Identification and metabolic characteristics of an apolipoprotein C-II variant isolated from a hypertriglyceridemic subject

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Abstract The very low density lipoprotein (VLDL) apolipoproteins from a Type IV hypertriglyceridemic Caucasian subject (plasma TG: 645 mg/dl) and his brother (plasma TG: 328 mg/dl) were separated by isoelectric focusing gel electrophoresis (IEF) and found to contain two isoforms of apoC-II, identified by immunoblot. These corresponded to normal apoC-II-1 (isoelectric point: pI 4.88) and a variant isoform (apoC-II-v, pI 4.74). The pI of C-II-v was not altered by neuraminidase treatment, indicating that it was not sialylated. The concentration of total immunoreactive C-II in VLDL was elevated (18 mg/dl vs normal; 5.0 ± 2 mg/dl) but similar to that in other Type IV subjects. In VLDL, which contained 90% of the plasma immunoreactive apoC-II, the ratio (by IEF) of C-II-1:C-II-v was 2:1, whereas in high density lipoproteins (HDL) the ratio was 1:1. VLDL apoB turnover was measured after the pulse injection of ^{125}I-labeled VLDL. VLDL apoB kinetic parameters for the proband and four Type IV subjects were similar: production rate, 28 mg/kg per day versus 30 mg/kg per day; fractional catabolic rate, 1.62 · day^{-1} versus 1.96 · day^{-1}; and pool size, 17 mg/kg versus 18 mg/kg. The decline in VLDL triglyceride (TG) after the infusion of heparin (9,000 IU over 4 h) was also similar to that observed in Type IV subjects. In VLDL, the fractional catabolic rates of apoC-II-1 and C-II-v were similar (C-II-1: 0.31 · day^{-1}, C-II-v: 0.29 · day^{-1}) whereas in HDL, although similar to each other, the rates were greater than in VLDL (C-II-1: 0.48 · day^{-1}, C-II-v: 0.44 · day^{-1}). VLDL and HDL from the proband were normal in their ability to activate bovine skim milk lipase, compared to Type IV VLDL and HDL without C-II-v. Purified apoC-II-1 and apoC-II-v activated the milk lipase to a similar extent (at 1 μg of C-II; C-II-1: 34 units/h, C-II-v: 35 units/h). Thus, apoC-II-v is a newly recognized isoform of apoC-II-1. It remains to be determined whether this mutation plays a role in the genesis of hypertriglyceridemia. —**Huff, M. W., A. J. Evans, B. M. Wolfe, P. W. Connelly, G. F. Maguire, and W. L. P. Strong.** Identification and metabolic characteristics of an apolipoprotein C-II variant isolated from a hypertriglyceridemic subject. *J. Lipid Res.* 1990. **31:** 385–396.

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The C apolipoproteins in human plasma are designated apolipoprotein C-I, C-II, C-III$_0$, C-III$_1$, and C-III$_2$ (1). These are well characterized small molecular weight proteins (6,000–9,000) that play an important role in the metabolism of triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins). ApoC-II is required for activation of lipoprotein lipase, an enzyme that hydrolyzes the triglyceride of chylomicrons and VLDL (2, 3). This enzyme is normally bound to endothelial cell surfaces but can be released by heparin administration resulting in a rapid clearance of VLDL triglyceride (4).

The function of apoC-III is not fully understood, although a low ratio of C-II/C-III has been demonstrated in hypertriglyceridemic subjects (5) and a low C-II/C-III ratio in triglyceride-rich lipoproteins has been shown to inhibit lipoprotein lipase in vitro (6) and inhibit the hepatic uptake of these lipoproteins by perfused rat livers (7).

In vitro studies have shown that the C apolipoproteins from VLDL are rapidly transferred to HDL during lipolysis of VLDL triglyceride (8, 9). Subsequently, the C apoproteins transfer back to newly secreted nascent VLDL (8). This concept has been supported by a number of kinetic studies in vivo, in both normal and hypertriglyceridemic individuals (10, 11).

There have been a number of reports of apoC-II variants in VLDL in human subjects, some of which are associated with hypertriglyceridemia. Connelly et al. (12,

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; FPLC, Fast Protein Liquid Chromatography; IEF, isoelectric focusing; FCR, fractional catabolic rate.

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13) have reported two structural variants of apoC-II, denoted C-II-Toronto and C-II-St. Michael, which do not activate lipoprotein lipase and are associated with severe hypertriglyceridemia. Other apoC-II variants have been described which also do not activate lipoprotein lipase (14, 15). Havel, Kotite, and Kane (16) and Menzel et al. (17) have described a C-II variant in a subject of African descent that possesses normal lipoprotein activation potential in vitro.

In this study we report the isolation, metabolism, and lipoprotein lipase activation potential of an apoC-II variant present in VLDL in a hypertriglyceridemic Caucasian subject. In addition, we have assessed the effect of this variant on the in vivo metabolism of VLDL apoB as well as the response of VLDL triglyceride to an infusion of heparin.

METHODS

Clinical data

The proband, a 44-year-old male, was referred to the Lipid Clinic, University Hospital in 1982 for hypertriglyceridemia. Subsequent lipoprotein analysis indicated a Type IV pattern as shown in **Table 1**. At the time of study the subject was not taking any lipid-lowering drug. Fasting serum glucose, serum alkaline phosphatase, serum gamma GT, serum glutamic oxaloacetic transaminase, and thyroxine levels were normal; urine analyses were also normal. To allow for comparison of the proband with other subjects with Type IV hyperlipidemia but without the apoC-II variant, seven male subjects (ages 32–66 years), with the Type IV lipid phenotype were also studied. All subjects were asked to avoid alcohol 1 week prior to study. One subject had a mild elevation of fasting serum glucose (6.45 mM) and a moderate elevation of serum gamma GT (68 units/l). Serum alkaline phosphatase and thyroxine levels were normal and urine analyses were normal. Thyroid indices were normal, and no subjects were taking any lipid-lowering drug during the course of study. Baseline lipid values are reported in Table 1. The proband's brother, age 41, was referred to the Lipid Clinic at University Hospital in 1986 for mild hypertriglyceridemia, which was found to be a Type IV pattern (Table 1). Serum and urine chemistry were found to be normal.

The in vivo studies were approved by the University of Western Ontario Health Sciences Standing Committee on Human Research and informed consent was obtained.

