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**Research Article**

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# Apolipoprotein B Metabolism in Subjects with Deficiency of Apolipoproteins CIII and AI

## Evidence That Apolipoprotein CIII Inhibits Catabolism of Triglyceride-rich Lipoproteins by Lipoprotein Lipase In Vivo

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## Abstract

**Previous data suggest that apolipoprotein (apo) CIII may inhibit both triglyceride hydrolysis by lipoprotein lipase (LPL) and apo E-mediated uptake of triglyceride-rich lipoproteins by the liver. We studied apo B metabolism in very low density (VLDL), intermediate density (IDL), and low density lipoproteins (LDL) in two sisters with apo CIII–apo AI deficiency. The subjects had reduced levels of VLDL triglyceride, normal LDL cholesterol, and near absence of high density lipoprotein (HDL) cholesterol. Compartmental analysis of the kinetics of apo B metabolism after injection of $^{125}$I-VLDL and $^{131}$I-LDL revealed fractional catabolic rates (FCR) for VLDL apo B that were six to seven times faster than normal. Simultaneous injection of [$^{3}$H]glycerol demonstrated rapid catabolism of VLDL triglyceride. VLDL apo B was rapidly and efficiently converted to IDL and LDL. The FCR for LDL apo B was normal.**

**In vitro experiments indicated that, although sera from the apo CIII–apo-AI deficient patients were able to normally activate purified LPL, increasing volumes of these sera did not result in the progressive inhibition of LPL activity demonstrable with normal sera. Addition of purified apo CIII to the deficient sera resulted in 20–50% reductions in maximal LPL activity compared with levels of activity attained with the same volumes of the native, deficient sera. These in vitro studies, together with the in vivo results, indicate that in normal subjects apo CIII can inhibit the catabolism of triglyceride-rich lipoproteins by lipoprotein lipase.**

## Introduction

In 1982, Norum et al. (1) reported that two sisters with extreme reductions in plasma high density lipoprotein (HDL) cholesterol and precocious coronary artery disease had deficiencies of apolipoproteins (apo)[1] CIII and AI. The parents and children of the probands had low plasma levels of apo CIII and apo AI, compatible with heterozygosity in those family members. Recently, Breslow and his co-workers have characterized the genetic mutation in these young women as a 6.5 kilobase-pair translocation from the apo CIII gene to the region of the apo AI gene (2, 3). Although interest in this disorder was focused initially upon the link between the absence of HDL and precocious coronary artery disease, these subjects also provided a unique opportunity to investigate the role of apo CIII in lipoprotein metabolism.

Apo CIII, a glycoprotein of molecular weight 8,500, may play an important regulatory role at two points in the metabolism of the triglyceride-rich lipoproteins, very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL). First, in vitro studies have suggested that apo CIII inhibits the hydrolysis of triglyceride by both lipoprotein lipase (LPL) (4, 5) and hepatic triglyceride lipase (HTGL) (6). Second, liver perfusion studies have suggested that apo CIII inhibits the removal of chylomicrons and VLDL by hepatocytes (7–9). These latter results have led investigators to propose that apo CIII and apo E may have opposing roles in the regulation of the removal of triglyceride-rich lipoproteins by the liver. Both of the actions of apo CIII described above would tend to prolong the residence time of VLDL in plasma and increase its concentration. Therefore, the absence of apo CIII might be associated with reduced plasma VLDL levels. The demonstration of markedly reduced plasma VLDL triglyceride concentrations in the two sisters with deficiency of apo CIII and AI (1, 10) suggested that the absence of apo CIII might have significant impact on lipoprotein metabolism.

To examine the role of apo CIII in the regulation of the metabolism of triglyceride-rich lipoproteins, we carried out studies of the turnover of VLDL triglyceride using [$^{3}$H]glycerol, and the turnovers of VLDL, IDL, and low density lipoprotein (LDL) apo B, using $^{125}$I-VLDL and $^{131}$I-LDL in these two subjects without apo CIII. If apo CIII were to function by either of the mechanisms described above, the fractional removal of VLDL triglyceride from plasma would be increased in the two sisters. If the predominant role of apo CIII were the inhibition of triglyceride hydrolysis, then its absence might be associated with a rapid turnover of VLDL apo B and an efficient conversion of VLDL and IDL to LDL. On the other hand, if apo CIII had, as its major effect, the inhibition of hepatic uptake of remnants of VLDL and IDL catabolism, then its absence might be associated with a rapid turnover of VLDL apo B associated with minimal conversion of apo B in VLDL and IDL to LDL. The results of our studies, although not eliminating a role for apo CIII in the regulation of hepatic catabolism of VLDL remnants in normal subjects, indicate that apo CIII plays a significant role in vivo in the regulation of LPL-mediated hydrolysis of lipoprotein triglyceride.

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1. *Abbreviations used in this paper:* apo, apolipoprotein; FCR, fractional catabolic rate; HTGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; RIA, radioimmunoassay.

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## Methods

*Subjects.* The clinical characteristics and case histories of the two subjects have been described in detail previously (1). At the time of study, Subjects 1 and 2 were 34 and 32 yr of age, respectively. Both subjects had evidence of stable coronary artery disease, but were able to carry out routine daily activities without difficulty. Subject 1 was receiving propranolol and nitrates, whereas subject 2 was taking digoxin and furosemide. In both cases, treatment regimens had been stable for several months prior to study and were continued during the study period. Neither subject had hepatic, renal, or thyroid disease. Both individuals were on a low fat, low cholesterol diet and this was maintained during the studies (see below). Informed consent was obtained from each subject and the studies were approved by the Institutional Review Board at our institution.

