Apolipoprotein C-II Deficiency Syndrome

Clinical Features, Lipoprotein Characterization, Lipase Activity, and Correction of Hypertriglyceridemia after Apolipoprotein C-II Administration in Two Affected Patients

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Abstract

Two patients (brother and sister, 41 and 39 yr of age, respectively) have been shown to have marked elevation of plasma triglycerides and chylomicrons, decreased low density lipoproteins (LDL) and high density lipoproteins (HDL), a type I lipoprotein phenotype, and a deficiency of plasma apolipoprotein C-II (apo C-II). The male patient had a history of recurrent bouts of abdominal pain often accompanied by eruptive xanthomas. The female subject, identified by family screening, was asymptomatic. Hepatosplenomegaly was present in both subjects.

Analytical and zonal ultracentrifugation revealed a marked increase in triglyceride-rich lipoproteins including chylomicrons and very low density lipoproteins, a reduction in LDL, and the presence of virtually only the HDL_3 subfraction. LDL were heterogeneous with the major subfraction of a higher hydrated density than that observed in plasma lipoproteins of normal subjects. Apo C-II levels, quantitated by radioimmunoassay, were 0.13 mg/dl and 0.12 mg/dl, in the male and female proband, respectively. A variant of apo C-II (apo $C\text{-}II_{Padova}$) with lower apparent molecular weight and more acidic isoelectric point was identified in both probands by two-dimensional gel electrophoresis.

The marked hypertriglyceridemia and elevation of triglyceride-rich lipoproteins were corrected by the infusion of normal plasma or the injection of a biologically active synthesized 44–79 amino acid residue peptide fragment of apo C-II. The reduction in plasma triglycerides after the injection of the synthetic apo C-II peptide persisted for 13–20 d. These results definitively established that the dyslipoproteinemia in this syndrome is due to a deficiency of normal apo C-II. A possible therapeutic role for replacement therapy of apo C-II by synthetic or recombinant apo C-II in those patients with severe hypertriglyceridemia and recurrent pancreatitis may be possible in the future.

Introduction

Human plasma apolipoprotein (apo)[1] C-II is a 79-amino acid protein secreted primarily by the liver and present on plasma chylomicrons, very low density lipoproteins (VLDL), and high density lipoproteins (HDL) (1). Apo C-II has a central role in triglyceride metabolism as a cofactor for lipoprotein lipase (2, 3), the enzyme that catalyzes the hydrolysis of triglycerides on plasma lipoproteins.

Recently, the gene for apo C-II has been cloned, and the complete complementary DNA (cDNA) sequence of apo C-II has been determined (4–6). Apo C-II is synthesized as a 101-amino acid precursor protein, preapo C-II, containing a 24-amino acid prepeptide that is cotranslationally cleaved during synthesis. Apo C-II has been localized to chromosome 19 (6–8), which also contains the genes for apo E (9, 10), and the low density lipoprotein (LDL) receptor (11). Solid-phase synthesis of the entire 79-amino acid apo C-II has been completed, and the synthetic apo C-II has full biological activity (12). Previous studies have established that the terminal synthetic fragment of apo C-II containing residues 44–79 will bind to lipoprotein lipase and activate the enzyme (13).

The apo C-II deficiency syndrome is a rare genetic disease characterized by a deficiency of plasma apo C-II, marked elevations of plasma triglycerides and chylomicrons, decreased LDL and HDL, and a type I phenotype (14). Since the first identification of this genetic disease by Breckenridge et al. (15–17) in 1978, additional affected subjects have been described in Canada (18), Japan (19), Italy (20, 21), England (22), the Netherlands (23), and the United States (24). The molecular defect(s) in the apo C-II deficiency syndrome is not known. Recent analysis of the apo C-II gene in the patients presented here and an independent kindred by restriction enzyme analysis established that the apo C-II gene was present in members of these two affected kindreds, and there were no major insertions or deletions in the apo C-II gene (25, 26).

To date only a few subjects with apo C-II deficiency have been thoroughly characterized with respect to the plasma lipoproteins. The purpose of this report is to provide a detailed characterization of the clinical features as well as the plasma apolipoproteins and lipoproteins of two patients with apo C-II deficiency and to definitely establish that replacement of apo C-II in these subjects will correct the metabolic defect in triglyceride metabolism. These results provide the base for future definitive treatment of this disorder by apo C-II administration or gene therapy.

Methods

Case report and clinical evaluation. Clinical, biochemical, and radiological evaluations of the two patients with apo C-II deficiency were performed in the Department of Internal Medicine, Padua, Italy. The clinical protocol was approved by the Clinical Review Committee of the University of Padua, Padova, Italy, and informed consent for all studies was obtained from both patients.