Plasma lipids and lipoproteins

The plasma lipid and lipoprotein profile was determined on plasma obtained after a 16-h fast. Plasma was ultracentrifuged through buffered saline (0.195 M NaCl; 1 mM Tris, pH 7.4, 1 mM EDTA, 3 mM NaN_3, 0.1 mM merthiolate, and 10 μM phenylmethane sulfonyl fluoride) in a Beckman Ti 50.3 rotor, in a Beckman L8-70 ultracentrifuge, for 18 h, at 40,000 rpm, 12°C, to isolate VLDL (S_f 20–400) (18). The cholesterol concentration of LDL and HDL was determined after precipitation of the infranatant with dextran sulfate-magnesium chloride (19). The cholesterol and triglyceride concentrations of plasma and the lipoprotein fractions were determined enzymatically (Boehringer-Mannheim, Montreal Quebec, cholesterol: CHOD-PAP, triglyceride: TG without free glycerol).

Plasma lipoproteins were also fractionated by sequential ultracentrifugation at 12°C in a Beckman Ti 50 rotor as follows: VLDL (S_f 60–400), 2 h, 40,000 rpm; IDL, (S_f 12–60), 16 h, 40,000 rpm; LDL, (S_f 0–12), 18 h, 50,000 rpm; and HDL (d 1.063–1.21 g/ml) 28 h, 50,000 rpm (18). Lipoprotein protein content was determined by a modification of the method of Lowry (20).

TABLE 1. Plasma and lipoprotein cholesterol and triglyceride concentrations in subjects with an apoC-II variant

		Proband	Brother	Type IV (n = 7)[a]
		mg/dl		
Triglyceride	Plasma[b]	645	328	652 ± 87
Triglyceride	VLDL	497	274	440 ± 80
Cholesterol	Plasma	290	205	249 ± 20
Cholesterol	VLDL	127	49	106 ± 22
Cholesterol	LDL	137	122	107 ± 17
Cholesterol	HDL	26	35	36 ± 3
Apoprotein E phenotype		E_3/E_3	E_3/E_3	—[c]

[a]Values are mean ± SEM.
[b]Values for VLDL (S_f 20–400) were obtained after ultracentrifugation, for HDL after precipitation of plasma VLDL and LDL by dextran-sulfate/magnesium chloride, and for LDL by difference, as described in Methods.
[c]None of the Type IV subjects were homozygous for E_2/E_2.

Electrophoretic analyses of VLDL apolipoproteins

The isolated VLDL (S_f 20–400) was dialyzed against 5 mM NH_4HCO_3, lyophilized, and delipidated with chloroform–methanol–diethyl ether 2:1:5 as described previously (21). Apoproteins were solubilized in deionized 8 M urea, 10 mM Tris (pH 8.2), 10 mM dithiothreitol. Aliquots containing 150–300 μg were focused in tubes (0.8 × 10 cm) containing 7.5% acrylamide, 2% ampholine (LKB, Sweden, 4 parts pH 4–6, 1 part pH 5–7), and deionized 6.8 M urea, as described previously (21). Gels were stained with Coomassie Blue G-250 and scanned by densitometry at 600 nm using an EC corporatron densitometer. The areas under the peak of each C and E apoprotein were determined by integration (21). The apoE phenotypes were determined according to Bouthillier, Sing, and Davignon (22). Immunoblotting was carried out after transfer of the apoproteins to nitrocellulose filters and blotted with rabbit anti-human C-II as described previously (23, 24).

Two-dimensional gel electrophoresis was carried out with 50-μg aliquots of VLDL protein by performing isoelectric focusing (3-mm diameter gels) for the first dimension and sodium dodecyl sulfate-glycerol gel electrophoresis in a second dimension, as described previously (25). The gels were either stained with Coomassie Blue G-250 or the proteins were transferred to nitrocellulose filters and immunoblotted with rabbit anti-human apoC-II as reported previously (23, 24).

The apoproteins were tested for the presence of sialic acid by treatment with neuraminidase by a method similar to that described previously (26). Delipidated VLDL apoproteins (400-μg aliquots) were dissolved in 200 μl of a buffer consisting of 0.1 M KH_2PO_4 (pH 4.5), 1 mM calcium chloride, and 2 U of neuraminidase V (*Clostridium perfringens*; Sigma). The pH was adjusted to 5.0 with HCl. The samples were incubated with shaking for 4 h at 37°C, dialyzed against 5 mM NH_4HCO_3, 2 mM EDTA, lyophilized, and analyzed by analytical isoelectric focusing as described above.

Electroimmunoassay of apoC-II

The concentration of apoC-II was determined by electroimmunoassay using modifications to the Laurell procedure (27) as reported previously (18, 28). Details of the standardization of this assay have been reported previously (18). Values were unaffected by triglyceride content of the samples, which were assayed after the addition of Triton X-100 (0.016% final concentration). The lower limit of detection was 10 ng per 10 μl assay volume.

VLDL apoprotein B and plasma apoprotein C-II turnover study

Most of the procedures used have been described previously (10, 11, 18, 29, 30). Four days prior to study, 200 ml of blood was obtained after a 14-h fast. VLDL (S_f 60–400) was isolated by ultracentrifugation as described above, washed once through buffered saline, and labeled with ^{125}I by a modification of the iodine monochloride technique (30). Isolation and labeling techniques were performed aseptically and the preparations were filtered through a 0.45-μm Millipore filter into a sterile vial containing gentamycin sulfate (100 μg/ml). Aliquots were checked for sterility and pyrogenicity. The VLDL preparation contained less than 1% free iodine; 39% of the label was associated with apoB, 8% with apoC-II, and 5% was associated with lipid. All radioiodinated lipoprotein injections were autologous. Tracers were injected in the morning after an overnight fast and blood samples were collected over a 48-h period. During the turnover study, a low fat diet was administered (<5% of calories) to limit the contribution of intestinally derived lipoproteins and, as found previously, VLDL concentrations remained constant throughout the 48-h study (10, 11). Potassium iodide (300 mg/day), for a period of 16 days starting from 2 days before injection, was given to prevent the thyroidal uptake of radioiodine. VLDL (S_f 60–400), IDL, (S_f 12–60), LDL (S_f 0–12), and HDL (d 1.063–1.21 g/ml) were isolated from 10 ml of plasma as described above. VLDL, IDL, and LDL apoB were precipitated with isopropanol and specific activities were calculated as described previously (29). The isolation of the C apoproteins from VLDL and HDL and the determination of their specific radioactivities by a polyacrylamide gel isoelectric focusing technique have been described in detail (10). Kinetic parameters for apoB were determined from VLDL specific activity disappearance curves and parameters for apoC-II from both VLDL and HDL specific activity curves. As described previously, the apoB and apoC-II kinetics in this hypertriglyceridemic subject were best described by a monoexponential (linear) function, over the 48-h study (10). Parameters for the size of the pool, the fractional catabolic rate, and the production rate or flux through the pool were calculated by conventional techniques (31).

