Abnormal activation of lipoprotein lipase by non-equilibrating apoC-II: further evidence for the presence of non-equilibrating pools of apolipoproteins C-II and C-III in plasma lipoproteins

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Abstract Using artificial triglyceride emulsions, we have demonstrated the presence of non-equilibrating pools of apolipoproteins C-II and C-III in human plasma lipoproteins. As the concentrations of acceptor triglycerides were increased, a greater fraction of both apoC-II and apoC-III shifted away from the native plasma lipoproteins to the artificial lipid emulsions. All of the apoC-II and apoC-III in very low density and high density lipoproteins (VLDL and HDL), however, could not be removed from native plasma lipoproteins. The percent of total plasma apoC-II and apoC-III that could be recovered in the VLDL and HDL density fractions varied when plasma from different individuals was used. When plasma samples from normotriglyceridemic subjects were used, HDL was the primary donor of apoCs. The percent of total plasma apoCs associated with HDL decreased from 60% to 25% for apoC-II and from 65% to 15% for apoC-III. When plasma samples from hypertriglyceridemic subjects were incubated with artificial lipid emulsions, VLDL was the primary donor of apoCs. HDL from hypertriglyceridemic subjects only accounted for 5–10% of total fasting plasma apoCs and did not contribute significantly to the final apoC contents of the artificial triglyceride emulsions. To evaluate the significance of the depletion of exchangeable apoCs from plasma HDL, we also examined the ability of control and apoC-depleted HDL to serve as activator for bovine milk lipoprotein lipase (LPL) in vitro. When HDL depleted of exchangeable apoCs were used as the source of plasma apolipoproteins for the activation of LPL in vitro, only 5–10% of the maximal activity obtained with native HDL was demonstrated. In fact, in the presence of comparable concentrations of HDL apoC-II, activation of LPL was the least with HDL which lacked exchangeable apoCs. Our data thus indicated that the presence of exchangeable apoC-II on HDL is necessary for the activation of LPL in vitro. This finding is consistent with our data that suggest that HDL from hypertriglyceridemic subjects do not stimulate LPL as well as HDL from normolipidemic subjects. **—Tornoci, L., C. A. Scheraldi, X. Li, H. Ide, I. J. Goldberg, and N-A. Le.** Abnormal activation of lipoprotein lipase by non-equilibrating apoC-II: further evidence for the presence of non-equilibrating pools of apolipoproteins C-II and C-III in plasma lipoproteins. *J. Lipid Res.* 1993. **34:** 1793–1803.

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The C apolipoproteins of human plasma are small polypeptides, ranging in molecular weight from 6000 to 9000, that play an important role in modulating the metabolism of several classes of plasma lipoproteins. ApoC-II is a required activator for lipoprotein lipase (LPL) (1, 2). Defects or deficiencies in apoC-II have been associated with hypertriglyceridemia in humans (3). Intravenous infusion of a synthetic polypeptide based on apoC-II amino acid composition has been reported to significantly reduce hypertriglyceridemia in these patients (4, 5). ApoC-III which is present both as non-glycosylated ($apoC\text{-}III_0$) and glycosylated isoforms containing either one ($apoC\text{-}III_1$) or two ($apoC\text{-}III_2$) moles of sialic acid (2, 6) inhibits LPL (7) and hepatic triglyceride lipase (HTGL, 8) activities in vitro. More direct evidence of the inhibitory effect of apoC-III was demonstrated in the kindred with familial apoC-III/apoA-I deficiency (9) who have very rapid fractional catabolic rates of VLDL triglyceride (TRIG) in vivo. The recent report of marked hypertriglyceridemia (10) and delayed TRIG clearance (10) in transgenic mice that overproduce human apoC-III is also consistent with the hypothesis that apoC-III may have an inhibitory effect on lipolytic activity. Inhibition of LPL has also been recently demonstrated using synthetic peptides of apoC-III (11). ApoCs as a group have also been shown to interfere with the receptor-mediated uptake of

Abbreviations: CHYLO, chylomicrons, $S_f > 400$; VLDL, very low density lipoproteins, S_f 20–400, $d < 1.006$ g/ml; [IDL+LDL], mixture of intermediate and low density lipoproteins, S_f 0–20, d 1.006–1.063 g/ml; HDL, high density lipoproteins, d 1.063–1.21 g/ml; LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase; apo, apolipoprotein; IL, Intralipid; TRIG, triglyceride; CHOL, cholesterol.

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certain lipoproteins (12, 13) and the content of apoC-III in plasma HDL, in particular, is correlated with the fractional catabolic rate of HDL apoA-I (14).

ApoCs are associated with several classes of plasma lipoproteins and are believed to exchange spontaneously among lipoprotein particles both in vivo and in vitro (15, 16). ApoCs shift from plasma HDL toward newly secreted TRIG-rich lipoproteins during postprandial lipemia (17) and return to HDL as TRIG are hydrolyzed (18–20). Earlier tracer kinetic studies have suggested that this exchange may be very rapid and complete (15, 16). Using immunoaffinity chromatography (21) which allows the direct determination of apoC-III specific activity, we have reported that there is a fraction of the apoC-III pool in VLDL as well as in HDL that does not participate in this spontaneous exchange process (22). We now report that apoC-II also fails to equilibrate completely among plasma lipoproteins. Furthermore, HDL that contain only non-exchangeable apoC-II and apoC-III fail to fully activate bovine milk lipoprotein lipase (LPL) in vitro. HDL isolated from hypertriglyceridemic subjects also failed to stimulate maximal activation of LPL as compared to HDL from normotriglyceridemic subjects. This difference appears to be due to the depletion of exchangeable apoCs from hypertriglyceridemic HDL.

METHODS

Materials

Intralipid (IL) (20% intravenous fat emulsion) was obtained from Kabivitrum Inc. (Alameda, CA). Lipoprotein fractionation was carried out using the TLA 100.3 rotor in the Beckman TL-100 tabletop ultracentrifuge (Beckman Instruments, Palo Alto, CA). Clear thick-wall polycarbonate tubes (3.0 ml capacity, Beckman Instruments) were used for ultracentrifugation. Lipid analyses were performed by enzymatic methods on the Hitachi 705 automatic chemistry analyzer using reagent kits from Boehringer-Mannheim Diagnostics (Indianapolis, IN). The laboratory was a participant in the CDC/NHLBI Lipid Standardization Program.

