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Lipoprotein ApoC-II Activation of Lipoprotein Lipase

MODULATION BY APOLIPOPROTEIN A-IV*

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Lipoprotein lipase (LPL)-mediated hydrolysis of triglycerides (TG) contained in chylomicrons requires the presence of a cofactor, apolipoprotein (apo) C-II. The physiological mechanism by which chylomicrons gain apoC-II necessary for LPL activation in whole plasma is not known. Using a gum arabic stabilized TG emulsion, activation of LPL by lipoprotein apoC-II was studied. Hydrolysis of TG by LPL was greater in the presence of serum than with addition of either high density lipoproteins (HDL) or very low density lipoproteins (VLDL). LPL activation by either VLDL or HDL increased with addition of the lipoprotein-free fraction of plasma. A similar increase in LPL activity by addition of the lipoprotein-free fraction together with HDL or VLDL was observed when another TG emulsion (Intralipid) or TG-rich lipoproteins from an apoC-II deficient subject were used as a substrate. Human apoA-IV, apoA-I, apoE, and cholesteryl ester transfer protein were assessed for their ability to increase LPL activity in the presence of VLDL. At and below physiological concentrations, only apoA-IV increased LPL activity. One hundred percent of LPL activity measured in the presence of serum was achieved using VLDL plus apoA-IV. In the absence of an apoC-II source, apoA-IV had no effect on LPL activity. Removal of >80% of the apoA-IV from the nonlipoprotein-containing fraction of plasma by incubation with Intralipid markedly reduced its ability to activate LPL in the presence of VLDL or HDL. Gel filtration chromatography demonstrated that incubation of the nonlipoprotein-containing fraction of plasma with HDL and the TG emulsion caused increased transfer of apoC-II to the emulsion and association of apoA-IV with HDL. Our studies demonstrate that apoA-IV increases LPL activation in the presence of lipoproteins. We hypothesize that apoA-IV is required for efficient release of apoC-II from either HDL or VLDL, which then allows for LPL-mediated hydrolysis of TG in nascent chylomicrons.

Chylomicron and very low density lipoprotein (VLDL)[1] triglyceride (TG) hydrolysis requires lipoprotein lipase (LPL) (1-3). This enzyme is synthesized in adipose, muscle, and several other tissues and is transported to the luminal surface of capillary endothelial cells where it can interact with circulating lipoproteins. Apolipoprotein (apo) C-II is a cofactor required for efficient actions of LPL. The importance of apoC-II for TG hydrolysis is illustrated by human disorders with genetic defects in the structure or production of this protein (4). These patients have high circulating levels of TG and are phenotypically indistinguishable from those with LPL deficiency. Transfusion of normal plasma into these patients results in rapid hydrolysis of the TG, presumably as LPL is activated by apoC-II.

A series of events in the lymph and the bloodstream is required to provide chylomicrons with the apoC-II needed for their eventual catabolism. Chylomicrons are synthesized in the intestine and secreted into mesenteric lymph. These nascent particles contain lipid and apoB-48, apoA-IV, and apoA-I (5-7) but are deficient in E and C apoproteins, including apoC-II required for activation of LPL. However, some E and C apoproteins are acquired from high density lipoproteins (HDL) after chylomicrons enter the lymph, and more are obtained after these lipoproteins enter the bloodstream (5-9). ApoE and -C are transferred to chylomicrons from plasma HDL which enters the lymph via the interstitial fluid compartment (9). As the chylomicrons lose apoA-I and apoA-IV they gain apoE and -C. During hydrolysis of chylomicron TG the C apoproteins are returned to the HDL. Thus, apoC-II needed for LPL activation is normally available for transfer to nascent TG-rich lipoproteins.

The mechanism involved in apoC-II release from HDL and its transfer to nascent chylomicrons is unclear. *In vivo*, C apoproteins exchange rapidly between VLDL and HDL (8), although, at least for apoC-III, there also is a nonexchangeable pool of apolipoprotein (10). The exchange mechanism is unknown but likely involves particle collision or an intermediate, *e.g.* nonlipoprotein-associated apoC-II. A similar exchange cannot occur between HDL and nascent chylomicrons, because these latter particles have no apoC-II associated with them. In this situation the exchange must involve other apolipoproteins or lipids on the chylomicron surface.

In this report, we describe studies of the activation of LPL by serum and apoC-II containing lipoproteins. TG emulsions have been used to study LPL interaction with substrates and to assess levels of LPL activity in tissues and plasma (11). LPL-catalyzed hydrolysis of two different TG emulsions and TG-rich lipoproteins from an apoC-II-deficient subject was

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[1] The abbreviations used are: VLDL, very low density lipoprotein; apo, apolipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; TG, triglyceride; PBS, phosphate-buffered saline.

markedly increased in the presence of apoC-II-containing lipoproteins if the nonlipoprotein-containing fraction of plasma was added to an LPL assay. Addition and elimination experiments demonstrated that this activation of LPL was due to apoA-IV. ApoA-IV alone without an apoC-II source had no direct effect on LPL activation. These data suggest that apoA-IV may act as a modulator of apoC-II transfer from lipoproteins which may affect the *in vivo* catabolism of TG-rich lipoproteins.

MATERIALS AND METHODS

Preparation of Lipoproteins—Plasma was obtained from normal subjects attending the Arteriosclerosis Research Center of the Columbia-Presbyterian Medical Center. The subjects had fasted for at least 12 h. The blood was drawn into tubes containing EDTA at a final concentration of 1 mg/ml. The plasma samples were refrigerated at 4 °C and the cells were separated within 2 h of collection. Plasma cholesterol, TG, and HDL cholesterol were quantitated by Lipid Research Program procedures using an Abbott Biochromatic Analyzer 100 (Abbott) (12). Any intrinsic lipolytic activity in the sera was inactivated by heating for 60 min at 57 °C. All samples were stored at 4 °C until used.