Heparin infusion

After an overnight fast, blood samples were drawn every 30 min for 2 h. An infusion of heparin was then administered (heparin sodium USP, Organon). For the initial 0.5 h, 2000 IU was given at a rate of 4000 IU/h (priming dose) followed by 2000 IU/h for 3.5 h. Blood samples were drawn at 0.5 h and then hourly for 6 h and again at 24 h after the initiation of the infusion. Blood samples were placed on ice, plasma was separated within 10 min, and *m*-aminophenylboronic acid (final concentration 0.4 mM) was added to inhibit lipolysis in vitro (32). VLDL was isolated from each sample by ultracentrifugation and the triglyceride content was determined as described above.

Purification of apoC-II

Apolipoprotein C-II was purified using a combination of high performance gel filtration and anion exchange chromatography and the FPLC system (Fast Protein Liquid Chromatography system, Pharmacia, Montreal Quebec) by a modification of a previously published method (33). Delipidated VLDL soluble apolipoproteins were dissolved in 0.5 ml of a buffer containing 0.01 M Tris, pH 8.6, deionized 6 M urea, which had been filtered and degassed. The sample was centrifuged at 2500 rpm for 10 min and the supernatant (approximately 1 mg of protein) was applied to a Superose 12 (Pharmacia) column (1.0 × 30 cm) previously equilibrated with the same buffer at 0.5 ml/min. Elution was monitored by following absorbance at 280 nm. One major peak was obtained at 20 min (10 ml) which contained all of the C apolipoproteins as assessed by analytical isoelectric focusing. Column fractions (up to 1 mg of C apolipoproteins) were pooled and, using the Super Loop (Pharmacia), were applied to a Mono Q (Pharmacia) anion exchange column (0.5 × 5 cm) that had been equilibrated to the Tris buffer (pump A) at a flow rate of 1.0 ml/min. A linear gradient was developed using the Tris buffer containing 1 M NaCl (pump B). Ten minutes after sample injection, the controller was programmed to change from 100% A to 80% A, 20% B over 40 min. This length of time was required to achieve resolution of the C-II variant from $C\text{-}III_0$. Elution was monitored by following absorbance at 280 nm. The run was complete by 50 min. Fractions were collected as controlled by peak deflection, using 20% of full scale as the cut off. Purity of each peak was assessed by analytical isoelectric focusing gel electrophoresis and specificity by rocket immunoelectrophoresis using antisera against human apoC-II and C-III as described above.

Tryptic digestion and isolation of apoC-II peptides by reverse-phase HPLC

Approximately 100 μg of apoC-II-1 and apoC-II-v were digested with trypsin (protein/trypsin; 50:1 wt/wt) by incubation in 0.1 M NH_4HCO_3, pH 8.2, 0.2% decyl sodium sulfate for 18 h at 37°C. HPLC was performed as described previously using a 15 × 0.46 cm Altex ODS column (particle size, 5 μm) (12).

Assay of lipoprotein lipase activation by apoC-II

Bovine skim milk lipase was obtained and partially purified by a modification of the method described by Bengtsson and Olivecrona (34). Approximately 400 ml of fresh, unpasteurized whole milk (Silverwood Dairies, London, Ontario) was centrifuged (1200 *g* for 30 min) and the skim milk infranatant was adjusted to 0.5 M NaCl, 1 mM phenyl methane sulfonyl fluoride. Heparin-Sepharose 4B, prepared as described previously (35), was added to the skim milk and the mixture was gently rocked for 2 h at 4°C. The gel, containing the bound enzyme, was collected by vacuum filtration and washed once with 300 ml of 0.005 M barbital buffer, pH 7.4, 0.5 M NaCl. The gel was then packed into a column, washed a second time with 40 ml of the barbital buffer (60 ml/h), and the enzyme activity was eluted with the barbital buffer containing 1.5 M NaCl elution at 4°C. The enzyme was stored in 50% glycerol at −20°C for at least 6 months without loss of activity.

The subjects' whole plasma, VLDL, and HDL, as well as purified native apoC-II, the apoC-II variant, and $apoC\text{-}III_1$, were tested for their ability to activate purified bovine milk lipase using a method described by Blache, Bouthillier, and Davignon (36) in which a [^{14}C]triolien-gum arabic emulsion was used as substrate. Each assay contained 1–20 μl of plasma, VLDL, or HDL from the same volume of plasma, or 0–10 μg of apoC-II or $C\text{-}III_1$; 100 μl of the substrate emulsion, 300 μl of 0.2 M Tris, pH 8.3, 0.15 M NaCl containing 15 μg fatty acid-free bovine serum albumin (fraction V, Sigma), and 50 μl of the bovine skim milk lipase (approximately 5 units of activity). Samples were incubated at 37°C for 30 min and the release of free fatty acid (as [^{14}C]oleic acid) was determined as described previously (36). One unit of activity is defined as micromoles of free fatty acid released per ml of enzyme solution per h.

RESULTS

The proband and his brother both presented with Type IV hyperlipidemia (Table 1) and both were homozygous for apoE3. No chylomicronemia was observed either following standing of the plasma at 4°C for 16 h or after ultracentrifugation ($S_f > 400$). The brother had lower values of triglyceride and cholesterol, possibly due to treatment with diet and weight reduction by the referring physician.

The apolipoprotein C-II variant in the proband was initially noted from the isoelectric focusing patterns of the C apolipoproteins as shown in **Fig. 1**. This apoC band focused with a pI between $C\text{-}III_1$ and $C\text{-}III_2$. Also shown is the apoC pattern observed in the proband's brother; and the pattern shows the presence of the same band. By comparison to isoelectric point standards, the pI of native C-II was found to be 4.88, whereas the new band was found to be 4.74. Immunoblots of the proband's isoelectric focusing gel indicated that this band reacted strongly with antisera against human apoC-II. This band showed no visible reaction with human anti-apoC-III. Thus, normal apoC-II was denoted as C-II-1, whereas the band with a pI of 4.74 was denoted apoC-II-v. The apoC-II-v was further characterized by two-dimensional

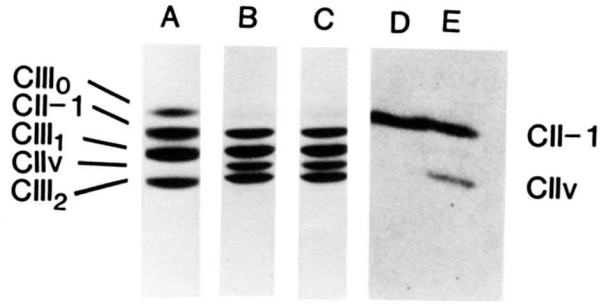

Fig. 1. Analytical isoelectric focusing gel electrophoresis of VLDL C apolipoproteins from: A, a normal subject; B, the proband; and C, the proband's brother. Immunoblot, after isoelectric focusing of VLDL C apolipoproteins, using anti-human apoC-II of a normal subject (D) and the proband (E).