Lipoprotein isolation and characterization. Plasma lipoproteins were isolated by preparative ultracentrifugation utilizing an L5-65 ultracen-

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Received for publication 13 November 1984 and in revised form 17 September 1985.

1. *Abbreviations used in this paper:* apo, apolipoprotein; CT, cholesterol; HL, hepatic lipase; IDL, intermediate density lipoproteins; LPL, lipoprotein lipase; $NaDodSO_4$, sodium dodecyl sulfate; PL, phospholipids; TG, triglyceride.

The Journal of Clinical Investigation, Inc.
Volume 77, February 1986, 520–527

trifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) and a Ti-50 rotor at densities of 1.006, 1.019, 1.063, and 1.120 g/ml to obtain chylomicron plus VLDL, intermediate density lipoproteins (IDL), LDL_2, and HDL_2, respectively (27). HDL_3 lipids were quantitated in the $d > 1.12$ g/ml infranatant. Chylomicron isolation was performed with a SW 40 Ti (Beckman Instruments, Inc., Fullerton, CA) (28) at 26,000 rpm for 30 min at 4°C. Chylomicrons that concentrated in a layer at the top of the centrifuge tube were removed by pipetting. The chylomicrons were resuspended and reisolated as outlined above. VLDL were isolated at $d < 1.006$ g/ml after the separation of chylomicrons.

Plasma lipoproteins were analyzed by rate zonal ultracentrifugation as previously described (29, 30). VLDL subfractions were isolated from whole serum while analysis of LDL and HDL were performed on the $d > 1.006$ g/ml infranate obtained by preparative ultracentrifugation. The effluent from the zonal rotor was monitored continuously at an absorbance of 280 nm, and 25-ml fractions were collected. The flotation properties (S_f) of the lipoproteins isolated by zonal ultracentrifugation were calculated on the basis of the zonal rotor calibration as reported (30). Cholesterol was quantitated directly in the effluent from the zonal rotor (31), appropriate lipoprotein fractions were pooled, and dialyzed against 100 mM NaCl containing 1 mM EDTA (pH 7.6) and 1 mM NaN_3. The isolated fractions were concentrated by ultrafiltration in Amicon cells.

Triglycerides (32), cholesterol (33), and phospholipids (34) were quantitated by standard procedures using buffers and enzymes obtained from Boehringer Mannheim Diagnostics, Mannheim, West Germany. Cholesterol esters were calculated from: (total − free cholesterol) × 1.68. Electrophoresis in agarose gel was performed by the method of Seidel et al. (35).

Apolipoprotein characterization. Apolipoproteins were qualitatively analyzed by 7.5% polyacrylamide gel electrophoresis in 8 M urea (pH 8.4) (36) and by analytical 3.5% $NaDodSO_4$ gel electrophoresis (37). Immunodiffusion (38) was performed with monospecific antibodies to apo A-I, apo B, and apo C-II. Radioimmunoassay of apo C-II was performed as previously described (39). Apo A-I and apo B were quantitated by rocket immunoelectrophoresis by the procedure of Laurell (40) as modified by Curry et al. (41). Two-dimensional gel electrophoresis was performed as recently reported (42) and immunoblotted by the procedure of Towbin et al. (43). Analytical isoelectrofocusing was performed as described by Marcel et al. (44).

Electron microscopic analysis of lipoproteins was carried out as previously reported (45). Analytical ultracentrifugation was performed on fresh samples (<48 h) as described (46).

Lipoprotein lipase and hepatic lipase activities were quantitated by the method of Greten et al. (47). The assay for lipoprotein lipase was performed with and without the addition of 100 μl of normal plasma containing apo C-II.

Infusion of normal plasma and synthetic apo C-II fragment. 4 wk before the study as well as during the entire experimental period, patients consumed a standardized Italian diet (20% protein, 50% carbohydrates, 30% fat). 300 ml of cross-matched normal human plasma were infused in both patients during a 30-min period. Blood samples were collected in 0.01% EDTA before the infusion, thereafter every 3 h for 3 d, then twice a day (8:00 a.m. and 8:00 p.m.) for the following 24 d. The study was begun at 8:00 p.m. after the consumption of a meal at 6:00 p.m. Plasma triglycerides and cholesterol were determined at all time points while lipoprotein isolation and characterization were performed before study and on the 12- and 36-h samples.

18 mg of a purified biologically active synthetic peptide of apo C-II (residues 44–79) dissolved in 50 ml of sterile saline were infused in one patient with apo C-II deficiency utilizing a protocol identical to that outlined above for the infusion of normal plasma. The synthesis and characterization of the apo C-II synthetic peptide has been previously reported (13).