*Turnover protocols.* Each subject underwent plasmapheresis of 2 U of blood 1 wk before admission to the General Clinical Research Center. On admission, the patients were placed on a solid food diet consisting of 50% carbohydrate, 30% fat, and 20% protein with a ratio of polyunsaturated to saturated fat of 1.0 and 150 mg of cholesterol per day. This diet was based on a history of what their intake had been at home. At midnight, on the second day, the subjects were switched to a liquid-formula diet consisting of 75% carbohydrate and 25% protein. This formula, which was consumed every 3 h, provided 60% of the daily maintenance calories and enabled us to maintain steady-state plasma triglyceride concentrations during the VLDL turnover study without introducing dietary fat (11). The morning after initiation of the liquid-formula feedings, each subject received an intravenous injection of autologous $^{125}$I-VLDL (50 $\mu$Ci) and [2-$^{3}$H]glycerol (300 $\mu$Ci). Eighteen timed blood samples were obtained during the next 48 h. After the 48-h sample, each patient received an intravenous injection of autologous $^{131}$I-LDL (25 $\mu$Ci). The subjects were returned to their full-calorie solid-food diets at this time, and blood samples were obtained at specified intervals during the next 14 d. A saturated solution of potassium iodide was administered for the duration of the study and for an additional 2 wk to prevent thyroidal uptake of radioactive iodide.

An aliquot of $^{125}$I-VLDL used in the study of subject 1 was injected into a cynomolgus monkey along with $^{131}$I-VLDL obtained from a normal control subject. Timed blood samples were obtained during the following 20 h. VLDL, IDL, and LDL were isolated from each time point and apo B-specific radioactivity was determined as described below.

*Laboratory.* VLDL ($d$ < 1.006) and LDL ($d$ 1.025–1.060) used for iodination were isolated from plasma by ultracentrifugation as previously described (11). Each ultracentrifugation was performed at 10°C for 20–24 h. All procedures were carried out using aseptic technique and sterilized equipment. The lipoproteins were iodinated by a modification (12) of the iodine monochloride method (13). The lipoproteins were then diluted in 0.15 M NaCl and human serum albumin (final concentration 5 g/dl) and passed through filters (VLDL 0.45 $\mu$m and LDL 0.22 $\mu$m) (Millipore Corp., Bedford, MA) prior to storage in sterile vials. [2-$^{3}$H]glycerol (New England Nuclear, Boston, MA) was diluted to a concentration of 300 $\mu$Ci/ml and passed through a filter (0.22 $\mu$m) prior to use.

VLDL, IDL, and LDL used for determination of $^{125}$I-apo B and VLDL-[$^{3}$H]triglyceride-specific activities were isolated by sequential ultracentrifugation from the 18 plasma samples obtained during the 48 h after injection of $^{125}$I-VLDL and [$^{3}$H]glycerol as previously described (11). The specific radioactivity of apo B in each lipoprotein class was determined utilizing 1,1',3,3'-tetramethylurea to separate apo B from the other apolipoproteins present (14). Each specific activity determination was carried out in duplicate or triplicate. The specific radioactivity of VLDL-[$^{3}$H]triglyceride was determined by the method of Grundy et al. (15). LDL were also isolated from plasma obtained at specific times during the 2 wk after injection of $^{131}$I-LDL by sequential ultracentrifugation at $d$ 1.020 and then at $d$ 1.063 in a 40.3 rotor at 39,000 rpm at 10°C for 24 h each (11). Apo B specific activity was then calculated using the protein determinations made by the method of Lowry et al. (16) and the gamma radioactivity. This method results in apo B specific radioactivities for LDL apo B that are identical to those obtained with 1,1',3,3'-tetramethylurea (14). Gamma and beta radioactivity were measured in Packard scintillation counters (Packard Instrument Co., Inc., Downers Grove, IL).

Fractionation of lipoproteins directly from fasting plasma was achieved by gel filtration over 2.5 × 100-cm columns of 4% agarose beads (Biogel A15M, 200–400 mesh) equilibrated in 0.01 M sodium barbital, 0.15 M sodium chloride, 0.01% EDTA, 0.02% sodium azide, pH 7.0, containing 500 kIU Trasylol (Mobay Chemical, New York) per ml (17). Fractions of ~10 ml were collected and analyzed for apolipoproteins and lipids. Exact volumes were determined gravimetrically.

Cholesterol and triglyceride concentrations were measured by specific enzymatic methods using an ABA-100 Analyzer (Abbott Laboratories, Chicago IL). Lipoprotein cholesterol levels were determined after ultracentrifugation according to Lipid Research Clinic methodology (18), except that dextran sulfate and $MgCl_2$ were used to precipitate the lower-density lipoproteins before measurement of HDL cholesterol (19). This method results in HDL cholesterol concentrations that are ~5–10% lower than those obtained using the Lipid Research Clinic method.

Apolipoproteins E, B, AI, CII, and CIII were measured utilizing specific radioimmunoassays (RIA). Details of these RIA have been reported elsewhere for apo E, B, AI, and apo CIII (17). The RIA for apo CII was essentially identical to that described for apo CIII (17). Apo AII was measured by specific RIA in the laboratory of Dr. Ronald Goldberg, University of Miami School of Medicine (20). Apo AIV was determined by RIA in the laboratory of Dr. Charles Bisgaier, Columbia University College of Physicians and Surgeons (21).

*In vitro studies.* Measurements of post-heparin plasma LPL and HTGL activities were carried out as previously described (5). Blood was withdrawn 15 min after injection of 60 U/kg heparin intravenously and the plasma stored at −70°C until assayed. LPL was measured in the presence of a monospecific antibody that inhibits HTGL (22), whereas HTGL was assayed in the presence of a high salt buffer.

In vitro studies of the activation of LPL by sera were carried out using purified bovine milk LPL, which had a specific activity of 55 mmol free fatty acid (FFA) released/h per mg protein. LPL activity was measured at 37°C in the following incubation mixture: 1.15 $\mu$mol glycerol-9,10($n$)-[$^{3}$H]trioleate (sp act 0.87 $\mu$Ci/$\mu$mol) emulsified with phosphatidylcholine (5% by weight of the triolein), 6 mg/ml bovine serum albumin, RPMI 1640, 15 mM Hepes, pH 8.0, and varying volumes of heat-inactivated serum as indicated, in a total volume of 0.5 ml (23). Released FFA were quantified by the method of Pittman et al. (24). Less than 10% of the substrate was hydrolyzed in 60 min at 37°C.