VLDL ($d < 1.006$ g/ml) or a mixture of VLDL, intermediate density lipoproteins, plus low density lipoproteins ($d < 1.063$ g/ml) were isolated by ultracentrifugation in density solutions of KBr, using a Ti-50.3 rotor in a model L8-M ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 40,000 rpm, 10 °C, for 24 h (13). After removal of the $d < 1.063$ g/ml lipoproteins, HDL was then isolated at $d < 1.21$ g/ml after centrifugation for 48 h. The infranatant nonlipoprotein fractions (1.21 bottom) were $d > 1.21$ g/ml. The lipoproteins and 1.21 bottoms were extensively dialyzed against PBS (0.15 M NaCl, 0.01 M sodium phosphate, 0.1% EDTA, pH 7.4) and adjusted with PBS to make their concentration equivalent to that found in the initial plasma prior to their inclusion in the LPL assays.

Sources of LPL—Bovine milk LPL was purified by affinity chromatography using heparin-Sepharose (Pharmacia LKB Biotechnology, Inc.) by the method of Socorro *et al.* (14) and stored at −70 °C until used. Bovine milk LPL preparations (330 μg/ml) were diluted 10-fold with PBS just prior to use. Depending on the preparation used, LPL activity in the presence of serum ranged from 0.23 to 2.8 μmol of free fatty acid/μg LPL/h. Some studies were also performed using human LPL from post-heparin plasma in which hepatic triglyceride lipase activity was inhibited using goat anti-human hepatic triglyceride lipase serum (15).

Purification of Apoproteins and Cholesterol Ester Transfer Protein—Human apoA-I and -A-IV were purified using a slight modification (16) of the method of Weinberg *et al.* (17, 18). Human apoE was purified as described by Blum *et al.* (19) and apoC-II was purified from delipidated human VLDL by reversed-phase chromatography using a fast protein liquid chromatography system (LCC 500, Pharmacia) (20). Human cholesteryl ester transfer protein, purified by the method of Hesler *et al.* (21), was a gift from Dr. Alan Tall. Apolipoprotein purity was assessed by sodium dodecyl sulfate-polyacrylamide or isoelectric focusing (for apoC-II) gel electrophoresis and mass determined by the method of Lowry *et al.* (22). ApoA-IV and -C-II in plasma, lipoproteins, or lipoprotein-free fractions were determined by radioimmunoassay (23, 24).

Preparation of Triglyceride Substrate Emulsions—A triolein-gum arabic substrate emulsion for LPL assays was prepared as described by Baginsky and Brown (25). LPL substrates were freshly prepared the day of the assay from stock solutions of [1-^{14}C]oleic acid (10 nCi/ml toluene) and tri[9,10-^{3}H]oleate (300 μCi/g) (Du Pont-New England Nuclear) plus trioleate (1 g) (Nuchek Prep, Elysian, MN) in 33 ml of heptane. The [^{14}C]oleic acid (2.5 μl/assay) and the triolein stock solution (75 μl/assay) were evaporated under N_2. To the dried lipids were added solutions of gum arabic (15 g/100 ml) in 0.2 M Tris-HCl, pH 8.2 (75 μl/assay), and 0.35 M Tris-HCl, 0.16 M NaCl in 3% bovine serum albumin, pH 7.4 (100 μl/assay). The emulsion was prepared by sonication on ice (10 s/ml) using a model S75 sonicator (Branson Instruments Inc., Danbury, CT).

For some studies, native lipoproteins or an alternative TG-containing emulsion were used for the LPL assay. TG-rich lipoproteins ($d < 1.006$ g/ml plasma fraction) from an apoC-II-deficient subject (26) were generously provided by Dr. Silvia Fojo, National Heart, Lung and Blood Institute, Bethesda, MD. Radiolabeled trioleate was incorporated into these particles by the method of Fielding (27). Briefly, tri[9,10-^{3}H]oleate in toluene (100 μCi) was placed in a glass tube, the solvent evaporated under N_2, and resuspended in dimethyl sulfoxide (1 ml). This solution was drawn up into a syringe, slowly dripped into a stirred solution (4 ml) containing the apoC-II-deficient TG-rich lipoprotein (6 mg of TG/ml), and then incubated for 4 h at 37 °C. The TG-rich lipoproteins were reisolated by centrifugation of the solution for 5 min at 14,000 rpm in a microcentrifuge. Over 90% of the radioactivity was recovered with the floating lipoprotein fraction. A second emulsion was prepared by sonication essentially as described by Eisenberg *et al.* (28). This emulsion contained 2 mg of Intralipid (KabiVitrum, Inc., Alameda, CA) TG and 0.2 μCi of tri[9,10-^{3}H]oleate/150 μl of 3% bovine serum albumin in PBS.

LPL Assays—The assay mixture consisted of 150 μl of substrate emulsion (*i.e.* 1.9 mg of TG for the gum arabic emulsion, 120 μg of TG for apoC-II-deficient lipoproteins, and 2.0 mg of TG for the Intralipid emulsion) and a potential source of apoC-II (inactivated serum, VLDL, $d < 1.063$ g/ml lipoproteins, or HDL) and 1.21 bottom added singly or in combination, in a total volume of 300 μl. In some assays purified proteins were substituted for the 1.21 bottom. The assay mixtures were incubated at 37 °C for 1 h. Then a source of LPL (330 ng in 10 μl) was added, and the reaction was allowed to proceed for a second hour at a reduced temperature of 27 °C as described by Baginsky and Brown (25). The reaction was terminated by addition of 3.5 ml of a solution of methanol/chloroform/heptane/oleic acid (1410:1250:1000:1, v/v/v/v) and 1 ml of 0.05 M potassium borate buffer, pH 10 (29). After centrifugation, 1 ml of the aqueous phase was removed, 3.5 ml of Hydrofluor (National Diagnostics, Manville, NJ) was added, and the amount of liberated free fatty acids hydrolyzed was determined by liquid scintillation spectroscopy in an LS-1000 β counter (Beckman Instruments). All assays were performed in triplicate. Procedural recovery of sodium [9,10-^{3}H]oleate during organic extraction was corrected for recovery of sodium [1-^{14}C]oleate internal standard in the aqueous phase. The results for each assay were converted to micromoles of free fatty acid liberated per μg LPL/h.