Results

Clinical history. S.A. and S.F. were the products of a nonconsanguineous marriage and were of normal birth weight. Neonatal development was normal, and both subjects were asymptomatic during adolescence on a normal Italian diet. S.A., a forest ranger, was married at age 28 yr and has no children due to azospermia. As a young adult S.A. consumed a normal Italian diet and drank ~2 liters of wine per day. The patient had his first attack of abdominal pain associated with nausea and vomiting at the age of 27 yr. Repeated attacks occurred in association with heavy meals and drinking over the next few years. During attacks the patient occasionally developed eruptive xanthomas. As a result of these attacks, the patient at age 33 yr began on his own initiative to restrict dietary fat as well as alcohol intake and to use analgesic drugs. At age 34 yr the patient was hospitalized because of increased frequency of episodes of abdominal pain. The patient was found to have plasma triglycerides and cholesterol levels of 5,000 and 450 mg/dl, respectively, and the patient was diagnosed as having type V hyperlipoproteinemia. Because of mild obesity the patient was placed on a carbohydrate-restricted hypocaloric diet. During the next 2 yr the patient lost 21 kg; however, triglyceride levels remained greater than 2,500 mg/dl. The patient was treated with several hypolipidemic drugs without effect, and at age 37 yr the patient was evaluated at the Lipid Clinic at the University of Padua, Padova, Italy. A diagnosis of type I hyperlipoproteinemia was established based on clinical history, elevated plasma triglycerides and cholesterol, agarose lipoprotein electrophoresis, and lack of change of the lipoprotein pattern on electrophoresis after heparin injection. The patient was placed on a 20 g/d fat diet supplemented with medium-chain triglycerides. At age 38 yr, the patient's plasma lipoprotein and heparin lipase activities were normal when assayed in the presence of apo C-II contained in normal plasma.

S.F., a housewife, has been entirely asymptomatic and was diagnosed by family screening at age 33 yr to have plasma triglycerides of 2,000 mg/dl and a type I lipoprotein phenotype. She has been asymptomatic with plasma triglycerides of ~2,500 mg/dl up to the present. The patient has two living healthy children; however, she had two spontaneous abortions, cause unknown, in the third and fourth months of gestations.

At age 38 and 36 yr, respectively, the two patients were diagnosed as having apo C-II deficiency. Both patients have had persistent hepatosplenomegaly over the last 5–7 yr. Renal, liver, hematologic, endocrine, and respiratory function were normal in both patients. S.A. had azospermia and testicular biopsy revealed mild atrophy of the germinal line; no obstruction of the vas deferens was demonstrated. S.F. had a single gallstone visualized by oral cholecystography and by abdominal radiography. Electromyographic analysis of both patients revealed mild abnormalities of both motor and sensory velocities in the ulnar and peroneal nerves in the extremities. There was no evidence of cardiovascular disease in either patient when evaluated by exercise testing, digital plethysmography, and Doppler ultrasound of the extracranial vessels.

Lipid and lipoprotein analyses. The plasma lipids of the two patients with apo C-II deficiency are shown in Table I. Plasma triglycerides were elevated, whereas cholesterol and phospholipids were normal.

The plasma lipoproteins in these patients were analyzed by preparative ultracentrifugation (Table I). The majority of the elevated plasma triglycerides was present in chylomicrons; however, VLDL were also elevated. LDL (d = 1.019–1.063 g/ml) and HDL ($d > 1.063$ g/ml) were reduced; the HDL_2 subfraction was extremely low. Electrophoresis of the isolated chylomicron fraction revealed lipoproteins at the origin and in the prebeta zone, and electron micrographs of these lipoproteins contained particles from 300 to 1,000 A in diameter.

Table I. Plasma Lipids and Lipoproteins in Patients with Apo C-II Deficiency

Subject		Plasma lipids	Plasma lipoproteins						
			Chylomicrons*	Chylomicrons + VLDL‡	VLDL‡	IDL‡	LDL‡	HDL_2‡	HDL_3‡
		mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
S.A.	CT	195	115	139	24	3	14	4	11
	TG	1,625	1,271	1,537	266	6	20	<1	<1
	PL	230	109	151	42	<1	27	4	39
S.F.	CT	183	88	106	18	2	24	8	14
	TG	1,633	1,314	1,490	176	5	25	<1	<1
	PL	283	87	133	46	<1	31	8	40

CT, cholesterol; PL, phospholipids; TG, triglycerides. * Chylomicrons separated in a SW 40 Ti rotor (see Methods). ‡ Plasma lipoproteins separated by sequential preparative ultracentrifugation in a 60 Ti rotor (see Methods).