Studies of the activation of LPL were also performed using human LPL. Human milk was obtained from the Human Milk Bank, Babies Hospital, Columbia Presbyterian Medical Center, New York City, and acetone-ether powders of the milk-cream were stored at −20°C until used. For these studies, LPL activity was measured using 175 $\mu$l of a gum arabic stabilized emulsion containing 2.5 $\mu$mols of triglyceride as described by Brown and Baginsky (5). Heat-inactivated serum from normolipidemic controls and the two probands (5–100 $\mu$l) was added to the emulsion which was then incubated at 37°C for 60 min. 10 $\mu$l of the LPL preparation was then added and the reaction was allowed to proceed at 37°C for 60 min. The FFA were extracted as described by Belfrage and Vaughan (25).

All assays were performed in triplicate. Less than 5% of the maximal activity of the human milk LPL preparation in the emulsion that contained control serum (12.2 $\mu$mol FFA released/ml per h) was observed when no serum was added. Additional studies were performed to assess the effect of addition of purified apo CIII and normal HDL to the probands' sera. Purified apo CIII was added to each patients serum to approximate levels present in normal plasma (17) and HDL was added at a final protein concentration 1 mg/ml. After a 30-min preincubation period, these sera were then used in the assay described above to activate human LPL. The maximum LPL activity obtained using the apo CIII-deficient sera was then compared with that achieved using the same amount of apo CIII- or HDL-repleted sera.

*Data analysis.* A multicompartmental model of the metabolism of VLDL, IDL, and LDL apo B, based on the simultaneous analysis and modeling program (26), was used to analyze the tracer data in the present

study. The model for the metabolism of VLDL apo B and its subsequent conversion to IDL and LDL was a modification of our earlier model (11) (Fig. 1). The decay of $^{125}$I-VLDL apo B from the plasma of each of the sisters was characterized by two kinetic components similar to those we have previously observed in other patients (11). In addition to these two kinetic subpopulations of plasma VLDL, a third kinetic subpopulation of VLDL particles characterized by an extremely rapid decay rate of $^{125}$I-apo B was detected in both subjects. A significant fraction of the apo B radioactivity associated with this third subpopulation was efficiently converted to IDL and LDL. This pathway could account for the rapid appearance of apo B radioactivity that we observed in IDL and LDL (see Results). In addition, the fraction of the injected VLDL apo B radioactivity recovered in LDL was consistently greater than the apo B radioactivity recovered in IDL. These findings suggested that some of the VLDL apo B radioactivity was directly converted to LDL without first contributing to the radioactivity associated with the IDL density fraction. This model was used to estimate the fractional catabolic rate (FCR) of apo B in VLDL, IDL, and LDL. Production rates of apo B in each lipoprotein were obtained by multiplying each FCR by the steady-state plasma pool of the appropriate lipoprotein apo B during the 48-h turnover period. The conversion of VLDL apo B to LDL was also estimated using these techniques.

The FCR for VLDL triglyceride in each subject was determined by calculating the area under the curves describing the multiexponential disappearance of VLDL-[$^3$H]triglyceride generated by injection of [$^3$H]glycerol. This approach was taken because each subject had an extremely rapid rising component in their curve, suggesting that plasma catabolism was rate limiting for VLDL triglyceride metabolism in these patients. Furthermore, both VLDL triglyceride specific activity curves displayed three-exponential components, kinetic characteristics that were not compatible with the usual compartmental models used by our group and by other investigators (11, 15). Normal patient data, presented for comparison, was determined using our previously described model (15).

## Results

The plasma lipid and apolipoprotein concentrations of the two subjects are presented in Tables I and II. Compared with values for normal subjects reported either in the literature or measured in our laboratory, the patients had reduced plasma levels of VLDL triglyceride and were almost completely deficient in HDL cholesterol. Plasma levels of total cholesterol were significantly reduced, but LDL cholesterol concentrations were normal. Plasma concentrations of apo E, apo CII, and apo AIV were decreased, whereas apo AI and apo CIII levels were not detectable. Plasma apo B concentrations were normal in the two subjects. These findings are all quite similar to those previously reported for these individuals (1, 10).

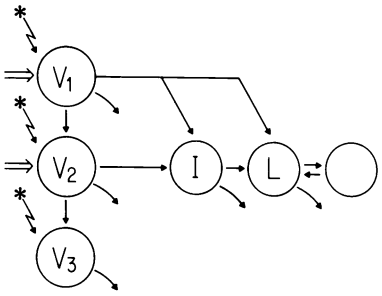


*Figure 1.* Schematic representation of multicompartmental model describing the metabolism of apo B in VLDL, IDL, and LDL in the two subjects with apo CIII–apo AI deficiency. The majority of apo B flux through VLDL occurs via $V_1$, with the majority of lipoprotein particles passing rapidly from $V_1$ to IDL and LDL. Some VLDL apo B enters $V_2$ and is mainly catabolized at a slower rate to IDL and LDL, with a minor fraction passing through $V_3$. Our previous model of apo B metabolism (11) did not require pool $V_1$ but did include a four-pool cascade to describe the kinetics of apo B metabolism from pool $V_2$ to IDL, and a pathway for transfer of apo B radioactivity from $V_2$ to the very slowly catabolized pool $V_3$. Apo B in pool $V_3$ is not converted to IDL or LDL in either the present or previous model (11). The latter model was used for the analysis of the data from studies in the normal subjects presented in this report.

*Table I. Plasma Lipid Levels*

| Lipid | Subject 1 | Subject 2 | Normal* |
|---|---|---|---|
| | *mg/dl* | *mg/dl* | *mg/dl* |
| Total triglyceride | 61.3±1.5 | 37.6±10.6 | 74.1±45.9 |
| VLDL triglyceride | 11.0±7.4 | 5.5±2.5 | 41.0±8.0 |
| Total cholesterol | 118.1±4.0 | 109.1±8.5 | 174.3±29.1 |
| LDL cholesterol | 100.8±4.4 | 101.7±5.5 | 108.9±25.9 |
| HDL cholesterol | 1.2±0.4 | 2.0±0.7 | 55.4±12.1 |

All values depicted are mean±SD.
* All values except VLDL triglyceride are the Lipid Research Clinic data (18). VLDL triglyceride values are from control subjects in our laboratory.