Removal of ApoA-IV from the 1.21 Bottom—ApoA-IV was removed from the 1.21 bottom by incubation with Intralipid and 4 M NaCl as described in detail by Weinberg *et al.* (17). The 1.21 bottom was separated from the Intralipid by ultracentrifugation (25,000 rpm, 35 min, 4 °C) in a Ti-50.3 rotor. The floating lipid layer was removed by slicing the centrifuge tube. The apoA-IV-deficient 1.21 bottom was dialyzed against PBS to remove the excess salt and the protein and apoA-IV concentrations were determined.

Exchange of ^{131}I-ApoC-II HDL—ApoC-II was gently iodinated with ^{131}I using lactoperoxidase and glucose oxidase (30), and the labeled protein was separated from free iodine by Sephadex G-50 gel filtration. ApoC-II-specific activity was approximately 8000 cpm/ng protein. Radiolabeled apoC-II (100 ng) was incubated with HDL (1 mg of protein) for 1 h at 37 °C. The ^{131}I-apoC-II-associated HDL was then reisolated by ultracentrifugation and dialyzed against PBS. ^{125}I-ApoA-IV was prepared by the chloramine-T method (31) as previously described (24). To determine the effect that the nonlipoprotein fraction of plasma had on transfer of HDL apoC-II to the emulsion, ^{131}I-apoC-II-labeled HDL (250 μg of protein) was incubated with the gum arabic emulsion (150 μl) in the absence or presence of the 1.21 bottom (50 μl) and ^{125}I-apoA-IV for 1 h at 37 °C in a final volume of 300 μl. Following incubation, aliquots of mixture were applied to Sephacryl S-300 columns (115 x 0.9 cm, Pharmacia LKB Biotechnology, Inc.) which had been equilibrated with 154 mM NaCl, 0.01% EDTA, 0.02% NaN_3 in 5 mM Tris-HCl, pH 7.4. Fractions of 1.6–1.75 ml were collected at 5-min intervals at room temperature and the distribution of ^{131}I-apoC-II and ^{125}I-apoA-IV was determined. Sephacryl S-300 fractionates proteins between molecular weights of 1×10^4 to 1.5×10^6; and emulsion particles elute in the void volume, while HDL (molecular weight = 2×10^5) and nonlipoprotein-associated apoA-IV (molecular weight = 4.6×10^4) and apoC-II (molecular weight ~ 8.8×10^3) separate from each other and the lipoprotein. The columns were previously calibrated and shown to have a void volume of 38.5 ± 0.5 ml, an apoA-I (*i.e.* HDL) elution volume of 54.2 ± 0.4 ml, and a human serum albumin volume of 60.5 ± 0.5 ml ($n = 21$).

RESULTS

Activation of LPL by Serum and Lipoproteins—Initial studies tested the effects of addition of serum or lipoproteins on the hydrolysis of a gum arabic-TG emulsion by purified LPL. A representative experiment is shown in Fig. 1. When inac-

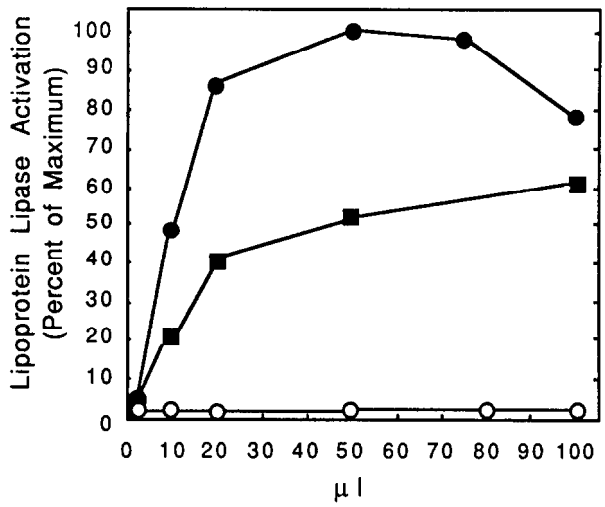

FIG. 1. **Activation of LPL with increasing amounts of inactivated human serum (●), HDL (■), and VLDL (○).** Bovine milk LPL (10 μl, 330 ng) was added to a triolein-gum arabic emulsion containing the indicated amounts of serum or lipoproteins or buffer resulting in a final volume of 300 μl. LPL activity was determined as described under "Materials and Methods." For the experiment shown, 100% activity was 0.23 μmol of free fatty acid/μg LPL/h. In replicate experiments, 100% activity ranged up to 2.5 μmol of free fatty acid/μg LPL/h and was dependent upon the bovine LPL preparation used. Data represent the average of triplicate determinations from a representative experiment.

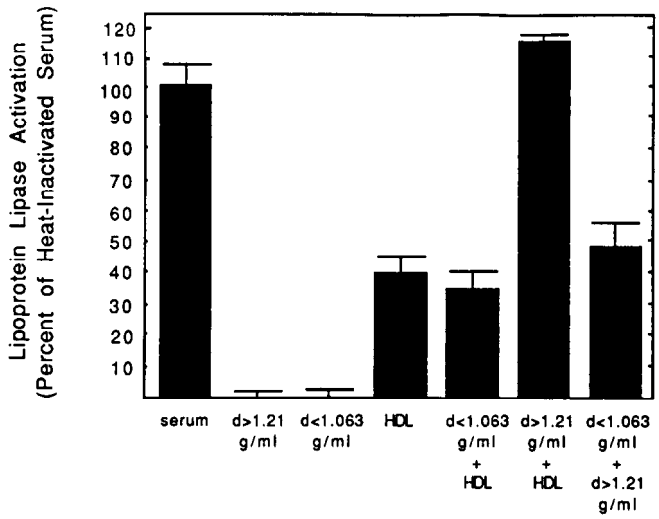

FIG. 2. **LPL activation using plasma lipoprotein or lipoprotein-free fractions.** Ten μl of bovine milk LPL was added to a triolein-gum arabic emulsion containing 50 μl of each of the indicated plasma fractions. LPL activity was determined as described under "Materials and Methods." One hundred percent activity, the amount of TG hydrolysis with serum, in the assay shown equaled 0.25 μmol of free fatty acid/μg LPL/h. Data represent the average of triplicate determinations ± S.D.