Analytical ultracentrifugal analysis of the plasma lipoproteins in the two apo C-II deficient patients is illustrated in Fig. 1. The marked increase in plasma triglycerides is reflected in the increase in the lipoproteins in the S_f 20–400 range (Fig. 1). Heterogeneity of the lipoproteins within the LDL density region (1.019–1.063 g/ml) is present in both patients with two major peaks at S_f 4 and 10. HDL_3 was the only HDL subfraction in S.A.; however, a small HDL_2 peak in addition to HDL_3 was present in S.F.

Plasma lipoproteins were also analyzed by zonal ultracentrifugation (Fig. 2 and Table II). There was a major increase in the $S_f > 200$ lipoproteins including chylomicrons and in the S_f 20–200 lipoproteins, consistent with an elevation of VLDL (Table II). A separate peak of IDL was observed, and two distinct lipoprotein peaks were present in the 1.019–1.063 g/ml density fraction, which were designated LDL_2 and LDL_3, respectively. LDL_2 is the predominant peak in normal subjects. HDL was present as a broad peak of HDL_3 in S.A. while the HDL of S.F. contained HDL_3 and a very small HDL_2 fraction (Fig. 2).

The protein and lipid percent composition of the lipoproteins separated by zonal ultracentrifugation are tabulated in Table II. Lipoproteins of $S_f > 200$ contained more triglycerides and less cholesterol esters when compared with these lipoproteins isolated from normal subjects (29–32). LDL_2, LDL_3, and HDL were also enriched in triglycerides. LDL_3 contained an increased and decreased percentage of protein and lipid, respectively, when compared with LDL_2 separated from normal subjects (29, 48).

Apolipoprotein characterization and lipase activities in patients with apo C-II deficiency. Apo A-I and apo B were in the lower range of normals, and apo A-II was normal in the apo C-II deficient patients (Table III). Apo C-II by radioimmunoassay was 0.13 mg/dl and 0.12 mg/dl in the male and female proband, respectively. Apo C-II was not detected by radioimmunodiffusion or urea polyacrylamide gel electrophoresis. Lipoprotein lipase activity assayed following heparin injection was not detectable but was normal when assayed in the presence of normal plasma containing apo C-II (Table III). Hepatic lipase activity was normal to increased in the apo C-II-deficient patients.

Two-dimensional gel electrophoresis followed by immunoblot with a monospecific antibody to apo C-II of normal VLDL and VLDL from one of the apo C-II deficient probands

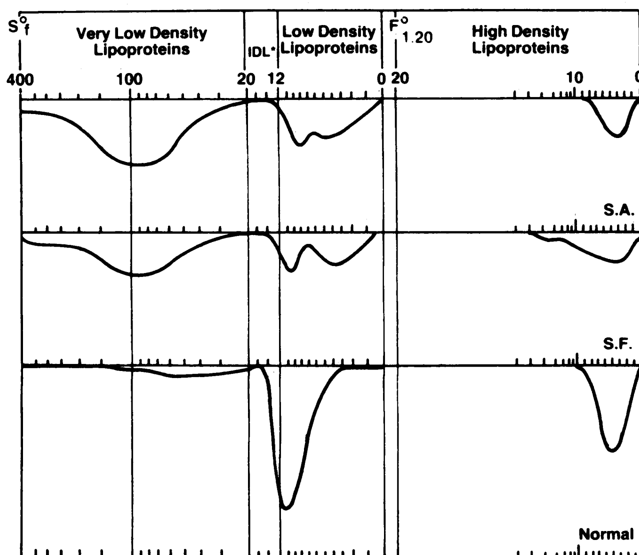

Figure 1. Computer-generated graphic representation of S_f 0–400 (*left*) and $F_{1.20}$ (*right*) plasma lipoprotein spectra of S.A. (*top*), S.F. (*middle*), and normal subject (*bottom*) separated by analytical ultracentrifugation. Plasma cholesterol and triglycerides of S.A. were 1,720 and 252 mg/dl, and those of S.F. were 1,440 and 226 mg/dl, respectively.