Apolipoprotein quantitation was performed by competition ELISA. ELISA procedures were carried out with a BioTek 706 ELISA system equipped with a 96-well washer. Flat bottom 96-well ELISA plates were obtained from Corning (Corning, NY). Specific rabbit antisera against human apoC-II and apoC-III were prepared in our laboratory (21) and alkaline phosphatase-linked goat anti-rabbit IgG was available from Bio-Rad Laboratories (San Francisco, CA).

Subjects

Table 1 presents the fasting plasma lipids for the subjects who participated in the various phases of this study.

TABLE 1. Clinical characteristics

Subject ID	Phenotype	TRIG	CHOL	HDL-C
		mg/dl	*mg/dl*	*mg/dl*
1a	N	55	185	52
1b		62	201	51
1c		104	176	52
2	N	52	171	62
3	N	73	211	95
4	N	84	221	72
5	HTG-IV	402	179	35
6	HTG-IV/IIb	652	420	32
7	HTG-V	1032	301	25
8	HTG-V	1134	344	27
9	HTG-V	1744	297	20
10	HTG-V	1785	430	17
11a	HTG-V	2334	436	18
11b		1032	301	25
11c		818	303	25
11d		326	277	27

Normal volunteers included staff from the Arteriosclerosis Research Center (Columbia University) and from Penn Med Labs (Medlantic Research Foundation). Patients with type IV and type V hyperlipidemia were recruited from the Arteriosclerosis Research Center of the Columbia-Presbyterian Medical Center (Table 1). Plasma samples from one patient with type V hyperlipoproteinemia (subject 11) were available at several visits over a 6-month period during which his hypertriglyceridemic condition was controlled by a combination of dietary fat restriction (15% of caloric intake from fat, saturates:polyunsaturates: monounsaturates = 1:1:1) and gemfibrozil (600 mg bid). All samples were collected after a 12–14 h fast in Vaccutainer containing liquid EDTA.

Incubations of whole plasma with Intralipid

A homogeneous preparation of Intralipid (IL) was prepared by ultracentrifugation as the stock solution of IL. Briefly, approximately 5 ml of IL was overlayered with 7 ml of 1.006 g/ml density solution (0.01% EDTA) and centrifuged for 30 min at 20,000 rpm using a swinging bucket SW40 rotor (Beckman Instruments) to obtain the stock solution of IL. Preparations of lipid emulsions containing different concentrations of TRIG (ranging from 50 mg/dl to 5000 mg/dl) were obtained by diluting stock IL with normal saline. Actual TRIG concentrations in these IL solutions were determined directly prior to any incubation. Incubations were carried out directly in individual ultracentrifuge tubes with 0.5 ml of plasma and 0.5 ml of the appropriate IL solutions calculated to provide the desired range of ratios of Intralipid-TRIG (IL-TRIG) to plasma TRIG (Plasma-TRIG). The normal distribution of apoCs among plasma lipoproteins was determined from the incubation of 0.5 ml of whole plasma and 0.5 ml of normal saline (d 1.006 g/ml). The mixtures were gently mixed and placed in a shaking water bath

(preheated to 37°C) for 1 h. In a number of initial studies, a separate set of tubes was allowed to incubate for an additional 15–18 h at 4°C prior to lipoprotein fractionation to ensure complete exchange.

Artificial lipid emulsions

In order to examine the effect of the acceptor particles on the redistribution of plasma apoCs, artificial lipid emulsions containing triolein, phosphatidylcholine, cholesterol, and cholesteryl oleate (50:32:2.5:17.8 mmol%) were prepared by sonication as previously described (23). The coarse emulsions were subjected to ultracentrifugation using the SW 41 swinging bucket rotor (Beckman Instruments) for 30 min at 20,000 RPM (15°C). The resulting lipid emulsions were removed from the supernate by aspiration and subsequently fractionated by column chromatography using Bio-Gel A-0.5m gel (Bio-Rad Laboratories). The eluted fractions were pooled into tertiles by TRIG contents corresponding to the largest subpopulation, mid-size, and smallest particles. Only fractions corresponding to the largest (Em-1) and smallest (Em-3) subpopulations were used at different concentration in incubation studies with plasma from a normotriglyceridemic donor (subject 1c).

Isolation of lipoprotein fractions

At the end of the incubation period, the mixtures were immediately cooled by immersion in an iced water bath followed by a brief incubation in the refrigerator for 30 min; they were then carefully overlayered with 2 ml of a d 1.006 g/ml solution. IL were recovered in the supernate by aspiration following ultracentrifugation at 50,000 rpm for 5 min (15°C) to isolate the artificial lipid emulsions as previously described (24). In some instances, due to the high concentration of TRIG present, a second 5-min centrifugation was required and all the tubes in that set would be subjected to a second spin and the two supernates were combined prior to composition analysis.

Approximately 0.75–1.0 ml of the intermediate fraction was removed by aspiration and discarded. The inner wall of the tubes above the residual infranate was carefully dried with a cotton swab and the volume in each centrifuge tube was brought up to 3 ml with d 1.006 g/ml density solution. The contents of the tubes were mixed prior to the next centrifugation step for the isolation of VLDL. One ml of the supernate was removed by aspiration as VLDL after a 2-h centrifugation at 100,000 rpm (15°C). An additional 1 ml of the middle layer was also removed by aspiration and discarded. The infranate was adjusted to a density of 1.063 g/ml using higher density solution for the isolation of LDL (d 1.006–1.063 g/ml, 100,000 rpm for 5 h at 15°C). The HDL fraction is defined as the infranate that includes the density fraction d > 1.063 g/ml remaining in the tubes.

TRIG and CHOL contents in each fraction were immediately determined by enzymatic methods. The fractions were diluted as necessary in order to keep the measured TRIG concentrations below 300 mg/dl. Aliquots of the individual lipoprotein fractions from each set were stored frozen at −70°C until they were needed for apolipoprotein measurements or LPL activation assays.