gel electrophoresis with isoelectric focusing in the first dimension and sodium dodecyl sulfate electrophoresis in the second dimension as shown in **Fig. 2**. The stained electrophoretogram of apoVLDL from a normal patient and an immunoblot, using anti-human apoC-II, of the same gel are shown in panel 1. The major isoform of apoC-II, C-II-1, was identified with a pI of 4.88 and an apparent molecular weight of 8,900. A minor isoform, designated C-II-la, with a slightly more acidic pI of 4.82 and a lower apparent molecular weight of 7,800, was also identified. This isoform appears to correspond to apoC-II-1/2, as reported previously by Fojo et al. (26). The pattern of apoVLDL from the proband is shown in panel 2. The stained gel and the immunoblot show that the variant, C-II-v, has the same apparent molecular weight as apoC-II-1, but a pI of 4.74. A minor isoform with an apparent molecular weight of 7,800 and a pI of 4.68 was identified, which is probably the cleaved form of C-II-v analogous to C-II-la. Since apoC-II-v had a more acidic pI than apoC-II-1, the C apolipoproteins were treated with neuraminidase to determine whether apoC-II-v was a sialylated form of apoC-II. The change in the electrophoretic migration is shown in **Fig. 3**. ApoC-II_1 and C-III_2 now appear to co-migrate with apoC-III_0, indicating the loss of one and two sialic acid residues, respectively, however, the migration of apoC-II-v was not altered, indicating that it is not sialylated. The concentrations of immunoreactive apoC-II in plasma, VLDL, and HDL of the proband are listed in **Table 2**. Total plasma values of 18 mg/dl, of which approximately 80% was in the VLDL fraction, were increased above normal. This pattern is similar to other Type IV subjects with similar plasma and VLDL triglyceride concentrations. As determined by isoelectric focusing, the ratio of the apoC-II isoforms differed in VLDL and HDL. Approximately twice the amount of C-II-1 compared to C-II-v

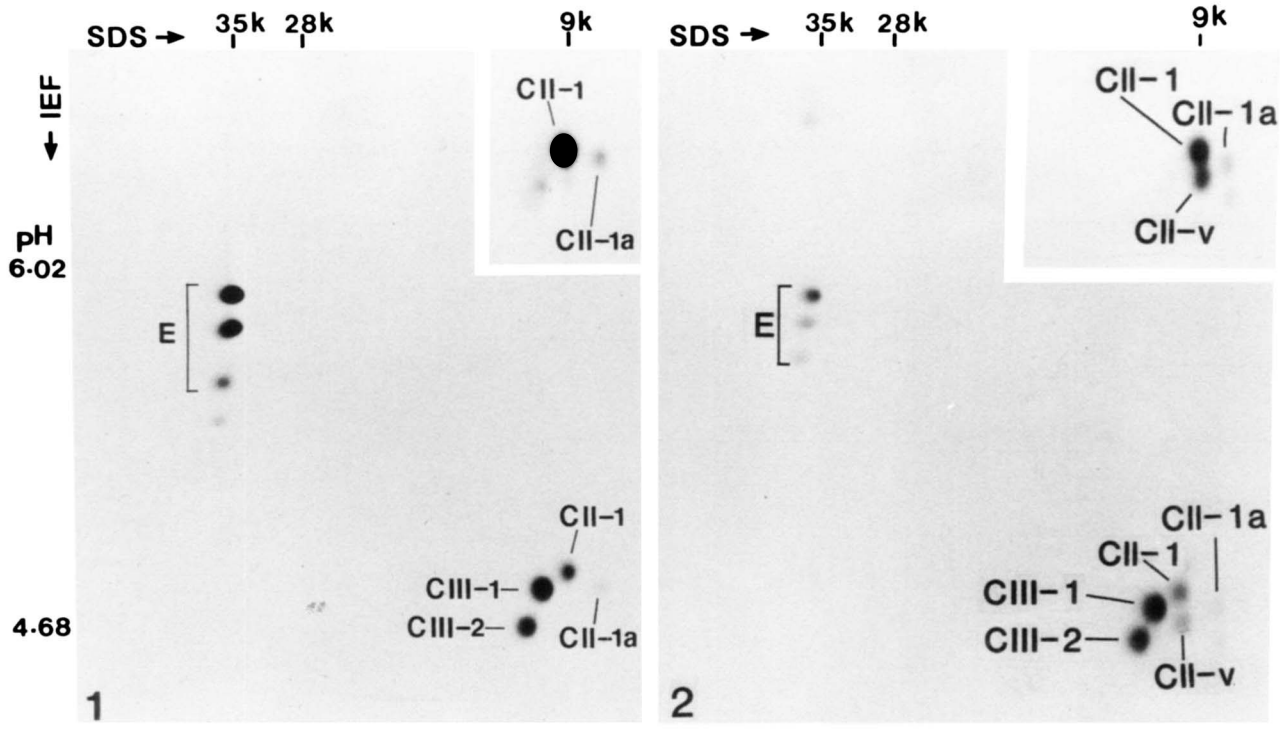

Fig. 2. Two-dimensional electrophoresis of normal apoVLDL (1) and of apoVLDL from the proband (2). The gels were stained with Coomassie Blue G-250. The insets in each panel are the results of immunoblotting with anti-human apoC-II; IEF, isoelectric focusing; SDS, sodium dodecyl sulfate.

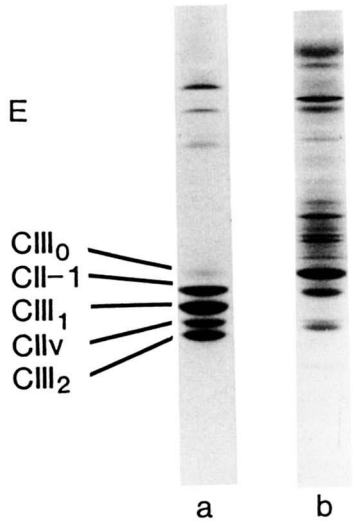

Fig. 3. Analytical isoelectric focusing gel electrophoresis of VLDL apolipoproteins after incubation without (A) and with (B) neuraminidase.

was present in VLDL, whereas the isoforms were present in about equal concentrations in HDL.

The specific activity-time curves of VLDL, IDL, and LDL apoB, after the injection of ^{125}I VLDL in the proband and a Type IV subject without C-II-v, are shown in **Fig. 4**. The decline in VLDL apoB specific activity was monoexponential in each subject. The appearance of radioactivity in IDL and subsequently LDL apoB showed no major differences between the two subjects. The kinetic parameters for VLDL apoB are shown in **Table 3**. Values for pool size (17 mg/kg), fractional catabolic rate (1.62 d^{-1}), and production rate (28 mg/kg per day) are similar to values observed in four Type IV subjects without C-II-v. Compared to normal subjects, the pool size is elevated due to a combination of both an increased production rate and a decreased fractional catabolic rate.