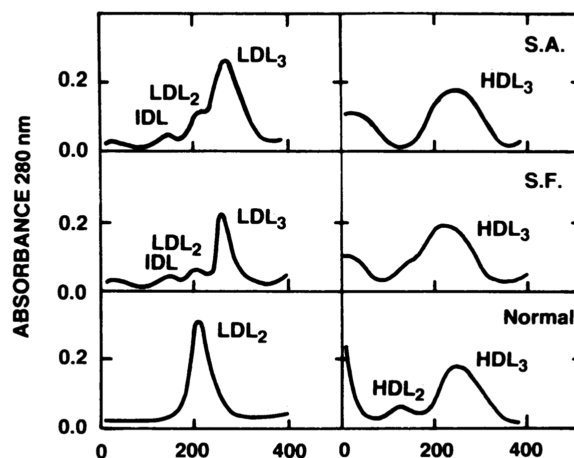

Figure 2. Absorbance profile at 280 nm of the zonal rotor effluent in S.A. (*top*), S.F. (*middle*), and in a normal subject (*bottom*). *Left,* profiles of the zonal ultracentrifugation of the fractions of $d > 1.006$ g/ml centrifuged for LDL analysis (90,000 *g,* 140 min, 15°C, linear gradient in the density range 1.00–1.30 g/ml); *right,* profiles of the HDL analysis (90,000 *g,* 22 h, 15°C, step gradient in the density range 1.00–1.40 g/ml). The direction of flotation is from the right.

Table II. Lipoprotein Composition of Plasma Lipoproteins Isolated by Zonal Ultracentrifugation in Patients with Apo C-II Deficiency

			Percent composition of lipoprotein fraction				
Subject	Lipoprotein fraction	Total cholesterol	Free CT	CT esters	TG	PL	Protein
		mg/dl					
S.A.	Whole serum	255					
	VLDL $S_f > 200$	86	5	5	76	10	4
	S_f 100–200	43	5	6	68	13	8
	S_f 60–100	29	6	6	57	19	12
	S_f 20–60	43	7	5	55	18	15
	IDL	10	14	22	20	22	22
	LDL_2	19	12	28	10	17	33
	LDL_3	33	8	31	12	13	36
	HDL_3	11	4	10	8	22	56
S.F.	Whole serum	339					
	VLDL $S_f > 200$	144	5	6	80	7	2
	S_f 100–200	97	7	4	79	8	2
	S_f 60–100	37	6	4	68	13	8
	S_f 20–60	34	7	5	67	13	8
	IDL	5	N.D.*	N.D.*	N.D.*	N.D.*	N.D.*
	LDL_2	9	13	19	19	19	30
	LDL_3	14	8	17	23	15	37
	HDL_3	14	4	10	14	23	49

* N.D., not determined because the quantity of the samples was not sufficient for a complete chemical analysis.

is illustrated in Fig. 3. No apo C-II could be detected in the VLDL of the apo C-II-deficient patient by Coomassie Blue protein staining. However, by immunoblot an apo C-II variant, designated apo $C\text{-}II_{Padova}$, with apparent lower molecular weight and more acidic isoelectric point could be detected (Fig. 3). Analysis by immunoblot of a mixture of VLDL from the apo C-II-deficient proband and a normal subject readily showed the difference in electrophoretic position of normal apo C-II and the apo $C\text{-}II_{Padova}$ variant (Fig. 3).

Infusion of plasma apo C-II. The infusion of 300 ml of normal plasma containing ~15 mg of apo C-II in the two apo C-II-deficient patients was associated with a significant reduction in plasma triglycerides within the first 12 h, which persisted for 4 d with a gradual return to preinfusion levels at ~10–12 d (Fig. 4). Both subjects exhibited a similar response to the infusion, and the lowest triglyceride level in S.F. was 200 mg/dl after 18 h, and in S.A. was 300 mg/dl after 38 h. Meals were consumed after 12, 16, 23, 36, 40, 47, 60, 64, and 71 h after the plasma injection, and the fluctuations in the triglyceride levels observed in the curve depicted in Fig. 4 in part may be related to meal consumption.

Quantification of the lipoproteins, lipids, and apolipoproteins during the first 36 h after the infusion revealed a significant reduction in plasma triglycerides within chylomicrons (1,272 to 39 mg/dl) with little change in VLDL triglycerides (Fig. 5 *A*). There was a two to threefold increase in the apo B and cholesterol within LDL (d = 1.019–1.063 g/ml) with only minimal changes in the phospholipids and triglycerides (Fig. 5 *B*).

The effect of plasma infusion on post-heparin lipoprotein lipase activity was assessed by agarose electrophoresis of plasma lipoproteins 12 h after the infusion (Fig. 6). A significant increase after heparin injection in lipoproteins within the prebeta and beta lipoproteins as well as the appearance of a typical free fatty acid albumin band in the pre-alpha position was observed in the electrophoretogram performed after heparin injection 12 h after the infusion. These results were interpreted as indicating that the infusion of normal plasma contained a cofactor(s) for lipoprotein lipase that resulted in lipolysis of triglyceride-rich lipoproteins after heparin injection.