Determination of apoC-II and apoC-III concentrations by ELISA

The concentrations of apoC-II and apoC-III were determined by competition ELISA using monospecific polyclonal antibodies previously characterized in our group (21). The density fraction d<1.020 g/ml isolated from pooled plasma was used as coating material. Aliquots of the coating material were stored at −80°C and were never refrozen. Purified apoC-II and apoC-III were used to calibrate the concentrations of apoC-II and apoC-III in a pooled plasma sample that was subsequently used as standard for all assays. The standard curve was fitted to a 4-parameter sigmoidal curve using the STATGRAPHICS package on IBM-compatible personal computers. The inter- and intra-assay variabilities for the apoC-II and apoC-III ELISAs were less than 11%. All apoC determinations from a series of incubations were performed in a single assay.

Source of LPL

Bovine milk LPL was purified by affinity chromatography using heparin-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) by the method of Socorro, Green, and Jackson (25), assayed for activity using an artificial substrate consisting of 1.15 μmol tri-[9,10(n)-^{3}H]oleoylglycerol emulsified with phosphatidylcholine (5% by weight), 6 mg/ml bovine serum albumin, pH 8.2 (26) and stored at −70°C until use. Bovine milk LPL preparations containing 330 μg protein/ml were diluted 10-fold with PBS just prior to use.

Activation of bovine milk lipoprotein lipase

In vitro studies of the activation of LPL by plasma or isolated lipoprotein fractions were carried out using purified bovine milk LPL with a specific activity of approximately 30 mmol free fatty acid (FFA) released per h per mg protein. LPL activity was measured at 37°C using gum arabic artificial emulsions as described by Baginsky and Brown (27). LPL substrate was freshly prepared the day of the assay from stock solutions of [^{14}C]oleic acid in toluene (10 nCi/ml, New England Nuclear Research Products, Boston, MA), and of triolein containing 300 nCi tri-[9,10,-^{3}H]oleoylglycerol (Amersham), plus 1 g unlabeled triolein (Nu-Chek Prep, Elysian, MN) in 33 ml of heptane. The [^{14}C]oleic acid (2.5 μl/assay) and 75 μl/assay of the triolein stock solution were evaporated under N_2. To this was added 75 μl/assay of a gum arabic preparation (15 g in 100 ml of 0.2 M Tris, pH 8.2) and 100 μl/assay of

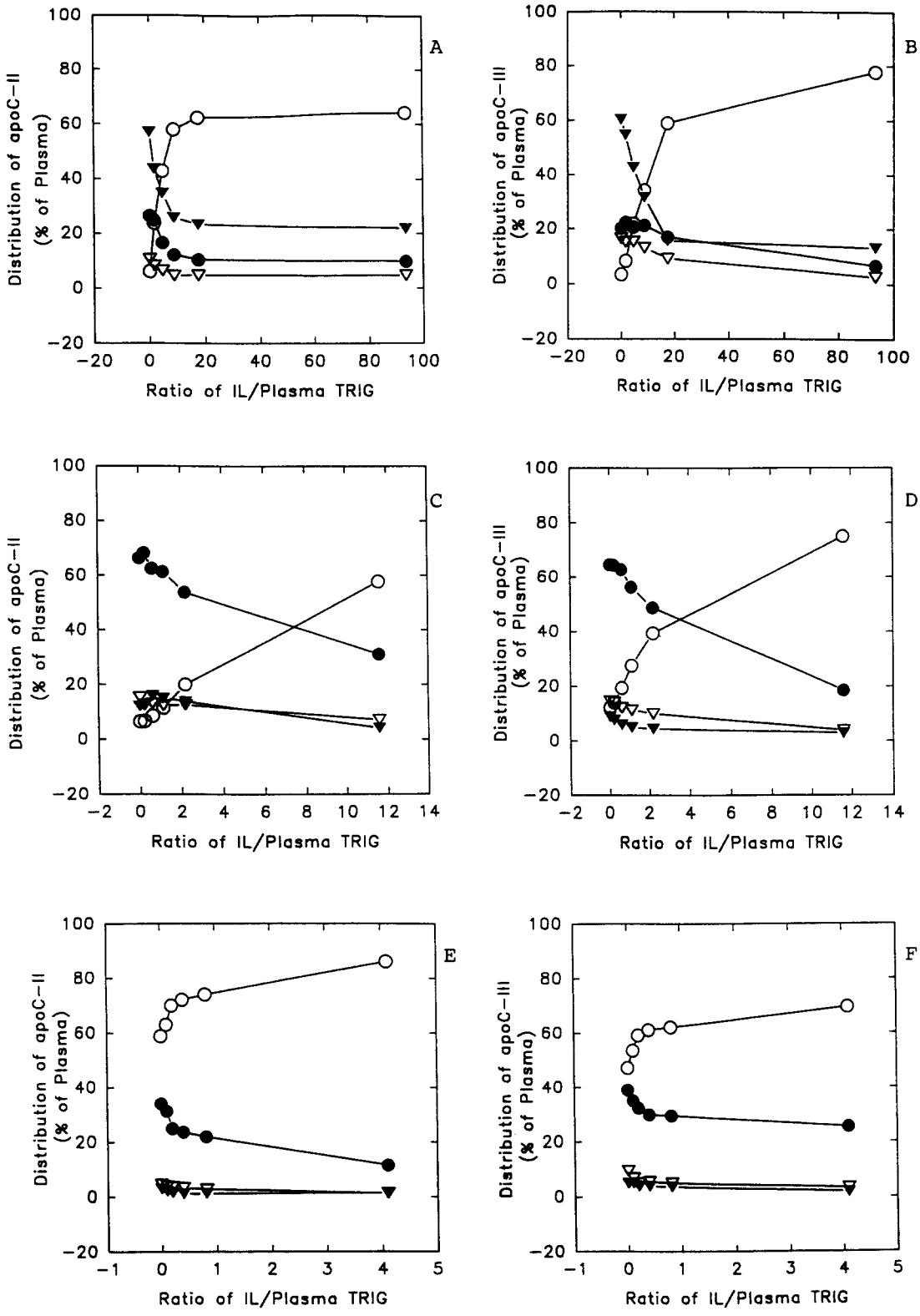

Fig. 1. Redistribution of apoC after incubations of fasting plasma with different concentrations of Intralipid (IL). The percent of total plasma apoC-II and apoC-III recovered in the $S_f > 400$ fraction (○), S_f 20–400 VLDL fraction (●), S_f 0–20 [IDL+LDL] fraction (▽), and $d > 1.063$ g/ml HDL fraction (▼) is presented on the vertical axis. The ratios of exogenous triglyceride (IL-TRIG) over the fasting plasma triglyceride in the incubation mixture are presented on the horizontal axis. A, B depict the redistribution of apoC-II and apoC-III with plasma from subject 1b, respectively. The redistribution of apoCs with plasma from subject 6 is presented in C, D and in E, F from subject 9.

a solution of 12.5% bovine serum albumin in 0.16 M NaCl, 0.35 M Tris, pH 8.2. The emulsion was sonicated on ice using a model S75 sonicator (Branson Instruments, Inc. Danbury, CT).

