrichment of HDL on selective HDL CE clearance has not been examined in vivo prior to this study. Selective HDL CE clearance has been identified as a major pathway in the "reverse" cholesterol transport of cholesterol from peripheral tissues to the liver and adrenal (21), and thereby is believed to play an important role in the cardioprotective effect of HDL. Recently, it has been shown in vitro in HepG2 cells that TG enrichment of HDL, with concomitant reduction in HDL CE content, impairs the process of selective CE uptake in a dose-dependent manner (11). However, that study, in contrast to the present study, was conducted in the presence of HL activity. HL is well known to play a key role in the process of selective HDL CE uptake. HL bound to proteoglycan sulfate at hepatocytes promotes the selective uptake of HDL CE by a number of mechanisms (14), including via the SR-B1 receptor (23, 27). Indeed, Collet et al. (12) demonstrated that HDL CE uptake in vitro is enhanced in TG-enriched human HDL upon treatment with HL.

CONCLUSION

Results of the present study suggest that TG enrichment of HDL without subsequent action by HL is not sufficient to alter the rates at which HDL apoA-I and CE are cleared from the circulation. In addition, selective HDL CE clearance is not altered with TG enrichment of HDL in an HL-deficient species. Therefore, changes in the TG content of HDL do not, by themselves, account for the deleterious effects of hypertriglyceridemia on HDL cholesterol metabolism. Future studies are required to determine more precisely how the structure and composition of HDL, HDL-modifying enzymes, and HDL receptors interact to alter HDL apoA-l, and CE clearance in hypertriglyceridemic states.

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