Infusion of synthetic apo C-II. To definitely establish that the hypertriglyceridemia associated with apo C-II deficiency could be corrected by apo C-II alone, an infusion of 18 mg of a

Table III. Plasma Apolipoproteins and Lipase Activities in Patients with Apo C-II Deficiency

	Apolipoproteins				Lipase activity	
Subject	Apo A-I	Apo A-II	Apo B	Apo C-II	LPL	HL
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*μmol/ml/min*	*μmol/ml/min*
S.A.	84	30	78	0.13	6.4	32.3
S.F.	95	34	77	0.12	4.5	17.4
Controls (n = 50)	117±17*	27±4*	85±19*	5.2±2.5‡	6±2	17±7

HL, hepatic lipase; LPL, lipoprotein lipase. * Values are means±SD. ‡ Normal range included in reference 39.

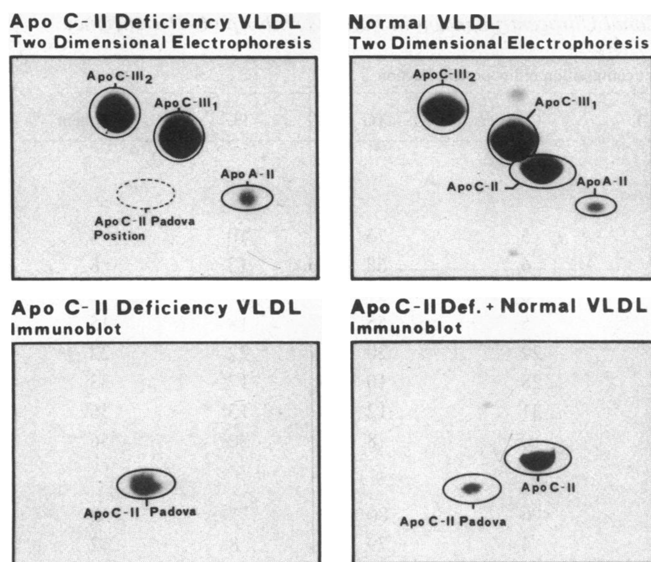

Figure 3. Two-dimensional gel electrophoretogram and immunoblot of VLDL from a normal subject and patient with apo C-II deficiency. *Top* panel contains electrophoretogram of apo C-II-deficient VLDL (180 μg protein) and normal VLDL (80-μg protein) stained with Coomassie Blue protein stain. An immunoblot of apo C-II-deficient VLDL (250-μg protein) and a mixture of apo C-II-deficient VLDL (180 μg protein) + normal VLDL (10 μg protein) are shown on the left and right in the lower panel, respectively. The location of the apo $C\text{-}II_{Padova}$ variant is indicated by the dashed circle in the electrophoretogram of the apo C-II-deficient VLDL.

biologically active synthetic fragment of apo C-II (residues 44–79) was performed in one apo C-II-deficient patient. After infusion the plasma triglyceride level decreased by >50% after 12 h and by 67% after 36 h (Table IV). The plasma triglyceride levels returned to preinfusion levels between days 13–20. In this study the triglycerides did not decrease to normal levels and

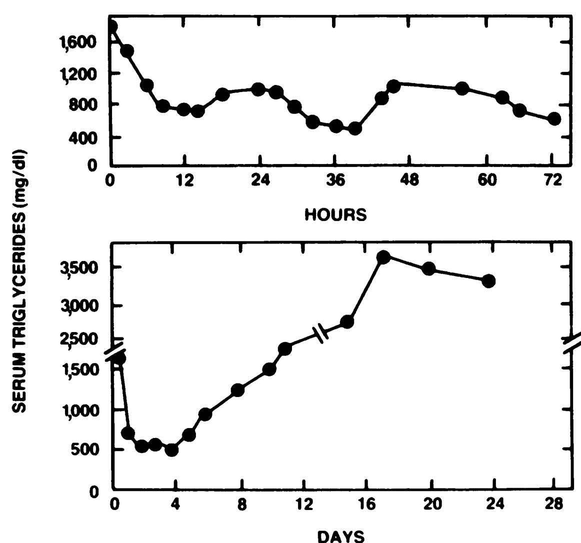

Figure 4. Serum triglycerides after infusion of 300 ml of normal human plasma in a patient (S.A., male) with apo C-II deficiency. The upper panel presents the results during the first 72 h and the lower panel contains the fasting triglyceride levels obtained over the total 24-d period.