tivated serum was used as a source of apoC-II, LPL activity increased in relation to the added serum. Maximum LPL activity was achieved using 40–80 μl of serum (13–27% of the total assay volume). However, further increases in the amount of serum added to the assay decreased TG hydrolysis. Similar results have been reported by other investigators (25). When either VLDL or $d < 1.063$ g/ml lipoproteins were added, the maximum LPL activity achieved was always less than 15% of the activity attained using serum. However, in all experiments when HDL in amounts equivalent to those in serum were added, 35–70% of the maximum LPL activity observed with serum was consistently achieved. For the study shown in Fig. 1 the apoC-II levels in the added serum, HDL, and VLDL were 35.6, 19, and 28.8 μg/ml, respectively. Thus, the differences in activation using different lipoproteins was not due to the concentrations of apoC-II added. In addition, similar results were found when human post-heparin plasma was used as the source of LPL activity (data not shown).

Effect of Nonlipoprotein Plasma Fraction on LPL Activation—As shown in Fig. 1, LPL activity in the presence of HDL or VLDL was less than that found using inactivated whole serum. These data suggested that other components present in whole serum might play a role in LPL activation. Addition of either 1.21 bottom or the $d < 1.063$ g/ml lipoproteins alone to the assay did not activate LPL (Fig. 2). Addition of $d < 1.063$ g/ml lipoproteins to normal HDL had no additional effect on LPL activity compared to that observed with HDL alone. However, when the 1.21 bottom was combined with HDL, activation of LPL was greater than that observed with HDL alone and was similar to that achieved with serum. Reconstitution of 1.21 bottom with $d < 1.063$ g/ml lipoproteins markedly increased LPL activity to 40–60% of the serum activity. Similar results were obtained when these experiments were repeated using Intralipid, rather than a triolein-gum arabic emulsion, as the LPL substrate. Typically 1.21 bottom combined with VLDL prior to its addition to the Intralipid emulsion plus LPL provided approximately twice as much TG hydrolysis as was achieved with VLDL alone.

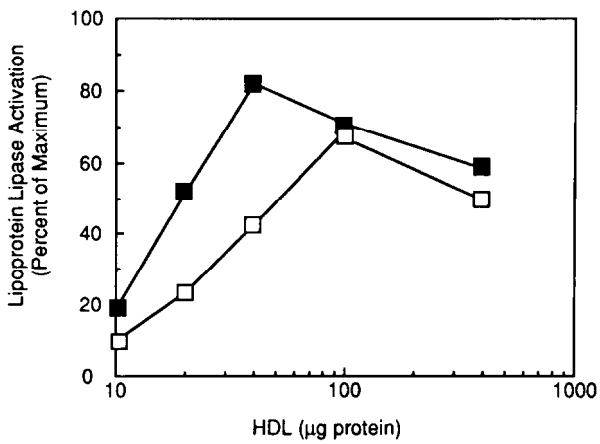

FIG. 3. **Activation of LPL by HDL and 1.21 bottom.** The amounts of LPL activity obtained with addition of the indicated amounts of HDL protein with (■) or without (□) 50 μl of 1.21 bottom are shown. In the study illustrated, 100% activity, the amount with addition of serum, was 2.8 μmol of free fatty acid/μg LPL/h. Data shown are the average of triplicate determinations.

Therefore, the effect of 1.21 bottom on activation of LPL in the presence of VLDL was confirmed using a second substrate.

Effect of Increasing Concentrations of HDL on LPL Activation—Previous studies using amounts of HDL (approximately 50–80 μg of HDL protein) which were equivalent to those found in serum did not produce LPL activity comparable to the maximal levels observed with serum addition (Figs. 1 and 2). Furthermore, unlike assays performed with higher concentrations of serum, no decrease of LPL activity was noted with the maximum amounts of HDL used. When concentrated HDL in amounts much higher than that in serum (up to 400 μg of HDL protein) were added to the assay, as shown in Fig. 3, LPL activity still failed to attain the level achieved with addition of serum. However, like serum, at higher concentrations HDL also decreased LPL activity. Addition of 1.21 bottom increased the amount of HDL-induced activation of LPL (Figs. 2 and 3), but this effect was most marked using lower amounts of HDL (<100 μg, Fig. 3). At HDL concentra-

tions which were greater than those required for maximal HDL-induced LPL activation, addition of 1.21 bottom no longer increased LPL activation by HDL. Thus, the effect of 1.21 bottom was most apparent using lower concentrations of HDL.

Effect of Nonlipoprotein Fraction of Plasma on Hydrolysis of ApoC-II-deficient Human Lipoproteins—To test whether the 1.21 bottom modulates hydrolysis of a natural substrate, TG hydrolysis of apoC-II-deficient lipoproteins was studied. Addition of HDL (20 μl, 56 μg of protein) to 20 μl of labeled apoC-II-deficient lipoproteins (120 μg of TG) resulted in a >2-fold increase in TG hydrolysis (8.1% of the TG) in 1 h. As shown in Fig. 4, at lower HDL levels (5 and 10 μl) addition of 1.21 bottom (20 μl) to LPL and apoC-II-deficient lipoproteins resulted in a marked increase in TG hydrolysis. With higher HDL concentrations (20–50 μl) addition of 1.21 bottom did not increase LPL activation further. These data are consistent with the observed loss of effectiveness of the 1.21 bottom at higher HDL concentration (Fig. 3). Similar studies using TG-rich lipoproteins obtained from normal and LPL-deficient humans showed that those lipoproteins did not require an added source of apoC-II for maximal hydrolysis (not shown). Presumably these TG-rich particles contain sufficient apoC-II and do not need an exogenous source (*i.e.* HDL or VLDL). Although we attempted to perform similar studies with the addition of VLDL, rather than HDL, substrate dilution led to such low levels of TG hydrolysis that we could not be confident of the results. However, our studies do show that at low HDL concentrations a component of the 1.21 bottom increased the LPL-mediated TG hydrolysis of apoC-II-deficient lipoproteins.