The assay mixture consisted of 150 μl of the triolein substrate emulsion and a potential source of apoC-II (inactivated serum, isolated VLDL, or HDL) in a total volume of 300 μl. The mixture was incubated at 37°C for 1 h. Then a source of LPL (10 μl) was added and the reaction was allowed to proceed for an additional hour at 37°C. The reaction was terminated by the addition of 3.5 ml of a mixture of methanol–chloroform–heptane–oleic acid 1410:1250:1000:1 (by volume) and 1 ml of borate buffer (0.05 M, pH 10) (28). Recovery of [^{3}H]FFA during organic extraction was determined from the amount of [^{14}C]oleic acid internal standard present in the aqueous phase. All assays were performed in triplicate and the results were converted to μmoles FFA liberated per ml of LPL per hour.

RESULTS

Redistribution of plasma apoCs by incubations with Intralipid

More than 95% of the exogenous TRIG (IL-TRIG) added in the form of Intralipid was recovered in the IL fraction defined as $S_f > 400$ with, in most cases, minimal TRIG enrichment of the VLDL, LDL, and HDL. In the presence of higher concentrations of IL-TRIG ([IL-TRIG]/[Plasma-TRIG] > 3.0), all lipoprotein fractions were found to be enriched in TRIG. TRIG increased from 15 to 36 mg/dl in VLDL, from 12 to 37 mg/dl in LDL, and from 1 to 51 mg/dl in HDL. Lipoprotein particles did not aggregate nonspecifically with the lipid emulsion particles; there was less than 1% of either plasma apoB or apoA-I in the IL fractions as isolated by ultracentrifugation in incubation studies using plasma from normo- and hypertriglyceridemic subjects (data not shown).

Fig. 1 illustrates the redistribution of apoC-III and apoC-II among plasma lipoproteins after the incubations of fasting plasma with different levels of IL-TRIG. Even with the highest concentration of IL-TRIG (4393 mg/dl as compared to 47 mg/dl for Plasma-TRIG or a ratio of [IL-TRIG]/[Plasma-TRIG] of 93.5), a significant portion of apoC-II and apoC-III remained in HDL and VLDL when plasma from a normolipidemic subject was used (Fig. 1A and 1B). HDL was the primary source of apoCs, losing over 70% of its apoC-III and 60% of its apoC-II to the artificial lipid emulsions. In contrast, HDL from hypertriglyceridemic samples did not contribute significantly to the apoCs recovered in the artificial triglyceride emulsions (Figs. 1C–F). One possible explanation for this phenomenon is the observation that, in fasting plasma obtained from hypertriglyceridemic subjects, apoCs were associated primarily with the density fraction $d < 1.006$ g/ml, 60–70% in subject 6 with TRIG of 652 mg/dl (Fig. 1C, D), and 65–80% in subject 9 with TRIG of 1774 mg/dl (Fig. 1E, F). Upon incubations of hypertriglyceridemic plasma with IL, VLDL was the primary donor of apoCs for the transfer to artificial lipid emulsions. The concentration of apoC-III in VLDL was reduced by 45% and by 80% for apoC-II in VLDL (Fig. 1C and 1D, respectively).

The contribution of VLDL as the source of apoCs for exogenous triglyceride emulsions is further illustrated in incubation studies of IL with whole plasma from a normolipidemic subject (**Fig. 2**). At low concentrations of acceptor emulsions, HDL was the primary donor of apoC-III, while at higher concentrations of Intralipid, additional apoC-III was contributed by fasting plasma VLDL (Fig. 2A). With respect to the redistribution of apoC-II toward the artificial lipid emulsions, on the other hand, the contribution of VLDL as a source of exchangeable apoC-II was demonstrable at very low concentrations

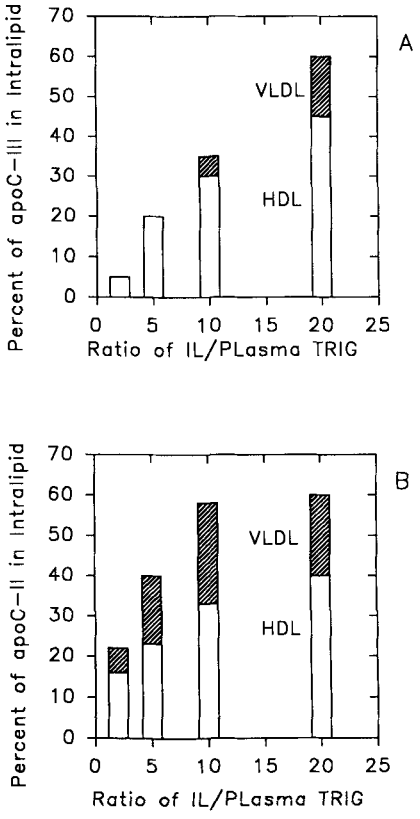

Fig. 2. Contribution of VLDL and HDL to the amount of apoC recovered in the artificial lipid emulsions after the incubations of plasma from subject 1a with different concentrations of Intralipid. The percent of plasma apoC-III (Fig. 2A) and apoC-II (Fig. 2B) recovered in the $S_f > 400$ fraction is presented on the vertical axis. The horizontal axis indicates the ratio of IL-TRIG to Plasma-TRIG in the incubation mixture. The solid bar represents the contribution from plasma HDL and the hatched bar, the contribution from plasma VLDL.

of Intralipid (Fig. 2B). However, HDL remained the major source of apoC-II in incubation studies in which plasma from normotriglyceridemic subjects was used.