The distribution of the apolipoproteins in fasting plasma from each sister was determined by gel-permeation chromatography using 4% agarose (Fig. 2 *A* and *B*). Although the samples were chromatographed over different columns with slightly different elution characteristics, comparison can be made using the cholesterol-apo B peaks in LDL as an internal marker. Plasma triglyceride was detectable only as a small peak concordant with LDL cholesterol (data not shown). The most striking finding was the presence of a single peak of apo E which was in the HDL region. This was in contrast to the normal distribution of apo E among three peaks (coincidental with VLDL, with a region overlapping small VLDL and IDL, and with large HDL) observed in most individuals (17). Of further interest was the demonstration that the peaks of apo E and apo AII in the HDL region did not completely coincide in either patient, suggesting that there might be discrete populations of particles containing one of these apolipoproteins without the other as well as populations with both of these apolipoproteins together. Finally, although there was a peak of apo CII in the region of triglyceride-rich lipoproteins in each subject, the overall distribution of apo

*Table II. Plasma Apolipoprotein Levels*

| Apolipoprotein | Subject 1 | Subject 2 | Normal |
|---|---|---|---|
| | *mg/dl* | *mg/dl* | *mg/dl* |
| Apo CIII | ND | ND | 11.4±2.7* |
| Apo AI | ND | ND | 133.5±24.7* |
| Apo B | 101.6 | 117.6 | 113.8±30.3* |
| Apo E | 2.9 | 1.7 | 4.7±1.6* |
| Apo CII | 0.14 | 0.33 | 3.7±2.9* |
| Apo AIV | 25.8 | 15.6 | 37.4±4.0‡ |

All values depicted are presented as mean±SD. ND, not determinable.
* Normal values obtained from a large group of free-living males (17).
‡ Normal values from Bisgaier et al. (21).

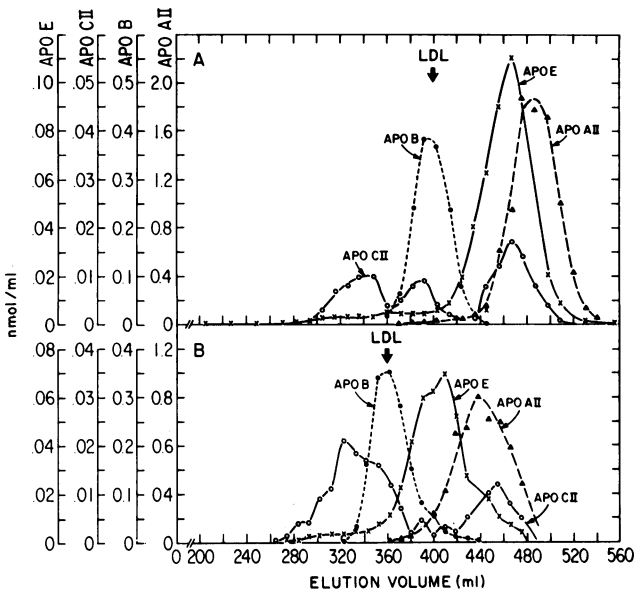


*Figure 2.* The distribution of apolipoproteins obtained after chromatography of plasma over 4% agarose. The notation, LDL, denotes the position of the low density lipoprotein cholesterol peak in each study, and can be used as an internal marker for comparison of the two studies. Although apo CII has a peak in VLDL, no significant accumulation of apo E is present in that range. In each subject, apo E is distributed in a single peak eluting just in front of the apo AII peak. (*A*) In subject 1, apo CII in HDL is mainly coincidental with the apo E peak; (*B*) in subject 2, HDL apo CII appears to be much more closely aligned with apo AII.

CII was different in the two sisters. There was a closer association of apo E with apo CII in the HDL region in subject 1 and better concordance for the distributions of apo AII and apo CII in subject 2. The basis for this heterogeneity is unknown. However, subject 2 has shown variability of this distribution over time, and a pattern more closely resembling that in subject 1 was observed several months later (27).

The results of apo B turnover studies are depicted in Fig. 3. After injection of autologous $^{125}$I-VLDL, there was a very rapid decay of radioactivity in VLDL apo B in both subjects followed by rapid conversion of labeled apo B to IDL and LDL (Fig. 3 *a* and *b*). In contrast to the characteristic biphasic decay of apo B specific activity in VLDL that is observed in a typical study of a normal subject (Fig. 3 *c*), each of the apo CIII–apo AI-deficient subjects appeared to have three-exponential components of their VLDL apo B curves with the additional component being a very rapid early decay. Normal subjects also have a characteristic delay of 3–5 h between the point at which the VLDL and IDL apo B specific activity curves cross and the maxima of the IDL apo B specific activity curve (Fig. 3 *c*), but this delay was nearly (Fig. 3 *a*) or completely (Fig. 3 *b*) absent in the two patients. Finally, in contrast to the normal time of 15–20 h before the peak LDL apo B specific activity is observed, LDL apo B specific activities reached their peak in <10 h in the studies of the two sisters.