The specific activities of apoC-II-1 and C-II-v in VLDL and HDL after the injection of radiolabeled VLDL are shown in **Fig. 5**. All four disappearance curves were found to be monoexponential. In VLDL, the fractional catabolic rates for apoC-II-1 and C-II-v were similar; 0.29 and 0.31 d^{-1}, respectively (Table 3). These rates were similar to those observed for normal C-II-1 in other hypertriglyceridemic subjects and lower than those observed in normal subjects. The fractional catabolic rates of C-II-1 and C-II-v in HDL were also similar (0.48 and 0.44 d^{-1}, respectively), however these values were higher than those observed in VLDL, suggesting a more rapid clearance of both apoC isoforms from HDL. Production rates into plasma of apoC-II-1 and C-II-v were not determined because we were not able to measure the exact plasma concentrations of the two isoforms, and total plasma FCR could not be estimated due to the difference in FCR between VLDL and HDL for both isoforms.

The changes in VLDL triglyceride after an infusion of heparin, known to release lipoprotein lipase into the circulation and initiate rapid lipolysis, are shown in **Fig. 6**. Within 1 h of the initiation of the infusion, VLDL triglyceride had declined to 60% of initial concentrations and by the end of the infusion had declined to 30%. A similar pattern was observed in a subject, with similar VLDL triglyceride concentrations, studied at the same time. The pattern for a normal subject is also shown and it indicates the greater extent of lipolysis in this sub-

TABLE 2. Plasma and lipoprotein concentrations of immunoreactive apoC-II and lipoprotein ratios of apoC-II-1 and apoC-II-v determined by isoelectric focusing

	Immunoreactive ApoC-II[a]		
	Proband[b]	Type IV Subjects[c]	Normal Subjects[c]
		mg/dl	
Total plasma	18	16 ± 2	5 ± 1
VLDL	16	11 ± 1	2 ± 0.4
HDL	2	4 ± 1	3 ± 0.8
	Isoelectric Focusing Ratio C-II-1:C-II-v[d]		
VLDL	1.97 ± 0.07:1		
HDL	1.01 ± 0.06:1		

[a]Concentrations of apoC-II were determined by electroimmunoassay as described in Methods. VLDL (S_f 60–400) and HDL (d 1.063 ± 1.21 g/ml) were obtained by ultracentrifugation. ApoC-II was not detectable in the d > 1.21 g/ml fraction.
[b]Determined at the time of the metabolic studies.
[c]Values in Type IV subjects (n = 7) and normal subjects (n = 10) from this laboratory, some of which have been published previously (18).
[d]Based on scanning 12 gels for each lipoprotein fraction.

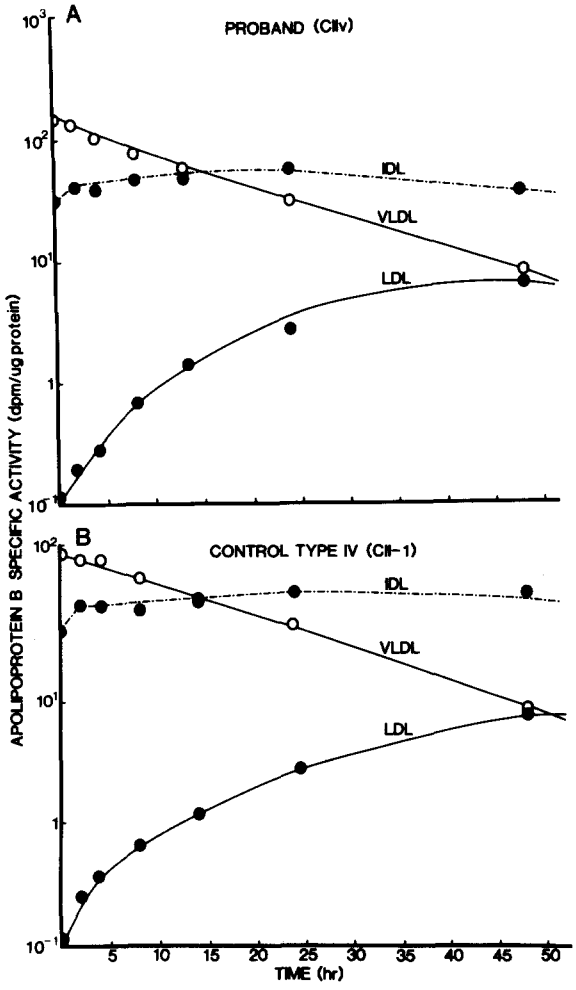

Fig. 4. Relationship between apolipoprotein B specific activity-time curves of VLDL, S_f 60–400 (○——○), IDL, S_f 12–60 (●···●), and LDL, S_f 0–12 (●——●) after the injection of ^{125}I-labeled VLDL. The top panel illustrates specific activity curves from the proband and the lower panel illustrates specific activity curves from a control hypertriglyceridemic subject.

ject. The results shown in Fig. 6 for the Type IV and normal subjects are similar to those for other Type IV subjects (= 7) and other normal subjects (n = 4), respectively, studied in this laboratory (data not shown). To determine whether the presence of apoC-II-v had any influence on the ability of VLDL or HDL to activate bovine milk lipoprotein lipase in vitro, VLDL and HDL isolated from the proband, from a hypertriglyceridemic subject with only apoC-II-1, and from a normal subject were tested as a source of activator. No differences were observed between the proband and the other two subjects for either VLDL or HDL (data not shown), with respect to the ability to activate bovine lipase.

ApoC-II-1 and apoC-II-v were isolated from VLDL of the proband by a combination of high-performance gel chromatography and anion exchange chromatography on the FPLC system. As shown in **Fig. 7**, apoC-II-1 and apoC-II-v were clearly separated from each other and from the C-III isoforms using anion exchange chromatography. These apolipoproteins were assessed for purity by analytical isoelectric focusing and found to be homogeneous as shown in Fig. 7. In addition, both apoC-II-1 and C-II-v reacted only with anti-human apoC-II and not anti-apoC-III in the electroimmunoassay (data not shown). The purified apoC-II-1 and C-II-v from the proband were tested for activation potential of bovine skim milk lipase. Using a constant concentration of lipase activity, there were no apparent differences between C-II-1 and C-II-v in activation potential over a range of 0.1 μg to 10 μg of apoC-II added as shown in **Fig. 8**. Determination of lipase activation was also carried out in the presence of apoC-II-1 from a normal individual. Using identical assay conditions, values for maximal enzyme activation were similar to those of C-II-1 from the proband (Fig. 8).