Table 2 illustrates the distribution of apoC-II and apoC-III in different subjects either in fasting plasma or in the presence of high concentrations of exogenous TRIG as artificial acceptors. The ratio of IL-TRIG to Plasma-TRIG varied in the different incubation studies and ranged from 0 (no Intralipid) to 90 in studies involving plasma from normolipidemic subjects, and from 0 (no IL) to 15 in studies using plasma from hypertriglyceridemic patients. Data from three separate incubation studies in the same normolipidemic subject (subject 1) performed over a 6-month period showed that the redistribution of apoC-II and apoC-III from plasma lipoproteins to intralipid emulsions in this in vitro system was quite reproducible.

Abnormal activation of bovine milk LPL by HDL

Fig. 3 illustrates the activation of bovine milk LPL by HDL (d 1.063–1.21 g/ml) isolated by ultracentrifugation from one hypertriglyceridemic subject (subject 11, Table 1) undergoing TRIG-lowering therapy. As hypertriglyceridemia was reduced by a combination of diet and gemfibrozil, HDL isolated from plasma collected during subsequent visits were found to be more effective in stimulating maximal LPL activity. HDL from each sample were concentrated by ultracentrifugation at density 1.063–1.21 g/ml and adjusted back to plasma concentration prior to the determination of LPL activity. Increasingly greater LPL activity was achieved with HDL isolated from plasma when TRIG was gradually reduced from 2334 to 326 mg/dl (Fig. 3A). Unfortunately, apoC-II concentrations in these HDL fractions were not available for this subject as TRIG levels were reduced.

In subsequent studies, apoC-II concentrations were available for all HDL fractions. When the ability of HDL isolated from normo- (subjects 2–4) and hypertriglyceridemic subjects (subjects 5–10) to activate LPL was adjusted for apoC-II contents, suboptimal activation could be shown with hypertriglyceridemic HDL, though less dramatic (Fig. 3B). At low concentrations of apoC-II (0.1–0.25 μg of apoC-II), there was minimal LPL activation (10% of maximal expected activity) and no difference could be seen between HDL isolated from hypertriglyceridemic and normotriglyceridemic individuals. At higher concentrations of apoC-II (1 μg of apoC-II), activation of LPL by normotriglyceridemic HDL was 50% higher than that obtained with hypertriglyceridemic lipoproteins ($P < 0.05$). In view of the data presented here on the minimal contribution of hypertriglyceridemic HDL to the redistribution of apoCs, in particular of apoC-II, we hypothesize that the observed lack of maximal activation of LPL by hypertriglyceridemic HDL may be due to the absence of exchangeable apoC-II from these lipoprotein particles.

To test this hypothesis, we used the density fraction d>1.063 g/ml isolated from a normotriglyceridemic individual (subject 1b) which was previously exposed to increasing concentrations of exogenous lipid emulsions (Fig. 1A and 1B) as source of apoC-II for the activation of bovine milk LPL. **Fig. 4** illustrates the activation of LPL by different volumes of HDL-containing fractions isolated by ultracentrifugation in d>1.063 g/ml after the incubations with exogenous lipid emulsions. As shown, the HDL fractions that were incubated with normal saline (i.e., in the absence of Intralipid) were the most efficient in activating LPL. In contrast, HDL fractions isolated from incubations that contained higher concentrations of IL-TRIG were less effective in activating LPL in a dose-dependent relationship (Fig. 4A). One possibility for the decreased activity may have been the low concentration of apoC-II in these HDL fractions. Fig. 4B presents the LPL activation curves from the same series of incubations after normalization for the total mass of apoC-II present in each LPL assay tube. As illustrated for a comparable

TABLE 2. Percent of non-exchangeable apoCs in VLDL and HDL

		% ApoC-II in VLDL[c]		% ApoC-II in HDL[c]		% ApoC-III in VLDL[c]		% ApoC-III in HDL[c]	
Subject[a]	Ratio[b]	Saline	IL	Saline	IL	Saline	IL	Saline	IL
1a	90.0	35.1	11.4	55.9	10.4	38.7	4.0	48.1	8.6
1b	93.5	26.5	9.6	60.2	13.1	20.2	6.7	56.9	11.7
1c	80.0	30.1	10.1	54.4	9.5	37.1	4.4	50.8	7.6
5	19.5	57.4	7.5	17.4	2.1	78.3	15.9	11.0	3.7
6	11.6	64.2	18.5	8.8	2.8	66.3	31.1	12.0	4.1
9	4.2	34.0	11.5	2.9	1.5	39.0	25.4	4.8	1.7

[a]Fasting plasma samples from subject 1 were used in three separate studies over a 6-month period. Data from Study 1b are presented graphically in Fig. 1A and 1B. HDL fractions from Study 1c were used in the study of LPL activation in Fig. 6. Data from subjects 6 and 9 are presented in Fig. 1 C and D and 1 E and F, respectively.
[b]Maximal ratio of Intralipid (IL) triglyceride to plasma triglyceride.
[c]Distribution of apoCs is expressed as percent of the total plasma concentration of the respective apoC.