Effect of Apoproteins on LPL Activation—To identify a factor in the 1.21 bottom which might increase LPL activity in the presence of VLDL or HDL, various purified components of 1.21 bottom (cholesteryl ester transfer protein, apoA-I, apoA-IV, or apoE) at several concentrations were added to the assay containing the TG emulsion, LPL, and VLDL. The maximum concentrations of these proteins were chosen to approximate or exceed their concentrations in serum. Compared to results obtained with VLDL alone there was minimal or no increase in measured LPL activity with any of the additions except for apoA-IV (Fig. 5). Addition of apoA-IV and VLDL to the assay mixture resulted in activation of LPL that was equal to or greater than that achieved using inactivated whole serum (Fig. 5). ApoA-IV, below and at physiologic levels, added to VLDL increased LPL activation in a dose-dependent manner (Fig. 6).

Effect of ApoA-IV on LPL Activation by Purified ApoC-II—As shown in Fig. 7 (*top*), when purified apoC-II was used as an activator of LPL, it resulted in increased LPL activity. To assess whether apoA-IV could directly augment apoC-II action, 0.2 μg of purified apoC-II, which resulted in less than maximal LPL activity, was added to the assay mixture containing various quantities of apoA-IV. The addition of purified apoA-IV alone or in the presence of submaximal amounts of apoC-II did not increase the amount of LPL activity achieved (Fig. 7, *bottom*). These data demonstrate that apoA-IV increased LPL activity only in the presence of a lipoprotein source of apoC-II.

Effect of Removal of ApoA-IV from the 1.21 Bottom—To determine whether apoA-IV was the primary protein respon-

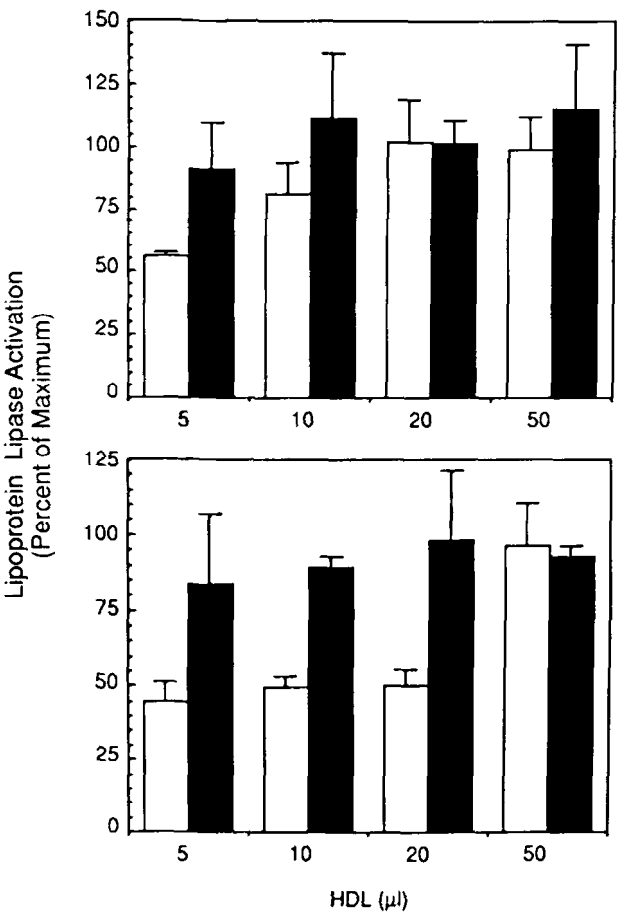

FIG. 4. **Hydrolysis of apoC-II-deficient human lipoproteins.** TG-rich lipoproteins from an apoC-II-deficient subject were radiolabeled by incorporation of tri[9,10-^{3}H]oleate and used as a substrate for LPL as described under "Materials and Methods." The percentages of TG hydrolysis by addition of HDL (2.8 mg/ml) in the presence or absence of 1.21 bottom (20 μl) are illustrated. Shown are data (average ± S.D.) of two separate experiments performed in triplicate. One hundred percent activation was found with addition of serum or HDL and equals hydrolysis of 8.1% of the substrate TG (6.5 nmol of free fatty acid/μg LPL/h). □, minus $d > 1.21$ g/ml; ■, plus $d > 1.21$ g/ml.

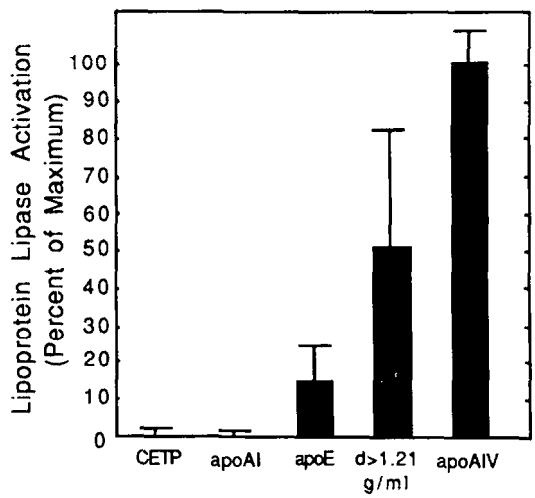

FIG. 5. **LPL activation using various components of the nonlipoprotein plasma fraction (1.21 bottom).** Bovine milk LPL (330 ng) was added to a triolein gum-arabic emulsion containing 50 μl of VLDL and 50 μl each of apoA-I (50 μg), apoA-IV (17.5 μg), apoE (2.5 μg), and cholesteryl ester transfer protein. These levels were chosen to approximate the amounts of each protein found in serum. The activity of cholesteryl ester transfer protein used, was equivalent to that found in 1.21 bottom. LPL activity was determined as described under "Materials and Methods." Data represent the average of triplicate determinations ± S.D. Maximum activity in the study illustrated, found with addition of apoA-IV, was 2.7 μmol of free fatty acid/μg/h.