To provide quantitation of the metabolism of VLDL apo B and its conversion to LDL apo B the data were subjected to compartmental analysis. The kinetic parameters describing the metabolism of apo B in these two subjects with apo CIII–apo AI deficiency are presented in Table III. Both subjects had markedly increased FCR for VLDL apo B based on analyses of the $^{131}$I-VLDL apo B specific-activity decay curves—49.1 and 42.0 pools per day, respectively. These rates were six to seven times faster than those present in normolipidemic males we have studied (28). The plasma VLDL apo B concentrations were concomitantly reduced (1.4±0.13 and 1.1±0.35 mg/dl for subjects 1 and 2, respectively). Reduced synthesis did not play a role in the reduced VLDL apo B because the calculated production rates of 30.3 and 21.2 mg/kg per d were at the upper end of

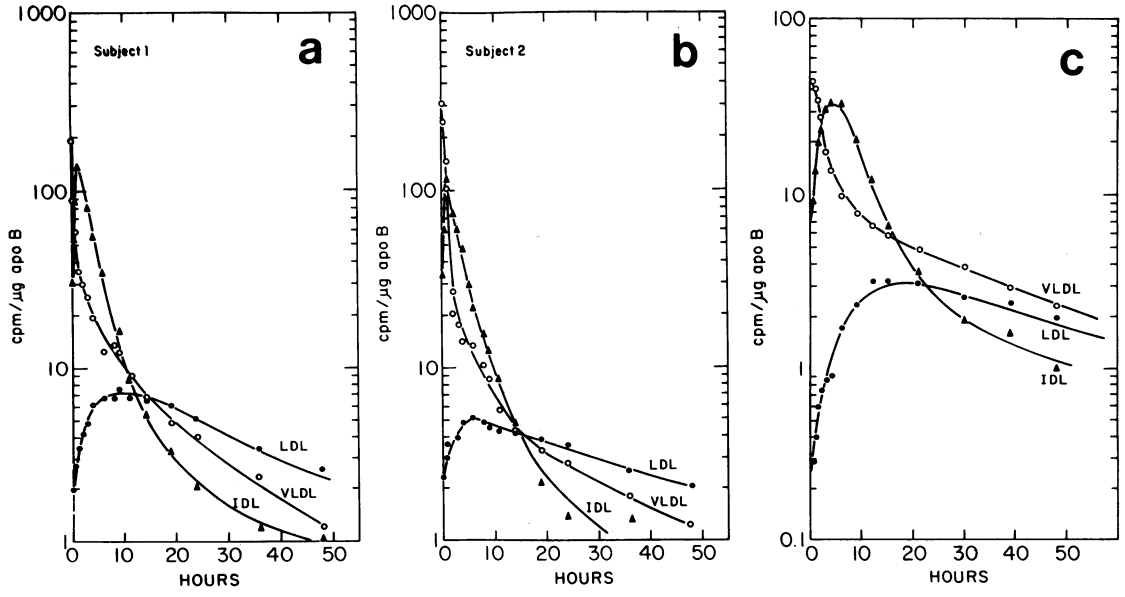


*Figure 3.* Curves describing the kinetics of apo B specific radioactivity in VLDL, IDL, and LDL during the 48-h period after injection of whole radiolabeled VLDL into (*a*) subject 1, (*b*) subject 2, and (*c*) a normal control subject. Apo B metabolism in both subjects 1 and 2 is characterized by an extremely rapid early component of VLDL decay; a rapid rise and fall of radioactivity in IDL, with a loss of the usual delay observed between the point that the IDL specific activity curve crosses the VLDL curve and the peak specific radioactivity in IDL; and a rapid rise and fall in LDL apo B specific activity.

*Table III. Apolipoprotein B Turnover*

| Subject (*n*) | VLDL | | | LDL | | | |
|---|---|---|---|---|---|---|---|
| | FCR | PR* | apo B | FCR | PR* | apo B | Conversion |
| | *day*$^{-1}$ | *mg/kg per day* | *mg/dl* | *day*$^{-1}$ | *mg/kg per day* | *mg/dl* | %‡ |
| Subject 1 | 49.2 | 30.3 | 1.4±0.13 | 0.52 | 20.1 | 85.7±7.8 | 62 |
| Subject 2 | 42.0 | 21.2 | 1.1±0.35 | 0.44 | 16.8 | 85.1±6.4 | 89 |
| Normals (5) (Mean±SD) | 6.3±3.1 | 15.2±5.1 | 5.8±1.4 | 0.52±0.10 | 12.9±1.2 | 57.0±11.3 | 48.0±25.9 |

* PR, production rate = FCR × apo B pool. ‡ %, percentage of VLDL apo B converted to LDL.

the range of values we have measured in normal control subjects (28).

Because the very rapid early decay of VLDL apo B in both subjects could have been the results of removal of damaged or "foreign" material by macrophages in liver and spleen, we screened the $^{125}$I-VLDL from subject 1 in a cynomolgus monkey. $^{131}$I-VLDL from a normal control subject was simultaneously injected into the monkey for comparison. The resultant curves for VLDL, IDL, and LDL apo B specific activity were essentially identical (Fig. 4), arguing against "damage" to the VLDL tracer as the basis for rapid removal in the two sisters.

The turnover of VLDL triglyceride in each subject, determined after the injection of [2-$^3$H]glycerol, was in accord with the rapid catabolism of VLDL apo B (Fig. 5). Each subject had an extremely rapid rise of radioactivity in plasma followed by a three-exponential decay curve. The peak VLDL triglyceride specific activity was attained by 30 min. This peak is reached in 2–3 h in normal subjects. The first decay component was very rapid in each subject, paralleling the rapid, first decay component present in their VLDL apo B specific-activity decay curves. The FCR for VLDL triglyceride was 2.6 $h^{-1}$ in subject 1 and 0.83 $h^{-1}$ in subject 2. Both of the values were significantly increased compared with values determined in normal subjects in our laboratory (0.33±0.07 $h^{-1}$) (28). The low steady-state levels of plasma VLDL triglyceride were due entirely to the rapid clearance in that the production rates were within normal limits.

In contrast to the rapid rates for VLDL catabolism, the FCR for LDL apo B were normal in both subjects (Table III). These rates were estimated from the apo B specific activity curves generated by the separate injection of $^{131}$I-LDL. They were in good agreement with the estimated fractional rates derived from analysis of the $^{125}$I-LDL apo B curves generated by the $^{125}$I-VLDL injection: 0.49 pools per day for both subjects. Using their respective LDL apo B levels of 85.7 and 85.1 mg/dl, the calculated production rates of LDL apo B were 20.1 and 16.8 mg/kg per d. These rates were moderately elevated compared with those present in our control subjects (28).