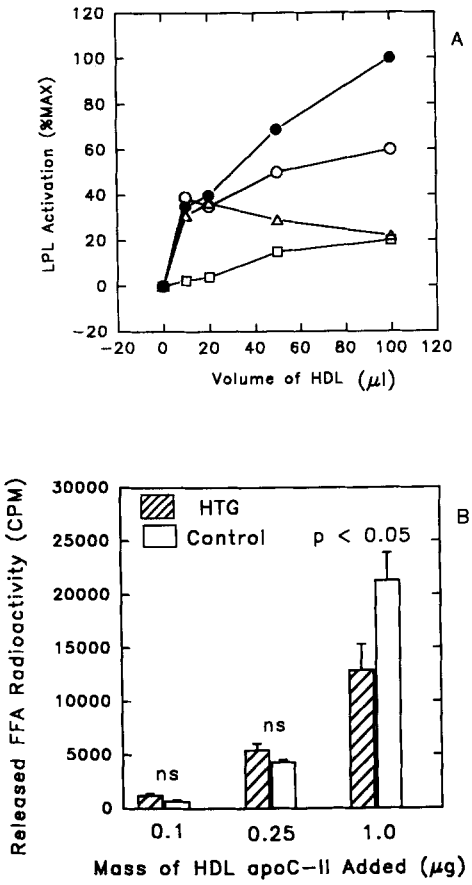

Fig. 3. Activation of bovine milk LPL by isolated HDL. Fig. 3A presents the activation of LPL by HDL isolated from plasma samples collected from subject 11 (see Table 1) at the different clinic visits as TRIG level was reduced by a combination of diet and gemfibrozil therapy. Minimum activation was observed with HDL from the pretreatment period (□) when TRIG was 2334 mg/dl. Fasting TRIG levels at the return visits were 1032 (△), 818 (○), and 326 (●) mg/dl. Activation of LPL by isolated HDL fractions was correspondingly increased. In Fig. 3B, the activity of bovine milk LPL was assessed in the presence of HDL that were isolated from fasting plasma of normotriglyceridemic subjects (subjects 2–4, open bars) and hypertriglyceridemic subjects (subjects 5–10, hatched bars). ApoC-II concentrations in HDL were determined by ELISA and appropriate volumes of HDL were used to provide the desired concentrations of apoC-II as source of activator for the determination of LPL activity. The vertical axis represents the actual radioactivity associated with free fatty acids that were released by LPL action.

mass of apoC-II, HDL that had been exposed to high concentrations of IL-TRIG, presumably with less exchangeable apoC-II, were less effective in activating LPL as compared to HDL that had retained more exchangeable apoCs after incubations with lower concentrations of IL-TRIG. Furthermore, the data in Fig. 4A would suggest that any inhibition of LPL by apoC-III present in the d>1.063 g/ml fraction would be minimal as increasing LPL activity could be demonstrated as the volume of the d>1.063 g/ml fraction added to the LPL assay was increased from 20 to 100 μl. It should be noted that in spite of the 50-fold increase in TRIG contents of HDL after incubations with Intralipid, LPL activity remained linear as a function of the volumes of HDL added, suggesting that the system had not been saturated.

Factors affecting the redistribution of apoCs

Several factors appear to affect the rate of transfer of apoC from plasma lipoproteins to the exogenous triglyceride-rich emulsions. To test whether additional transfer of lipoprotein apoCs could occur, in some studies a separate set of tubes was allowed to incubate for an additional period of 16 h at 4°C. No further exchange of either apoC-II or apoC-III could be demonstrated, suggesting that the equilibration was complete within the first hour of incubation at 37°C (data not shown). **Fig. 5** illustrates the difference in the redistribution of apoC-II to Intralipid when plasma samples from subjects with varying

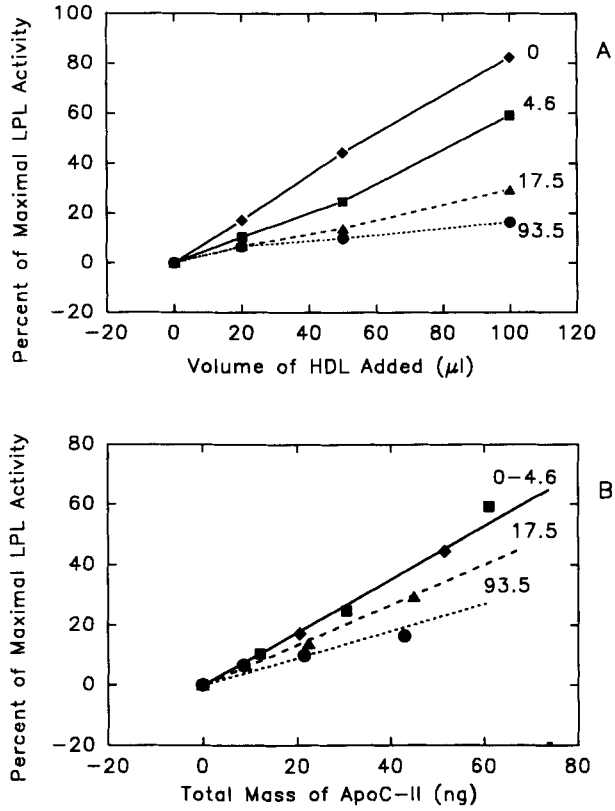

Fig. 4. Effect of non-exchangeable apoC-II on the activation of bovine milk LPL. The fractions of d>1.063 g/ml were isolated after the incubations of fasting plasma from a normolipidemic subject (subject 1b) with different concentrations of Intralipid and used to activate LPL in vitro either in terms of adjusted volumes of HDL (Fig. 4A) or in terms of HDL apoC-II masses (Fig. 4B). As the ratio of exogenous TRIG to plasma TRIG, [IL-TRIG]/[Plasma-TRIG], was increased from 0 (◆, no intralipid) to 4.6 (■) to 17.5 (▲) and to a maximum of 93.5 (●), the activity of LPL was reduced at comparable volumes of HDL (Fig. 4A) or at comparable concentrations of HDL apoC-II (Fig. 4B). The presence of apoC-III in these fractions was not sufficient to demonstrate inhibition of LPL given the volumes of HDL used in these assays.

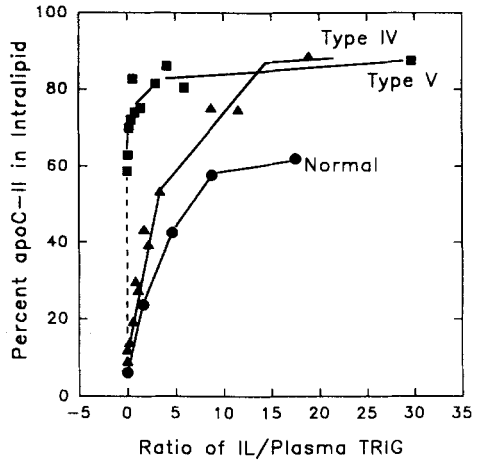

Fig. 5. Effect of donor lipoproteins on the redistribution of apoC-II. Plasma from one normolipidemic subject (●, subject 1a), two subjects with type IV hyperlipidemia (▲, subjects 5 and 6) and two subjects with type V hyperlipidemia (■, subjects 9 and 10) were incubated with different concentrations of exogenous TRIG. The percent of plasma apoC-II recovered in the $S_f > 400$ fractions is presented on the vertical axis. The horizontal axis represents the ratio of exogenous TRIG (IL) to plasma TRIG in an attempt to normalize the amount of TRIG in the acceptor particles as compared to the concentration of TRIG in fasting plasma samples from different subjects.

degrees of hypertriglyceridemia were used as donor lipoproteins. Each curve represents composite data from incubation studies using plasma from different subjects; the range of ratios of Intralipid TRIG to plasma TRIG varied with each subject. As illustrated, as much as 55–60% of the apoC-II was associated with plasma lipoproteins isolated in $S_f > 400$ in the absence of artificial lipid emulsions when plasma from subjects with type V hyperlipidemia was used. Furthermore, for similar increases in exogenous TRIG, the shift of apoC-II from plasma lipoproteins to the artificial triglyceride emulsions was greater when hypertriglyceridemic plasma was used as a source of apoCs. Similar results were observed with apoC-III (data not shown).