Tryptic peptides of apoC-II-1 and C-II-v, isolated from the proband, were separated by reverse-phase

TABLE 3. Kinetic parameters of apoB and apoC

Kinetic Parameter	Proband	Type IV[a]	Normal[a]
VLDL apoB			
Pool size (mg/kg)	17	16 ± 5	1.3 ± 0.2
Fractional catabolic rate (per day)	1.62	1.96 ± 0.38	12 ± 1.2
Production rate (mg/kg/day)	28	30 ± 3	15 ± 2
ApoC			
Fractional catabolic rate (per day)			
VLDL C-II-1	0.29	0.32 ± 0.02	1.8 ± 0.4
VLDL C-II-v	0.31		
HDL C-II-1	0.48	0.32 ± 0.02	1.8 ± 0.4
HDL C-II-v	0.44		

[a]Values (± SEM) for Type IV subjects (n = 4) and normal subjects (n = 6) were determined previously. Kinetic parameters for apoB have been published previously (18). The mean ± SEM for total and VLDL triglycerides for the Type IV subjects and normal subjects were 678 ± 65, 450 ± 40, 125 ± 10, and 63 ± 5, respectively.

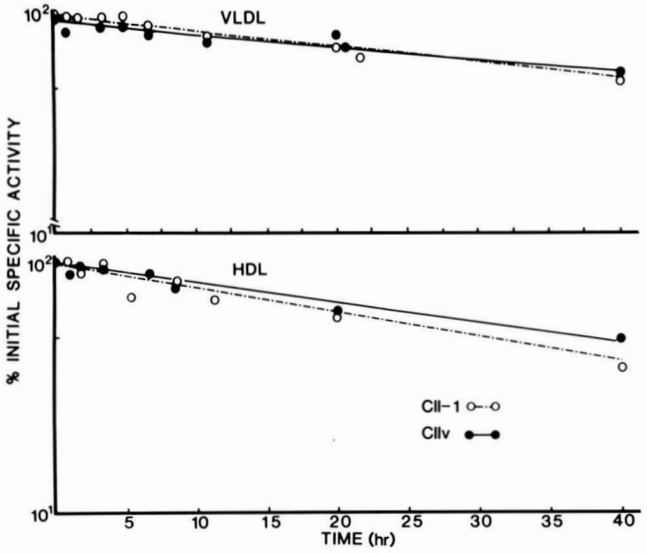

Fig. 5. Specific activity-time curves of ^{125}I-labeled apoC-II-1 and C-II-v in VLDL (S_f 60–400) (top panel) and in HDL (d 1.063–1.21 g/ml) (lower panel) after the injection of ^{125}I-labeled VLDL in the proband.

HPLC (**Fig. 9**). Six major peaks were identified in the profile of C-II-1 (Fig. 9A), which, based on retention times and peak heights, was identical to the pattern for normal C-II-1 reported previously (12). In the profile for C-II-v (Fig. 9B), peaks 1, 2, 3, and 6 were observed with

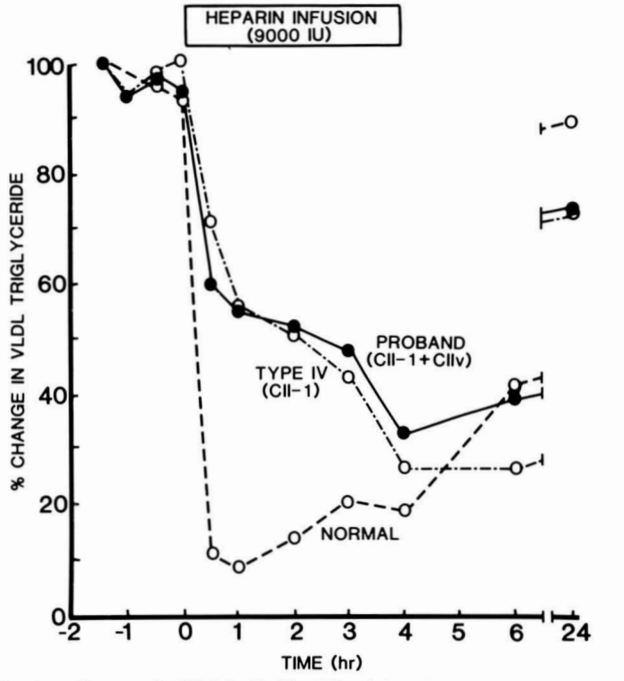

Fig. 6. Changes in VLDL (S_f 60–400) triglyceride during an infusion of heparin (9000 IU over 4 h). The results are expressed as percent of pre-infusion concentrations. Results are shown for the proband (●——●), a control hypertriglyceridemic subject (○···○), and a control normal subject (○——○). The total and VLDL triglyceride concentrations for the Type IV and normal subject were 794 and 512 and 114 and 68 mg/dl, respectively.