Compartmental analysis of the flow of apo B radioactivity from VLDL through IDL into LDL demonstrated that 62 and 89% of VLDL apo B was converted to LDL in subjects 1 and 2, respectively (Table III). These values were in the upper range for normal subjects in our laboratory (unpublished data). The more efficient conversion of VLDL apo B into the LDL density range was sufficient to explain the high rates of LDL production in these patients.

Post-heparin plasma LPL and HTGL activities were determined in both subjects. The levels of LPL activity (4.6 and 12.0 μmol FFA/ml of plasma per h for subjects 1 and 2, respectively) were within the normal range for our laboratory (20.2±8.0). HTGL activity was similarly normal (38.8 and 31.0 vs. 36.6±16.6 μmol FFA/ml of plasma per h). These results suggested that the rapid catabolism of triglyceride-rich lipoprotein in the apo CIII–

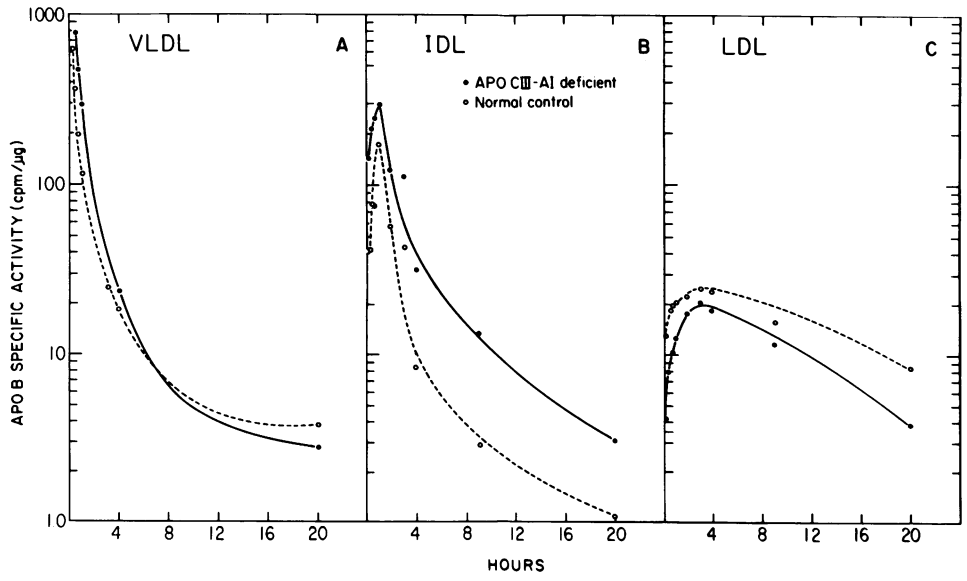


*Figure 4.* Curves describing the kinetics of apo B specific activity in (*A*) VLDL, (*B*) IDL, and (*C*) LDL after injection of differentially radiolabeled VLDL from subject 1 (●) and from a normal control subject (○) into a cynomolgus monkey. It is apparent that the metabolism of apo B in each trace was very similar.

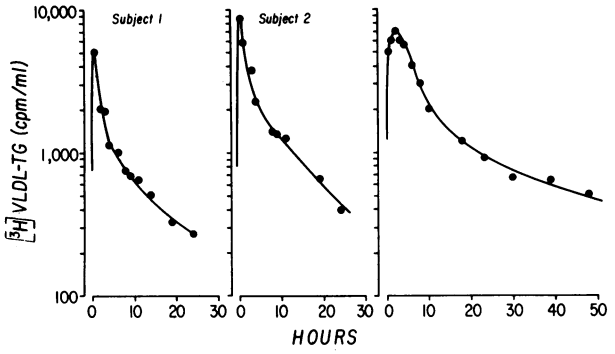


*Figure 5.* Plasma radioactivity curves for VLDL-[$^3$H]triglyceride (TG) after injection of 300 $\mu$Ci [$^3$H]glycerol into each of the apo CIII–apo AI-deficient subjects and a normal control subject. The patients' curves are characterized by a very fast early decay component followed by a biexponential fall in radioactivity. In contrast, the normal curve has a much broader peak of radioactivity followed by a biexponential decay curve.

apo AI patients was not the result of increased presence of LPL or HTGL on the luminal surfaces of endothelial cells.

In vitro experiments were carried out in order to investigate more directly the role of apo CIII deficiency in the rapid catabolism of VLDL observed in vivo. Normal serum, when mixed with an artificial or a natural substrate for measurement of LPL activity, produces activation of the enzyme that reaches a maximum at a volume characteristic of the substrate emulsion and the serum itself. Larger volumes of serum inhibit the hydrolysis of the substrate (4, 5). When sera from the apo CIII–apo AI patients were compared with normal sera, the results (Fig. 6 *A* and *B*) indicated that the inhibition of LPL activity was markedly reduced or absent when the sera from the apo CIII–apo AI-deficient subjects were used. Equivalent absolute levels of activity were attained when similar optimal volumes of patient or control serum were added to the substrate prior to addition of the purified bovine milk LPL, demonstrating that there was sufficient apo CII to maximally activate the enzyme even though the apo CII concentrations in the two patients were only about 10% of that in the control serum. However, whereas increasing volumes of normal sera above the level required for maximal activation resulted in inhibition of LPL activity, larger volumes of apo CIII–apo AI-deficient serum had no inhibitory effect. Identical results were obtained in several experiments utilizing either purified human or bovine milk LPL. Addition of purified apo CIII to the apo CIII–apo AI-deficient sera, prior to their use as activators, resulted in maximal levels of LPL activity that were 20–50% less than those observed when the native apo CIII–apo AI-deficient sera were used (Fig. 6 *C*). In addition, when greater than optimal volumes of apo CIII reconstituted sera were used as activators, further inhibition of LPL activity was demonstrated. In contrast, when normal HDL was added to the apo CIII–apo AI-deficient sera, no effect upon the ability of these sera to activate LPL was demonstrable (Fig. 6 *D*).