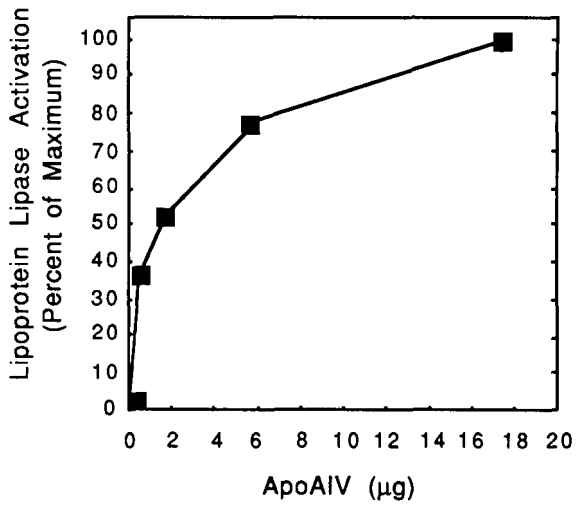

FIG. 6. **Dose-response curve of apoA-IV activation of LPL.** VLDL (50 μl) isolated from normal plasma (TG, 110 mg/dl), was incubated in a triolein-gum arabic emulsion with the indicated quantities of apoA-IV. Bovine milk LPL (330 ng) was added and LPL activity was determined as described under "Materials and Methods." Data represent the average of triplicate determinations. One hundred percent activity equals 2.3 μmol of free fatty acid/μg/h.

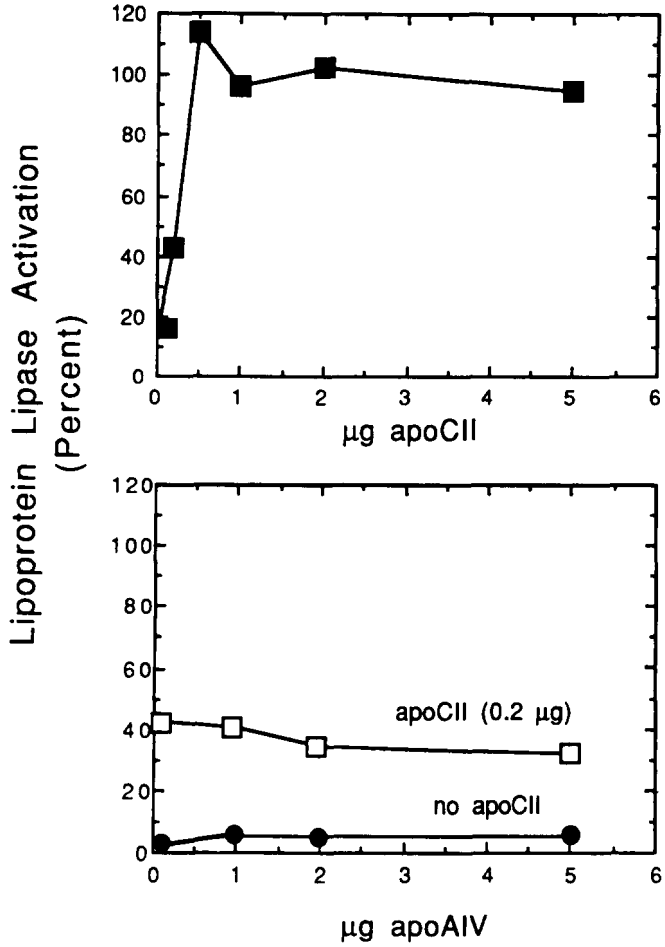

FIG. 7. **LPL activation using increasing concentrations of purified apoC-II.** Bovine milk LPL (330 ng) was added to triolein-gum arabic emulsion containing the indicated amount of apoC-II (*top*). LPL activity achieved using increasing quantities of apoA-IV alone (●) or with 0.2 μg of apoC-II (□) (*bottom*) are shown. LPL activity was measured as described under "Materials and Methods." One hundred percent activity equals 2.7 μmol of free fatty acid/μg LPL/h. Data represent the average of triplicate determinations.

sible for the activation of LPL in the presence of lipoproteins, 87% of this apoprotein (as determined by radioimmunoassay) was removed from the 1.21 bottom using Intralipid. Native and apoA-IV-deficient 1.21 bottoms were normalized for total protein concentration (17.5 mg/ml) and used in the assay; the apoA-IV levels were 52.8 and 6.7 μg/ml, respectively, in the control and apoA-IV-deficient 1.21 bottoms. As shown in Fig. 8, using either VLDL or HDL as a source of apoC-II, addition of apoA-IV-deficient 1.21 bottom resulted in less LPL activity. As expected from the dose-response curve shown in Fig. 6, as higher concentrations of 1.21 bottom were added the effect of removal of apoA-IV from the 1.21 bottom was less evident. This may be due to the residual apoA-IV still present in the apoA-IV-deficient 1.21 bottom. These data suggest that removal of apoA-IV decreased the activation of LPL by 1.21 bottom in the presence of lipoproteins.

Immunological methods were also employed to selectively remove apoA-IV from 1.21 bottom. Immunoprecipitation and use of anti-apoA-IV affinity chromatography were inefficient in removal of apoA-IV from 1.21 bottom unless detergents were present. This requirement for detergents to immunoprecipitate apoA-IV has been previously reported (24). However, using this approach we were unsuccessful in demonstrating a requirement for apoA-IV by depletion. Presumably, we were unable to completely remove Triton X-100 from the apoA-IV-deficient fractions, a condition that obscures the LPL activation assay. Addition of Triton X-100 alone to the LPL assay produced maximal amounts of LPL activity using lipoproteins without the addition of 1.21 bottom. Therefore, the Triton X-100 may have solubilized the apoC-II and dissociated it from the lipoproteins, negating the requirement for the 1.21 bottom effect.