To examine the effect of acceptor particles, artificial TRIG emulsions of different size were prepared and incubated with normotriglyceridemic plasma (**Fig. 6**). When subpopulations of lipid emulsions differing in size were used as acceptor particles in incubations with normotriglyceridemic plasma, a higher percent of apoC-II was shifted toward the larger size artificial emulsions (Em-1) as compared to the smaller emulsions (Em-3) (Fig. 6A). The larger emulsions also exhibited a higher affinity for apoC-III than smaller particles but the difference was not as pronounced (Fig. 6B). The volumes of emulsion preparations were adjusted to provide identical concentrations of TRIG in the incubations with large and small emulsions and the total volumes of the incubation mixtures were also kept constant.

DISCUSSION

Upon their entry into the bloodstream, TRIG-rich lipoproteins, chylomicrons, and VLDL require the transfer of apoCs from circulating plasma lipoproteins, primarily HDL in normolipidemic individuals, to achieve their normal apolipoprotein composition (17). As triglycerides are hydrolyzed, apoCs redistribute themselves back to HDL. ApoC-II is required for the activation of LPL which is the major enzyme responsible for the hydrolysis of triglycerides for subsequent storage in peripheral tissues (1–3). ApoC-III, on the other hand, may have an inhibitory effect on lipolytic activity (7–10). ApoCs have also been reported to interfere with the rate of receptor-mediated uptake of apoE-containing triglyceride emulsions (12, 13). The contents of apoC-III in HDL have also been shown to be inversely correlated with the fractional clearance rate of HDL apoA-I (14). The rate

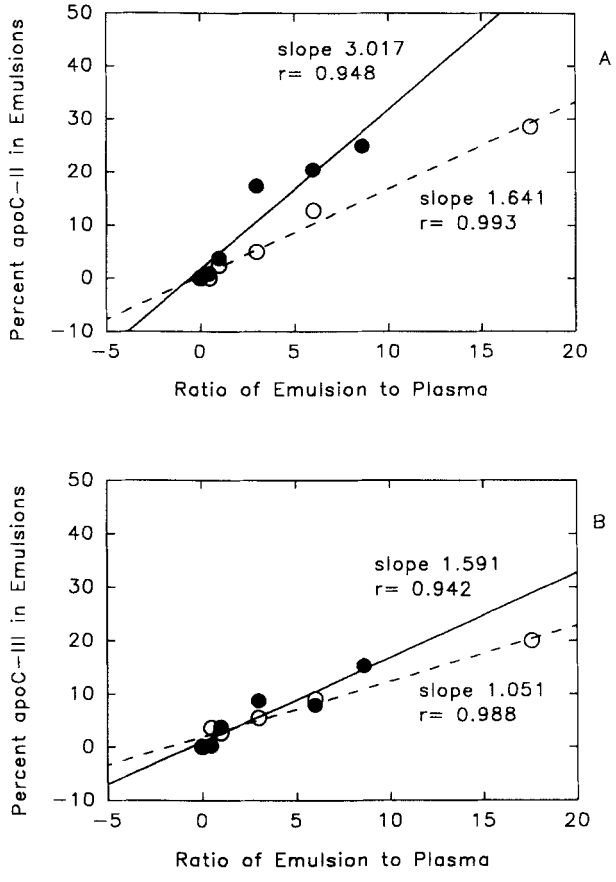

Fig. 6. Effect of acceptor particles on the redistribution of apoCs. Two subpopulations of artificial liposomes were used as acceptors of apoC-II (Fig. 6A) and apoC-III (Fig. 6B) in incubation studies using plasma from a normolipidemic subject (subject 1c). The size distribution of the artificial emulsions was assessed by column chromatography and pooled into tertiles by TRIG contents. Only the first tertile corresponding to the largest fraction (●) and the third tertile corresponding to the smallest subpopulation (○) were used as acceptor of apoCs. The intermediate tertile was discarded.

and extent of the redistribution of apoC from circulating plasma lipoproteins to exogenous triglyceride-rich particles may thus have a significant impact on the in vivo metabolism of the TRIG-rich lipoproteins as well as that of HDL.

Earlier studies have suggested that the equilibration of apoCs among plasma lipoproteins is spontaneous and complete (15, 16). In contrast, using radioiodinated lipoproteins and directly measuring apoC-III specific activity, we have reported the presence of non-equilibrating pools of apoC-III in both VLDL and HDL (22). There were some concerns that the lack of complete equilibration seen in these earlier studies may have been due to alterations in the apolipoproteins induced by the labeling techniques. Using artificial lipid emulsions at different triglyceride concentrations as acceptor for non-radiolabeled plasma apoCs, we have demonstrated in the present report that a portion of apoC-III and apoC-II pools in both VLDL and HDL could not be removed by a simple exchange process. In this study, the masses of transferred apolipoproteins were directly measured by immunoassays and were not exogenously labeled. The factors that determine the fractions of apoCs in VLDL and HDL that remain tightly associated to the native particles, and thus not available for participation in the equilibration process, are not known.

In studies using plasma from normolipidemic subjects, the present data set showed that HDL was the major donor of apoCs to artificial lipid emulsions. Approximately 10–15% of total plasma apoC-III and apoC-II, respectively, remained associated with the non-equilibrating pools of HDL. A significant portion of apoC-II recovered in the artificial lipid emulsions was actually derived from normotriglyceridemic VLDL. In fact, the apoC-II pool in VLDL was decreased by 60% (from 30.6% of the total plasma pool to 10.4%) by exposure to Intralipid. Of interest is the fact that the contribution of VLDL apoC-II to the artificial lipid emulsions was demonstrable at lower Intralipid concentrations as compared to those concentrations required for apoC-III shift. In patients with fasting chylomicronemia, as much as 60% of plasma apoC-II was found in $S_f > 400$ (Fig. 5) as compared to only 35% of plasma apoC-III (data not shown). Taken together, these data would suggest that apoC-II may have a higher affinity for lipid than apoC-III, a conclusion consistent with the data from Segrest et al. (29) indicating the presence of two class A amphipathic helixes on apoC-II as compared to only one for apoC-III.