## Discussion

In vitro and in vivo evidence from various laboratories has clearly demonstrated unique roles for several of the plasma apolipoproteins in the regulation of VLDL, IDL, and LDL metabolism. Genetic disorders of lipoprotein structure and metabolism have given further insights into the functions of these lipid-binding proteins. The role of apo B in VLDL secretion is evident from studies of the recessive disorder abetalipoproteinemia, and its importance in receptor interaction for uptake and degradation of LDL is demonstrated by familial hypercholesterolemia. The lack of receptor for apo B, as in the homozygous state of familial hypercholesterolemia, results in severe premature atherosclerosis (29). Studies by Rall et al. (30) and Havel (31) have amply demonstrated the importance of apo E for VLDL remnant catabolism, whereas type III hyperlipoproteinemia provides the in vivo model for ineffective interaction of apo E with cell membrane receptors. In vitro studies have also demonstrated the need for apo CII activation of LPL and the absence of this apolipoprotein in certain families with severe hypertriglyceridemia provided the in vivo confirmation for those in vitro studies (32, 33). Finally, in vitro and in situ studies have suggested two roles for apo CIII. The first is that of an inhibitor of hydrolysis of tri-

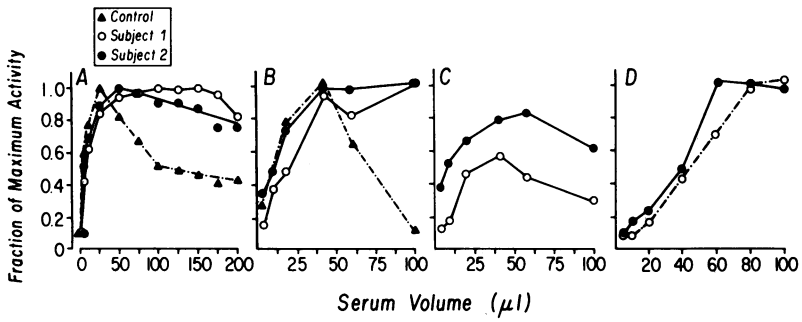


*Figure 6.* Activation of LPL by normal and apo CIII–apo AI-deficient serum. (*A*) Curves describing the effect of increasing volumes of serum upon the activity, in vitro, of purified bovine milk LPL. The data are plotted as the fraction of maximal activity vs. the serum volume added (see Methods). Although the absolute maximal activities obtained were quite similar (data not shown), it is clear that increasing volumes of normal sera inhibited LPL activity while similar volumes of apo CIII–apo AI-deficient sera did not. (*B*) Curves describing the effect of increasing volumes of serum upon the activity, in vitro, of purified human milk LPL. The data are plotted as in *A*. It is again apparent that apo CIII–apoAI-deficient serum activated but did not suppress LPL activity. The experiment shown is typical of three studies carried out under identical conditions. (*C*) Curves describing the effect of preincubation of apo CIII–apo AI-deficient serum with purified apo CIII upon activation of purified human milk LPL by that serum. The final concentration of apo CIII in each serum was 50 $\mu$g/ml. The data, representing only the activation curves for each reconstituted serum are plotted as the fraction of the maximal activity attained with each native apo CIII–apo AI-deficient serum in the same experiment. Each curve is the mean of results from two experiments. Compared with the curves depicting activation by the native apo CIII–apo AI-deficient serum optimal volumes of the apo CIII-reconstituted serum resulted in lower maximal levels of LPL activity. Significant inhibition of LPL activity occured with volumes of sera greater than the optimal volume. (*D*) Curves describing the effect of preincubation of apo CIII–apo AI-deficient serum with normal HDL upon activation of purified human milk LPL by that serum. The final concentration of HDL was 1 mg/ml. The data are plotted as described in *C*, and demonstrate the lack of any effect of HDL upon the ability of apo CIII–apo AI-deficient sera to activated LPL.

glyceride-rich lipoproteins by LPL and HTGL (4–6), and the second, as an inhibitor of apo E-mediated hepatic uptake of chylomicron remnants and VLDL (7–9).

The identification of two sisters with apo CIII–apo AI deficiency (1) provided us with a unique opportunity to observe the effects of the absence of apo CIII upon the in vivo metabolism of the triglyceride-rich lipoproteins, VLDL and IDL. The results of the kinetic studies presented in this report indicate that apo CIII acts as an inhibitor of in vivo hydrolysis of triglyceride in VLDL and IDL of normal subjects. This inhibitory activity appears to act, at least in part, upon LPL. This conclusion is based on several aspects of our studies.

First, the FCR of both VLDL triglyceride and VLDL apo B were significantly increased in the apo CIII-deficient subjects compared with normal controls. The decay curves of both VLDL apo B and VLDL triglyceride demonstrated very rapid early decay components that have not been observed in previous studies of normal or hyperlipidemic individuals. Major proportions of both apo B and triglyceride flux in VLDL occurred via this rapid catabolic pathway. Although the apo CIII–apo AI-deficient subjects had VLDL apo B concentrations that were significantly lower than the levels in our normal subjects, our control subjects were very "normal," with total triglyceride levels near the 10th percentile for their ages. In addition, in a study by Nestel et al. (34), in which plasma VLDL apo B concentrations were reduced by dietary fish oils to levels comparable to those in our patients, the FCR of VLDL apo B were quite similar to those of our control subjects. It appears quite likely, therefore, that the very rapid FCR for VLDL in the two patients were the cause, rather than the result, of their severely reduced plasma VLDL pools. Increased triglyceride hydrolysis in these subjects is also suggested by the observation of Forte et al. (10) who noted a less than normal increase in plasma triglyceride concentration after a 100-g fat meal in subject 1. Those investigators also noted the appearance of abnormal, nonspherical particles that floated in the LDL density range after the fat meal and suggested that these particles might be abnormal remnants of accelerated hydrolysis of chylomicron particles (10).