Transfer of HDL ApoC-II to TG Emulsions—To identify the mechanism whereby apoA-IV increased LPL activation by lipoprotein apoC-II, studies were performed to assess the effect of 1.21 bottom on the transfer of apoC-II from HDL to gum arabic-TG emulsions. Incubation of the ^{131}I-apoC-II HDL with the TG emulsion in the presence or absence of 1.21 bottom affected the transfer of radioactivity to the TG emulsion (Fig. 9). With the addition of 1.21 bottom, the transfer of radioactive apoC-II to the TG emulsion increased by 49% from 23 to 44% of the total recovered radioactivity. In the complete system, ^{125}I-apoA-IV gel-filtered primarily with HDL (23.1%) and with TG emulsion (62.5%). In the presence of HDL but in the absence of the emulsion, ^{125}I-apoA-IV was primarily (89.2%) associated with both HDL and the nonlipoprotein-containing fractions. These studies demonstrate that inclusion of 1.21 bottom in the incubation mixture containing HDL and TG emulsion increased the amount of apoC-II transferred from HDL to the TG emulsion.

DISCUSSION

Our studies demonstrate that maximal activation of LPL by lipoprotein associated apoC-II is modulated by other factors found in plasma. Only in the presence of the lipoprotein-free plasma fraction, was HDL capable of activating LPL to the levels achieved using serum. VLDL was less effective than HDL in activating LPL when assayed using the gum arabic emulsion. Perhaps VLDL apoC-II was less readily transferred to the emulsion. However, inclusion of 1.21 bottom markedly increased LPL activity in the presence of VLDL. When apoC-II-deficient lipoproteins were utilized as the substrate for LPL, addition of 1.21 bottom to HDL increased LPL activity. This suggested that the effect of 1.21 bottom may be important for normal hydrolysis of chylomicron TG. As expected, because these assays were performed using only 6% of the TG

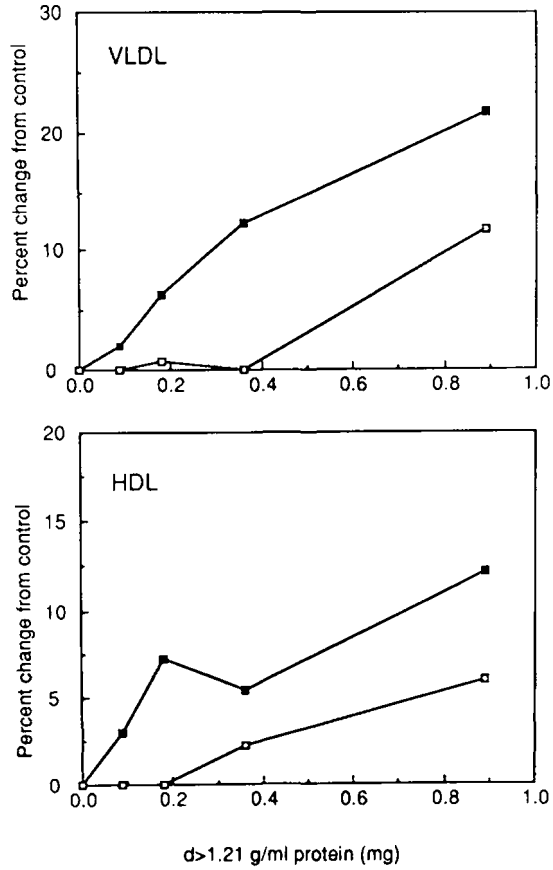

FIG. 8. **Activation of LPL using apoA-IV-deficient nonlipoprotein plasma fraction.** ApoA-IV was removed from 1.21 bottom as described under "Materials and Methods." ApoA-IV-deficient 1.21 bottom (□) (17.5 mg of protein/ml; 6.7 μg of apoA-IV) and 1.21 bottom (17.5 mg of protein/ml; 52.8 μg/ml apoA-IV) (■) were included in the LPL assay in the presence of VLDL (14 μg of protein) or HDL (139 μg of protein). Shown are the average values for four separate experiments. Control LPL activity without the addition of 1.21 bottom was 0.87 and 1.70 μmol of free fatty acid/μg/h, respectively, for VLDL and HDL.