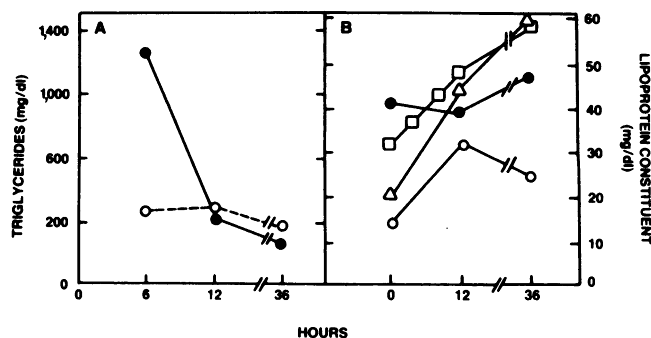

Figure 5 (*A*) chylomicrons (●) and VLDL-triglycerides (○) (mg/dl) in the density fraction < 1.006 g/ml before and 12 and 36 h after infusion of normal human plasma. (*B*) phospholipids (●), total cholesterol (△), triglycerides (○), and apoprotein-B (□) within the density range 1.019–1.063 g/ml before, 12, and 36 h after infusion of normal human plasma.

chylomicrons did not completely disappear from plasma samples. No change was observed in the lipoprotein constituents within LDL during the infusion of synthetic apo C-II.

Discussion

The clinical syndrome of apo C-II deficiency is characterized by elevated plasma levels of triglycerides, cholesterol, chylomicrons, VLDL, and reduced levels of LDL as well as HDL (14). The available data on the clinical features and lipoprotein analyses are variable in apo C-II-deficient patients, which indicates apparent heterogeneity in the severity of the clinical syndrome. The diagnostic biochemical feature of this syndrome is the deficiency of plasma apo C-II. To date only limited data are available on the plasma lipoproteins and apolipoproteins in these patients.

The clinical features of the two apo C-II patients described in this report that appear to be at variance with previous reports (14–19, 21, 22) include persistent hepatosplenomegaly in both

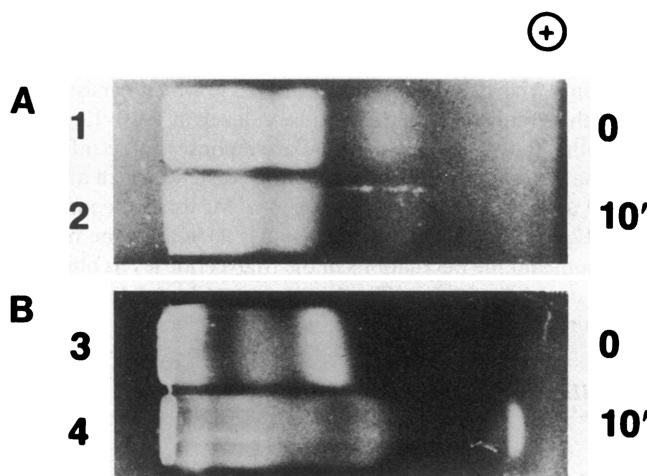

Figure 6. Whole serum lipoprotein electrophoresis in agarose before (1, 3) and 10 min after (2, 4) injection of 100 U/kg of Na-heparin in a patient with apo C-II deficiency (S.A.): 1 and 2 contain the electrophoretograms performed before the plasma infusion; 3 and 4 contain the electrophoresis performed 12 h after the plasma infusion. In panel 4, the mobility of the lipoprotein bands as well as the appearance of a pre-alpha migrating band corresponding to free fatty acids bound to albumin may be observed.

Table IV. Triglycerides in Whole Serum, Chylomicrons, and VLDL Before and After Infusion of a Synthetic 44–79 Residue Fragment of Apo C-II in a Patient (S.A., male) with Apo C-II Deficiency

Sample	Days after infusion					
	0	0.5	1.5	4	12	20
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
Whole serum	3,100	1,360	1,045	1,575	1,900	3,000
Chylomicrons	2,614	974		1,077		
VLDL	341	213		292		

patients and the virtual absence of symptoms with only minor dietary restriction in fat in the female proband. The male patient has had recurrent bouts of abdominal pain and eruptive xanthomas due to lapses in dietary fat restriction. He has minimal symptoms if he adheres to a low fat diet. The mild peripheral neuropathy in both patients and the single gallstone as well as the azospermia in the individual patients are difficult to ascribe to the deficiency of apo C-II. Of particular interest in this kindred as well as in the previously reported kindreds (14–19, 21, 22) is the absence of significant atherosclerosis despite severely elevated plasma triglyceride.

The plasma lipids and lipoproteins isolated by preparative ultracentrifugation were similar to previous reports (15–19, 21, 22). >90% of the plasma triglycerides were in the $d < 1.006$ g/ml density fraction. The other major classes of lipoproteins including LDL and HDL were reduced in concentration in plasma and had a change in composition with a relative increase in triglyceride content. Plasma apo A-I and apo B are low normal, which agrees with data reported by other authors (24).