Second, the rapid catabolism of VLDL was associated with an efficient conversion of VLDL to IDL and ultimately to LDL. This indicated that the rapid turnover of VLDL was not the result of removal of the entire particle from plasma by an endocytic process, but the hydrolysis of the triglyceride core with generation of a more dense product. The demonstration of increased conversion was not simply the result of injection of a VLDL tracer consisting of small VLDL, inasmuch as our patients have a normal size distribution of their VLDL (10). Third, the similarity between the plasma decay curves of apo CIII–apo AI-deficient VLDL and normal VLDL in the monkey suggested that monkey apo CIII was able to exchange into the apo CIII-deficient tracer and normalize (slow) its fractional catabolism. Fourth, although studies of VLDL metabolism have demonstrated a correlation between the fractional catabolism of this lipoprotein and LPL activity (35, 36), assay of post-heparin plasma LPL and HTGL activities did not support the possibility that the two subjects had increased quantities of either of these enzymes available to circulating lipoproteins. Finally, the in vitro studies utilizing the subjects' apo CIII–apo AI-deficient sera as activators for purified bovine and human milk LPL provided strong evidence in support of the role of apo CIII present in normal serum as an inhibitor of LPL induced triglyceride lipolysis.

Although these studies support the proposal that apo CIII inhibits LPL activity in vivo, it is more difficult to draw conclusions concerning the possible role of apo CIII as an inhibitor of apo E-mediated hepatic uptake of triglyceride-rich lipoproteins. On the one hand, the extremely rapid and efficient conversion of VLDL and IDL to LDL speaks against increased apo E-mediated receptor removal of triglyceride-rich lipoprotein particles in the two subjects. However, the high degree of conversion to LDL in our two subjects does not rule out the possibility that in normal subjects with slower hydrolysis of VLDL and IDL, the uptake of apo E-enriched remnant particles may be modulated by their apo CIII content. The near absence of any VLDL remnant pool in our two subjects (10) would make it very difficult to demonstrate the effect of the absence of apo CIII upon the metabolism of such a pool. Thus, nearly all of the apo E was in the HDL region (Fig. 1). In addition, Forte and co-workers (10) demonstrated that a large proportion of apo E in HDL was complexed to apo AII. This might reduce the apoE available to the patients' VLDL particles and limit apo E-mediated uptake of their VLDL by hepatocytes (10). The issue is complicated further by the demonstration that apo CI and apo CII also inhibit hepatic uptake of chylomicron and VLDL remnants (37). The apo CI levels in VLDL were not measured in this study but plasma apo CI levels have been reported to be ~40% of normal in the apo CIII–apo AI-deficient subjects. Plasma apo CII concentrations were 10% of normal (Table II). It is possible that the presence of these apolipoproteins on the subjects' VLDL could have minimized the consequences of the absence of apo CIII relative to hepatic uptake of VLDL remnants. Our results also do not directly address possible inhibition, in vivo, of HTGL by apo CIII. Although Kinnunen and Enholm (6) have demonstrated that apo CIII inhibits HTGL activity in vitro, apo CI and apo CII appear to be equivalent to apo CIII in this regard (6).

Whether or not apo CIII regulates remnant removal in normal subjects, the rapid and efficient hydrolysis of VLDL triglyceride and the subsequent efficient conversion of those VLDL to LDL in these apo CIII-deficient subjects, may have contributed to their precocious arteriosclerosis. Thus, in addition to the near absence of HDL in the two sisters, they had decreased direct removal of VLDL by hepatocytes. This catabolic pathway, which exists in both normal (38) and hypertriglyceridemic (11, 38) subjects, restricts the percentage of VLDL that is converted to LDL. The high LDL production rates present in the apo CIII-deficient subjects as a consequence of more efficient conversion of VLDL may have further potentiated their risk for vascular disease associated with the near absence of HDL (39). In addition, the presence of cholesterol-poor LDL in the two sisters with quite average apo B levels was similar to the LDL composition found in men with coronary artery disease and hyperapobetalipoproteinemia (40).

The studies in these patients have also provided unique information relative to the roles of apo CII in the regulation of VLDL metabolism. Thus, although apo CII levels in plasma were 5–10% of normal, in vivo fractional catabolism of VLDL was very rapid. In vitro studies, furthermore, indicated that similar volumes of serum from patients and the control subjects produced similar maximal hydrolysis by bovine milk LPL. These data support the view that very little apo CII is needed to maximally activate LPL. This view is also supported by the studies of kindreds with apo CII deficiency in which heterozygotes, with 30–50% normal levels of apo CII had normal plasma triglyceride concentrations (32, 33).

Finally, the two sisters had very rapid turnovers of VLDL and IDL in the presence of severely reduced HDL concentrations. In vitro studies have suggested that the presence of HDL is necessary for the orderly progression of lipolysis of triglyceride-rich lipoproteins by lipoprotein lipase. Our results indicate clearly that HDL containing apo AI are not necessary for that process. On the other hand, the abnormal HDL profile present in these subjects suggests that apo AI may be necessary for orderly HDL metabolism. The chromatographic studies demonstrated the presence of populations of particles in the HDL size range with apo E, apo CII, and apo AII in varying proportions. In fact, there appeared to be some particles with only apo E and apo CII present, others with only apo CII and apo AII present, and even others with all of these three apolipoproteins as components. The source of the apo E and apo CII in the HDL region would appear to be the hydrolysis of VLDL and IDL with concomitant transfer of these two apolipoproteins and surface lipids to an HDL particle containing apo AII as the major protein. However, the significant reductions in the plasma levels of apo CII, apo E, and apo AII in the two sisters, together with the very rapid fractional removal of their autologous, radiolabeled HDL (unpublished observations), indicate that apo AI and/or apo CIII may be necessary to stabilize plasma HDL particles and prolong their residence time in the extracellular space. Alternatively, the rapid catabolism of their HDL may result from the presence of apo E on a large majority of particles (41). Regardless of the mechanism, the markedly shortened time of residence of plasma HDL in these two sisters was associated with severe, premature arteriosclerosis.

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