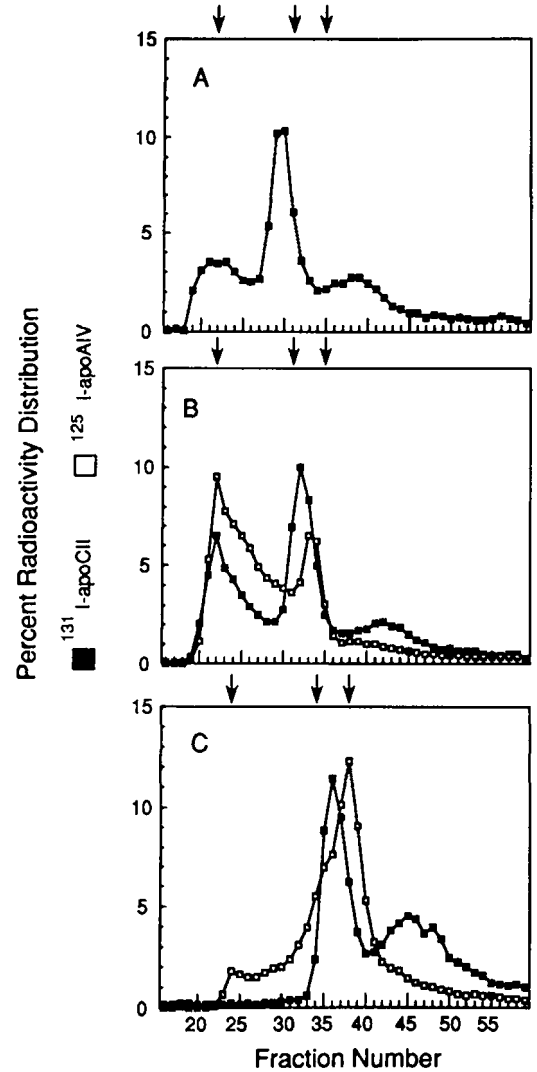

FIG. 9. **Gel filtration of ^{131}I-apoC-II HDL alone and after incubation in the presence or absence of TG emulsion and 1.21 bottom.** HDL was labeled with ^{131}I-apoC-II as described under "Materials and Methods" and 140 μg of HDL (0.03 μCi ^{131}I-apoC-II) was incubated alone at 37 °C for 1 h with or without 150 μl of gum arabic-TG emulsion, and with or without 50 μl of 1.21 bottom (8.50 μg of protein) containing ^{125}I-apoA-IV (9.8 ng, 0.12 μCi). Shown are the amounts of radioactivity after gel filtration of HDL plus TG emulsion (*A*), HDL plus TG emulsion and 1.21 bottom (*B*), and HDL plus 1.21 bottom (*C*). *Arrows* (*left to right*) indicate void, HDL, and albumin elution volumes, respectively. The area under the void volume peak in *B* demonstrated a 49% increase in ^{131}I-apoC-II over that found in *A*.

used in the gum arabic assays, lower amounts of HDL were required to show the effect of 1.21 bottom. A component in the 1.21 bottom which mimicked this effect was shown to be apoA-IV. When physiologic concentrations of purified apoA-IV were substituted for the lipoprotein-free plasma fraction a comparable increase in the amount of LPL activity was observed in the presence of lipoproteins. In addition, removal of apoA-IV from 1.21 bottom markedly reduced activation of LPL. However, other hydrophobic proteins are undoubtedly removed from 1.21 bottom during an incubation with Intralipid. Thus effects of these other hydrophobic proteins on LPL activation could not be ruled out. However, taken together, our studies make a strong argument for an effect of apoA-IV on increasing LPL activation in the presence of lipoproteins.

Other proteins have been described which may activate LPL. β_2-Glycoprotein, also known as apoH, has been reported to both directly activate LPL and to also augment LPL activity in the presence of purified apoC-II (32, 33). ApoA-IV clearly does not operate in a similar manner, in that it is only effective in the presence of lipoprotein apoC-II. Thus, a different mechanism, as suggested, other than direct interaction of apoA-IV with apoC-II or LPL must be operative. ApoE has been reported to increase or decrease LPL-mediated hydrolysis of TG. Studies performed by Clark and Quarfordt (34) demonstrated that apoE addition increases the binding and hydrolysis of a TG emulsion applied to a column of LPL bound to heparin-Sepharose. The increased hydrolysis of TG was postulated to occur because apoE increased the association of the TG particles with heparin, placing them in greater proximity to the LPL. By contrast, McConathy and Wang (35) reported recently that the receptor-binding region of apoE may inhibit LPL activity. ApoC-III will also inhibit LPL activity *in vitro* (36) and this apoprotein may be responsible for the decrease in LPL activity found using higher concentrations of serum or HDL.

In humans, essentially all apoA-IV is synthesized by intestines (37) and the average concentration in plasma is 14–37 mg/dl (24, 38). The vast majority (approximately 75–90%) of apoA-IV is found in the nonlipoprotein plasma fraction (24, 38–40). Intestinal apoA-IV synthesis and secretion is increased by a fat meal and primarily enters the plasma on

chylomicrons (24, 38, 41, 42). The observation that human chylomicron apoA-IV is transferred to HDL *in vivo* (40) and that HDL apoA-IV mass is elevated postprandially (24) suggest that our *in vitro* findings may have physiologic significance in regulation of postprandial lipoprotein metabolism. Experiments by Weinberg and Spector (18), in which apoA-IV was adsorbed onto TG-rich Intralipid particles and then incubated with HDL, demonstrated apoA-IV displacement with transfer of HDL apoC. Other experiments in which rat plasma was incubated with either LPL or postheparin plasma showed that apoA-IV shifted from HDL to the nonlipoprotein plasma fraction (42). This redistribution may be due to transfer of VLDL surface components onto HDL (43). These studies demonstrated that apoA-IV and apoCs can exchange on the surface of lipoproteins and suggested that the mechanism by which apoA-IV increased LPL activity in our assay was secondary to the displacement of apoC-II from lipoproteins. The ineffectiveness of apoA-IV to increase LPL activity in the presence of free apoC-II, compared to its effect in the presence of lipoprotein bound apoC-II, supports this displacement mechanism. We further confirmed that addition of 1.21 bottom led to an increase in the transfer of HDL apoC-II to a gum arabic-TG emulsion. The displaced apoC-II would then be able to activate LPL.

The mechanism by which apoC-II activates LPL is not fully defined. LPL appears to bind to phospholipids (44) which are found on the surface of TG-rich lipoproteins. Perhaps preexisting apoC-II on the surface of these TG-rich lipoproteins activates LPL. Alternatively, apoC-II may transfer from other lipoproteins and bind directly to the LPL enzyme (45). Extrapolation of our *in vitro* finding to the *in vivo* situation would suggest that apoA-IV facilitates apoC-II transfer to chylomicrons in the circulation or in the lymph. Although C apoproteins are not known to be synthesized in the intestine to appreciable amounts, lymph chylomicrons do contain these proteins. In the mesenteric lymph, chylomicron or free apoA-IV could affect the apoC-II transfer from HDL filtered from plasma. The addition of apoC-II to chylomicrons entering the circulation would then allow for some initial hydrolysis by LPL located in capillaries in the pulmonary bed. In the circulation, chylomicron surface components including apoA-I and apoA-IV are transferred to HDL during TG hydrolysis. Perhaps, during postprandial lipemia, when apoA-IV levels are markedly elevated, additional apoC-II on VLDL or HDL is displaced, thus augmenting LPL-mediated chylomicron hydrolysis.

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