Characterization of the lipoproteins by zonal ultracentrifugation revealed an increase in plasma chylomicrons and, in addition, increased VLDL of S_f 100–200, 60–100, and 20–60. Therefore, there was an elevation of triglyceride-rich lipoproteins including chylomicrons as well as VLDL, the latter which varied in size from relatively small to very large particles. HDL were reduced in concentration, and HDL_3 was the predominant HDL subfraction.

Of particular interest in the zonal ultracentrifugal analysis of the plasma lipoproteins was the presence of two major subfractions within LDL. The major LDL fraction was LDL_3, which has a lower hydrated density than the LDL_2 present in normal subjects (29, 31, 48). Increased LDL_3, with an increased protein-to-lipid ratio when compared with the predominant LDL_2 fraction of normal subjects, has been observed in patients with type V hyperlipoproteinemia (48) and in type I hyperlipoproteinemia due to lipoprotein lipase deficiency (49). The presence of LDL with an increased hydrated density seems to be characteristic of disorders of triglyceride metabolism and may represent a small remnant of triglyceride-rich lipoproteins the composition of which may be due to the action of plasma lipid exchange proteins or to defective lipolysis. Alternatively, it may represent a lipoprotein particle synthesized directly from the liver or intestine and independent of the lipoproteins secreted into VLDL and converted to LDL.

The infusion of normal plasma into the patients with apo C-II deficiency resulted in a prompt reduction in plasma triglycerides and chylomicrons. A two- to threefold increase in apo B and cholesterol occurred in LDL; however, the majority of chylomicrons and VLDL particles were not converted to LDL. An increase in <1 mg/dl in LDL cholesterol could be accounted for by the infusion of 300 ml of normal plasma. This result is similar to the results reported by Stalenhoef et al. (50) in which large triglyceride-rich lipoproteins isolated from patients with lipoprotein lipase deficiency were injected into normal subjects, and only a few percent of these lipoprotein particles were converted to LDL.

To definitely establish that apo C-II alone could correct the metabolic defect in apo C-II deficiency, a biologically active synthetic peptide of apo C-II (13) was injected in one apo C-II-deficient patient. A prompt reduction in plasma triglycerides occurred, and the reduction in triglycerides persisted for up to 20 d. The triglycerides did not normalize with the injection of 18 mg of the synthetic apo C-II fragment (equivalent to approximately threefold more micromoles of apo C-II than were present in the 300 ml of plasma utilized in the infusion study), which may indicate that the synthetic fragment does not contain the full biological activity of the full-length native apo C-II or that the synthetic fragment may not have the same lipid-binding properties and/or metabolism as normal apo C-II.

These results provide important documentation that a deficiency of apo C-II is responsible for the hypertriglyceridemia associated with the apo C-II deficiency syndrome. No additional cofactor(s) is required. The dramatic response of patients with apo C-II deficiency to injected apo C-II, and the prolonged reduction in triglycerides associated with treatment, suggest potential replacement therapy in these patients may be possible.

Recent studies have established that the molecular defect in patients with apo C-II deficiency is heterogeneous and may be responsible for the variability in clinical manifestations. In the present report, a variant of apo C-II, apo $\text{C-II}_{\text{Padova}}$, was identified, which is of apparent lower molecular weight and more acidic isoelectric point. The apo $\text{C-II}_{\text{Padova}}$ variant is present in extremely low plasma concentrations. A different apo $\text{C-II}_{\text{Bethesda}}$ variant has been identified in a separate kindred with apo C-II deficiency (51). Recently, Maguire et al. (52) reported the characterization of two mutant forms of apo C-II (apo C-IIX and apo C-IIY) in the original kindred reported with apo C-II deficiency (17, 18). The mutant apolipoproteins are present at near normal concentrations in plasma; however, they were nonfunctional and unable to activate lipoprotein lipase (52).

The combined information on the cloning and processing of apo C-II (4–6), and the identification of several variant forms of apo C-II in patients with apo C-II deficiency, provide new insight into structure and physiological function of this unique apolipoprotein in lipoprotein metabolism. Improved diagnostic methods as well as more definitive therapy of patients with defects in apo C-II function will now be possible.

Acknowledgments

The authors wish to thank Dr. A. M. Gotto and Dr. J. Sparrow (Baylor College of Medicine, Methodist Hospital, Houston, TX 77030) for supplying the 44–79 residue synthetic apo C-II fragment.

This work was supported by grants from Progetto Finalizzato National Council of Research: Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie; Sottoprogetto Basi Molecolari delle Malattie Ereditarie.

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