In studies using plasma from hypertriglyceridemic subjects, VLDL was the major donor of both apoC-II and apoC-III with less than 5% of plasma apoCs being transferred from HDL to the lipid emulsions. When plasma samples from subjects with different fasting TRIG levels were examined with the same artificial emulsions as acceptors, the rate of redistribution of apoCs was dependent on the composition of the donor lipoproteins. Available data in the few subjects studied suggest that apoCs from hypertriglyceridemic plasma were more susceptible to transfer to Intralipid as compared to apoCs from normotriglyceridemic plasma. It is possible that these exchangeable apoCs might be less tightly bound to TRIG-rich lipoproteins.

It is not clear whether the presence of increased number of small acceptor particles would be more effective in pulling apoCs away from plasma lipoproteins than a few large TRIG-enriched acceptor particles. A priori, we might expect the larger TRIG-enriched particles to be better acceptor for apoCs if one of the primary functions of these apolipoproteins is to stabilize these TRIG-rich particles with greater surface area. Indeed, when artificial lipid emulsions differing in size were used as acceptors of apoCs from normotriglyceridemic plasma, our data indicated a preferential shift of apoC-II toward larger particles. This preference for larger particles was less significant in the case of apoC-III, another piece of evidence in support of the hypothesis that apoC-II, as required activator of LPL, has higher affinity for TRIG-rich particles. Additional studies using liposomes that contain significantly less core lipids would be required to clearly assess the effect of particle size as compared to that of particle number on the redistribution of apoCs.

One may criticize that the ultracentrifugation steps used for the isolation of the various lipoprotein fractions in this report may strip the apoCs from native lipoproteins, resulting in inaccurate redistribution of apoCs. If a significant fraction of apoCs could be stripped from TRIG-rich lipoproteins during the ultracentrifugation steps, we would expect an enrichment of apoCs in the $d > 1.063$ g/ml density fraction. In fact, data presented in this study would suggest that the percent of apoCs recovered in the $d > 1.063$ g/ml fraction was significantly lower than that obtained in VLDL when hypertriglyceridemic plasma was used in these incubations studies. Furthermore, we have previously reported that the lack of complete equilibration of apoC-III can be demonstrated when lipoprotein classes were isolated using column chromatography (22). Thus the residual apoCs recovered in the $d > 1.063$ g/ml fraction as non-equilibrating apoCs do not appear to reflect the pool of free apoCs that were stripped off native lipoproteins by ultracentrifugation.

The data presented in this work would suggest that the rate at which apoCs exchange between fasting plasma lipoproteins and newly secreted TRIG-rich lipoproteins will depend on the composition of the circulating lipoproteins as well as the size of the acceptor lipoproteins. Thus, while the redistribution of apoC-III towards the newly secreted triglyceride-rich lipoproteins may be necessary to stabilize these particles, excessive enrichment in apoC-III may not be desirable. The shift of apoC-III from HDL to the newly secreted TRIG-rich lipoproteins

would result in the accumulation of a subpopulation of HDL particles that are depleted of apoCs, though transiently. If we hypothesize that apoCs have a protective effect on the catabolism of HDL (13), the variability in the extent of apoC depletion during postprandial lipemia would determine why transient reduction in plasma HDL-cholesterol can be noted in some individuals but not in others.

The depletion of apoC-II from HDL in the presence of hypertriglyceridemia may have another impact on the normal metabolism of plasma lipoproteins, specifically with respect to the ability of HDL to activate LPL. Bier and Havel (30) have reported that HDL isolated from normolipidemic plasma were most effective in activating LPL in vitro. Data presented here clearly demonstrated that the activation of bovine LPL by hypertriglyceridemic HDL was suboptimal as compared to the activation seen with normotriglyceridemic HDL. Could this observation be explained by the presence of some inhibitory factor that has been reported in the d>1.21 g/ml fraction of hypertriglyceridemia (31)? This is unlikely because our data on the activation of bovine milk LPL (Figs. 3 and 4) were based on HDL isolated by ultracentrifugation from plasma of normolipidemic and hypertriglyceridemic subjects. Furthermore, studies of activation of milk LPL by d>1.063 g/ml fractions after incubations with Intralipid did not demonstrate inhibition of LPL as the volume of the d>1.063 g/ml fraction was increased from 20 to 100 μl per assay. The mechanisms that allow apoC-II to transfer between lipoproteins and attach to LPL, activating this enzyme, are not fully defined. LPL appears to bind to phospholipids (32) on the surface of TRIG-enriched lipoproteins, and the apoC-II required for LPL activation may be donated from the complement of apolipoproteins present on the surface of these TRIG-rich particles. Alternatively, apoC-II may transfer from other lipoproteins and bind directly to LPL (33). The absence of exchangeable apoCs in hypertriglyceridemic HDL would interfere with its role in the activation of LPL in spite of the high concentration of HDL apoC-II that may be present in the assay.

The intermittent appearance of TRIG-rich lipoproteins after the consumption of a fat-containing meal and the delayed clearance of these TRIG-rich postprandial lipoproteins may result in transient but significant alterations in the metabolism of VLDL and HDL by affecting the redistribution of plasma apoCs. Studies examining the effect of fat meals differing in fat and cholesterol contents as well as in fatty acid composition on the redistribution of apoCs during postprandial lipemia may provide some better understanding of the role of postprandial lipoproteins in atherosclerosis. As the data from our preliminary study (Fig. 6) would suggest, the rate of transfer of apoCs from circulating lipoproteins to newly secreted TRIG-rich lipoproteins may be regulated by size and lipid composition of these particles. This would in turn have a direct effect on the rate of catabolism and metabolic fate of these TRIG-rich lipoproteins. The processes that could cause these non-equilibrating, poorly exchangeable pools of apoCs to become separated from VLDL, which they must do as these particles are converted to LDL, warrant more careful examination.

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