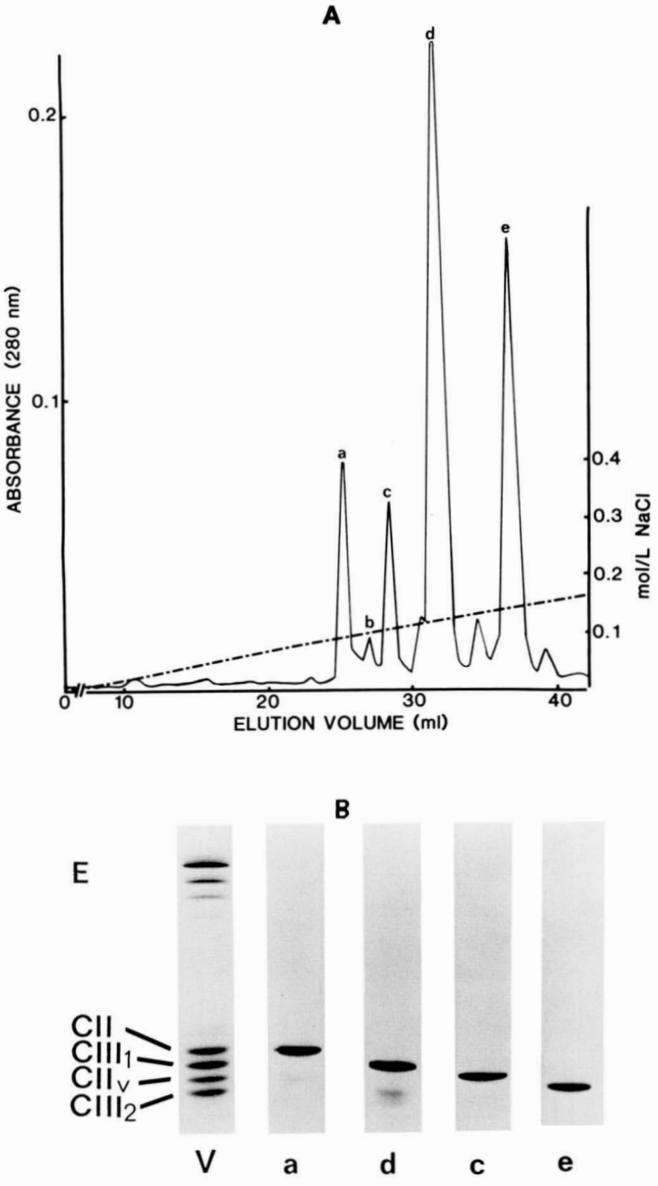

Fig. 7. A: separation of VLDL C apolipoproteins from the proband by anion exchange chromatography (Mono Q) on the FPLC system. The gradient was developed by switching from 100% of buffer A (0.01 M Tris, pH 8.6, 6 M urea) to 20% buffer B (0.01 M Tris, pH 8.6, 1 M NaCl, 6 M urea) over 40 min starting 10 min after sample injection. B: Analytical isoelectric focusing gel electrophoresis of whole VLDL from the proband and the peaks from the chromatogram in Fig. 7A. Based on isoelectric focusing, the peaks were identified as follows: a, C-II-1; b, C-III$_0$, (gel not shown); c, C-II-v; d, C-III$_1$; and e, C-III$_2$.

their characteristic retention times. Peaks P4 and P5 were missing and an additional peak P6', with a retention time 2.5 min longer than P6, was observed. Fig. 9C shows the relationship of peptides P1 through P6 to intact C-II-1, as determined previously (12). Examination of this arrangement indicates that the loss of P4 and P5 from the C-II-v profile is consistent with the loss of lysine 19 from C-II-v. It is possible that P6' represents uncleaved P4 plus P5.

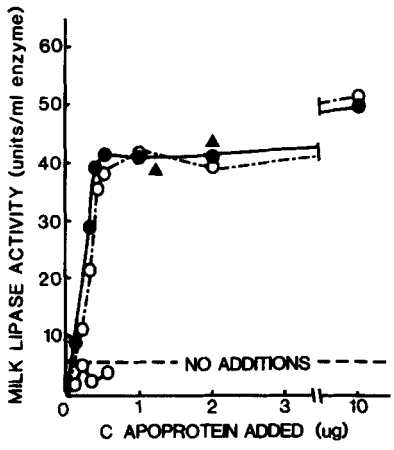

Fig. 8. Assay of apoC-II-1 (●——●), apoC-II-v (○– · –○), and apoC-III (○——○) from the proband and apoC-II-1 from a normal subject (▲) for cofactor activity for bovine milk lipoprotein lipase using a [^{14}C]triolein-gum arabic emulsion as substrate. One unit of activity was defined as μmol of free fatty acid released per h per ml of enzyme solution.

DISCUSSION

In this study we describe the identification of a variant of apoC-II-1 in a hypertriglyceridemic individual that we have designated apoC-II-v. This variant was also present in the proband of the brother but not in his mother. The father was deceased and other first degree relatives were not available for study. This apoC-II variant had an isoelectric point that was more acidic than normal C-II (4.74 vs 4.88). Two-dimensional gel electrophoresis indicated that the variant has a molecular weight similar to that of normal apoC-II-1. This protein was identified as a variant of apoC-II based on immunoblotting with anti-human apoC-II and its reaction with anti-C-II after purification by anion exchange chromatography. We determined that the more acidic pI was not due to sialylation of apoC-II-v as neuraminidase treatment did not alter the migration of this protein on analytical isoelectric focusing gels. Therefore, this protein is not a sialylated form of apoC-II. Fojo et al. (26) have identified a minor sialylated form of apoC-II in human plasma which has a pI similar to C-II-v.

As determined by electroimmunoassay, the concentration of total apoC-II, which would include both normal C-II-1 and C-II-v, was elevated in plasma and VLDL, consistent with elevated apoC-II concentrations reported in hypertriglyceridemic subjects who possessed only apoC-II-1 (10, 37). Since both apoC-II-1 and C-II-v were found in the proband, the simplest interpretation is that he is heterozygous for the two isoforms. Assuming that synthesis of C-II-1 and C-II-v were similar and that catabolism from plasma was similar, one would predict that equal amounts of each peptide would be observed. However, in the proband, the ratio of apoC-II-1 to C-II-v in VLDL was 2:1 (where about 90% of total plasma C-II was found) and 1:1 in HDL. The reason for this is not known but may be related to the nature of the molecular defect in apoC-II-v as discussed below.

ApoC-II-v possessed normal lipoprotein lipase activity in vitro. Plasma, VLDL, and HDL from the proband were all normal when assessed for activation potential in a bovine skim milk lipoprotein lipase assay. Purification of apoC-II-1 and apoC-II-v from VLDL showed identical activation potential (Fig. 8) in the activation assay. ApoC-II-1 from the proband gave maximal values for lipase activation which were similar to those for C-II-1 from a normal subject. The maximal activation for C-II-1 from the proband was approximately ninefold above baseline, a value similar to that reported previously by Jackson et al. (38) for normal intact apoC-II. Therefore, in vitro, C-II-1 and C-II-v from the proband have normal lipase activation activity.

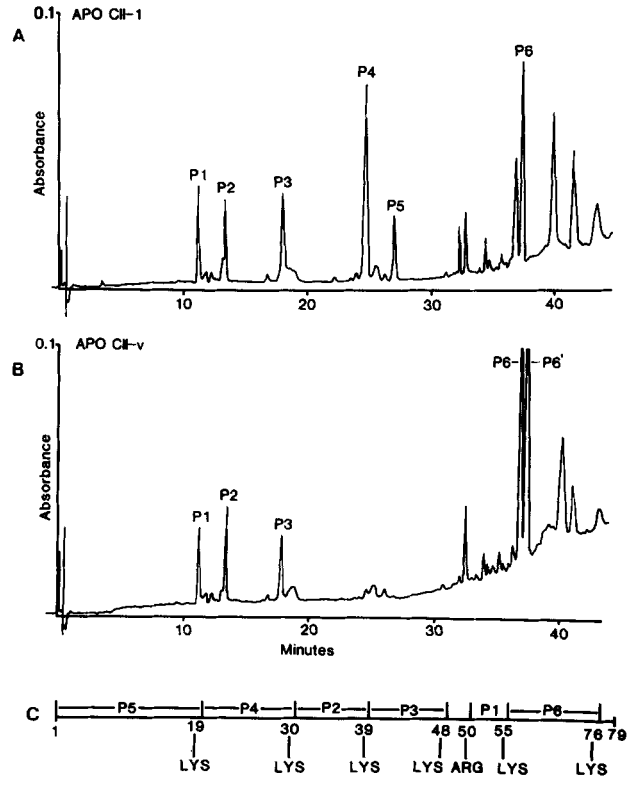

Fig. 9. A and B are the elution profiles of tryptic peptides of apoC-II-1 and C-II-v, respectively, isolated from the VLDL of the proband after separation by reverse-phase HPLC. The peptides were detected by their absorbance at 226 nm using an absorbance range of 0.1. In profile B, peaks P4 and P5 were missing; peak P6', which eluted with a retention time of 37.5 min, was not present in profile A. C represents the arrangement of peptides P1–P6 in apoC-II-1 from the amino terminal, amino acid 1, through to the carboxyl terminal, amino acid 79.

A number of nonfunctional variants of apoC-II have been found in VLDL from human subjects. Connelly et al. (12, 13) have described two different variants of apoC-II in two families, apoC-II-Toronto and apoC-II-St. Michael, both of which have little detectable lipoprotein lipase activation potential and result in hyperchylomicronemia in affected individuals. The molecular defects of these nonfunctional variants have been reported (12, 13) and both involve amino acid substitutions within the putative lipoprotein lipase binding region (38, 39). C-II-Toronto is characterized by a base deletion in the codon for amino acid 68 (40) resulting in a translation reading frame shift and a protein two amino acids shorter than native C-II (12). C-II-St. Michael is characterized by a base insertion in the codon for amino acid 69 or 70, a reading frame shift, and an abnormally long, 96 amino acid, apoC-II molecule.

Nonfunctional apoC-II variants have been described in four other kindreds and have been designated apoC-II-Bethesda and apoC-II-Padova (14, 15), C-II-Nijmegen (41), and C-II-Hamburg (42). The molecular defect in C-II-Bethesda has not been reported. Normal DNA and mRNA coding for apoC-II have been demonstrated in C-II-Padova (43); however, a recent report indicated that a substitution in the codon for amino acid 37 resulted in a termination codon, and hence a nonfunctional, truncated form of apoC-II (44). In the C-II-Hamburg kindred, a base substitution at a splicing site in intron II of the apoC-II gene results in abnormal splicing of apoC-II mRNA and ultimately a deficiency of apoC-II (42). In the C-II-Nijmegen kindred, a deletion of a guanosine in the codon for amino acid 18 results in a termination codon and a truncated nonfunctional 17 amino acid apoC-II (41).

A variant of apoC-II, apoC-II-2, which is characterized by a substitution of glutamine for lysine at residue 55, was identified in four hypertriglyceridemic subjects of African descent (16, 17). In a survey of 50 normal lipidemic African descendants, apoC-II-gln55 was found in 12% of these individuals, but none or trace amounts were detected in 500 Caucasian subjects (17). ApoC-II-gln55 has an isoelectric point similar to apoC-II-v reported in this study, and also activates bovine milk lipoprotein lipase in vitro as efficiently as normal apoC-II-1 (16). However, it is unlikely that apoC-II-v has the same molecular defect as apoC-II-2. The observation that tryptic fragments of apoC-II-v (P4 and P5), coding for amino acids 1 to 19 and 20 to 30 of normal C-II-1, are absent and that C-II-v has a molecular weight similar to C-II-1 is consistent with a substitution of a neutral amino acid for lysine at amino acid 19 (Fig. 9). In addition, fragments coding for residues 50–55 (P1) and 56–76 (P2) are present and show the same HPLC chromatographic characteristics as normal apoC-II-1, indicating the presence of the tryptic cleavage site, lysine 55. Studies with synthetic peptides of apoC-II have provided evidence for three functional domains: a lipid-binding domain (residues 44–52), an LPL activation domain (residues 56–67), and an LPL-binding domain (residues 65–75) (38, 39). Jackson et al. (38) have shown that a synthetic apoC-II peptide (residues 44–79) in an in vitro system resulted in only 60% activation of LPL compared to native C-II, indicating that residues 1–43 are important in lipid binding required for maximal activation of LPL. Catapano et al. (45) reported that sequences between the amino terminal and residue 43 contribute to lipid binding. Amino acid 19 is within this domain of apoC-II and thus may explain the reduced amount of apoC-II-v, relative to C-II-1 associated with VLDL. It is possible that the proposed defect does not influence binding to HDL, resulting in a ratio of apoC-II-1 to apoC-II-v of 1:1. If apoC-II-1 and C-II-v are synthesized and secreted in equal proportions, the reduced ability to bind to VLDL may result in apoC-II-v in plasma in the lipoprotein-free form. However, only trace amounts of immunoreactive apoC-II were detected in the $d > 1.21$ g/ml fraction of plasma.

Although apoC-II-v was able to activate lipoprotein lipase normally in vitro, it was possible that, in vivo, apoC-II-v contributed to the hypertriglyceridemia observed in the proband and his brother. It has been suggested that apoC-II has other roles in triglyceride metabolism, distinct from its cofactor activity for lipoprotein lipase (17). Therefore, we conducted kinetic studies of VLDL apoB and apoC in VLDL and HDL. In addition, we documented the response of VLDL triglyceride to an infusion of heparin.

In the proband, kinetic parameters of VLDL apoB differed from those in normolipidemic subjects, but were similar to those in other Type IV hypertriglyceridemic individuals. We speculated that if apoC-II-v affected VLDL metabolism in vivo, by reducing the efficiency of lipolysis, that the primary defect would be a reduced fractional catabolic rate, with little change in VLDL apoB synthesis. However, VLDL apoB synthesis increased twofold in addition to the sixfold reduction in the fractional catabolic rate. This suggests that in the proband an overproduction defect was present, which cannot be readily attributed to a defect in apoC-II, in addition to a catabolic defect. An increased VLDL apoB production and reduced FCR is characteristic of Type IV subjects as reported by others (10, 46, 47). The rate of clearance of VLDL triglyceride in the proband following an infusion of heparin, although slower than normal, did not appear to be different from that observed in another Type IV subject studied at the same time. This indicated that, once lipoprotein lipase was liberated into plasma by heparin, apoC-II-v probably did not adversely affect the rate of VLDL triglyceride hydrolysis.

Finally, determination of apoC-II-1 and apoC-II-v kinetic parameters revealed that the fractional catabolic rates in VLDL and HDL were similar. This indicates that the molecular defect responsible for apoC-II-v does not appear to alter its catabolism relative to apoC-II-1. However, metabolic studies after the re-injection of isolated and radiolabeled apoC-II-1 and C-II-v are required to confirm this point.

We have described a new variant of apoC-II, designated apoC-II-v, found in two Caucasian brothers with hypertriglyceridemia. ApoC-II-v has normal lipoprotein lipase activation potential in vitro and its presence does not appear to cause changes in VLDL apoB or triglyceride metabolism that differ substantially from other subjects with Type IV hypertriglyceridemia. These results would suggest that the hypertriglyceridemia in these subjects is a primary disorder and not secondary to the presence of C-II-v. However, it remains possible that, in association with familial hypertriglyceridemia, the presence of apoC-II-v could influence the expression of this disorder.

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