Expression of a novel human apolipoprotein (apoC-IV) causes hypertriglyceridemia in transgenic mice

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Abstract The human apolipoprotein (apo) C-IV gene has been recently identified: it is closely linked to the promoter region of the apoC-II gene (Allan, C. M., D. Walker, J. Segrest, and J. M. Taylor. 1995. *Genomics.* **28:** 291–300). To determine the effect of apoC-IV gene expression on lipoprotein metabolism, transgenic mice were generated using a human apoC-IV cDNA construct. Human apoC-IV was found associated with plasma lipoproteins (d < 1.21 g/ml), mainly in very low density lipoproteins (VLDL), and higher molecular mass isoforms were present, due to N-linked glycosylation and variable sialylation of apoC-IV. Human apoC-IV transgenic mice were hypertriglyceridemic compared to nontransgenic controls; the accumulated plasma triglycerides were present mainly in VLDL. There was little change in plasma cholesterol levels, although apoC-IV expression redistributed cholesterol to VLDL and larger particles in low density lipoprotein/large high density lipoprotein fractions. By immunoblot analysis, apoC-IV was not detected in normal adult human plasma or isolated plasma lipoproteins, a finding consistent with our previous observation of very low levels of human apoC-IV mRNA in human liver. However, our analysis of transgenic mice provides unequivocal evidence that human apoC-IV is a lipid-binding protein belonging to the apolipoprotein family and that it has the potential to alter lipoprotein metabolism.—**Allan, C. M., and J. M. Taylor.** Expression of a novel human apolipoprotein (apoC-IV) causes hypertriglyceridemia in transgenic mice. *J. Lipid Res.* 1996. **37:** 1510–1518.

Supplementary key words apolipoprotein C-IV • plasma lipoprotein • transgene • triglyceride

The genes encoding human apolipoprotein (apo) E, apoC-I and apoC-II are located within a 45-kb cluster on chromosome 19 (1, 2). The similar exon–intron arrangement of these genes suggests that they evolved from a common ancestral gene (3, 4) and that they are evolutionarily related to the apoA-I, apoA-IV, and apoC-III genes located in a cluster on chromosome 11 (4–6). We recently characterized a new human gene designated the apoC-IV gene (APOC4), which is located in the apoE, apoC-I, and apoC-II gene locus (7). Its 3′ terminus lies 555 bp upstream of the apoC-II gene, giving both genes the same transcriptional orientation. The structure and location of the apoC-IV gene, as well as the characteristics of its predicted protein sequence, suggested that it belongs to the apolipoprotein gene family.

Apolipoproteins E, C-I, and C-II are components of plasma lipoproteins that have evolved distinct roles in lipid metabolism. Apolipoprotein E mediates lipoprotein clearance from the plasma by acting as a ligand for the low density lipoprotein (LDL) receptor (8, 9), LDL receptor-related protein (10), and cell-surface proteoglycans (11). Apolipoprotein C-II is the cofactor for lipoprotein lipase, playing an obligatory role in the hydrolysis of lipoprotein triglycerides (12). The precise function of apoC-I is uncertain, but in vitro studies show that it may inhibit the apoE-mediated cellular uptake of lipoproteins (13, 14), suggesting a role for apoC-I in modulating lipoprotein catabolism. The proposed amino acid sequence of human apoC-IV shares a limited homology to the human apoC-I and apoC-II sequences (7), indicating that it also may function in lipid metabolism.

Apolipoproteins associate with lipid through the repeated amphipathic α-helical structures that are characteristic of these proteins (for review, see refs. 4, 15). The human apoC-IV gene was predicted to encode a protein of 127-amino acid residues that contains two amphipathic structures that belong to types characteristic of apolipoproteins; a class A1 domain between residues 47–64 and a class Y domain between residues 95–112 (7). These potential lipid-binding domains suggested that human apoC-IV may associate with lipoproteins. In addition, comparison of the exon sequences encoded by the human and mouse apoC-IV genes revealed that the

Abbreviations: apo, apolipoprotein; bp, base pairs; d, density; FPLC, fast performance liquid chromatography; kb, kilobase; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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apoC-IV coding sequence is evolutionarily conserved, with a 70% identity of nucleotide positions (7). Furthermore, comparison of the proposed human and mouse apoC-IV proteins reveals that apoC-IV is conserved at a level (57%) similar to that of human and mouse apoC-II (57%) and apoC-I (61%), which are encoded by the two neighboring genes that are actively involved in lipoprotein metabolism (7). This degree of sequence conservation in the apoC-IV gene and protein sequences suggests that apoC-IV may have a function in lipid metabolism.

In the present study, we have investigated this possibility using transgenic mice expressing human apoC-IV. We found that apoC-IV associates with plasma lipoproteins, and its overexpression resulted in a phenotype similar to those reported for transgenic mice expressing the small molecular weight human C apolipoproteins, apoC-I, apoC-II, and apoC-III (16–19). In addition, we show that apoC-IV is not expressed in normal adult human plasma, which suggests it may not participate in adult human lipoprotein metabolism.

MATERIALS AND METHODS

Generation of transgenic mice

A human apoC-IV cDNA fragment was isolated from pC-IV (7) by digestion with *Bam*HI and *Bgl*II and subcloned into pLITMUS 38 (New England Biolabs, Beverly, MA). The cloned cDNA was isolated by digestion with *Mlu*I and *Mun*I and inserted into pLIV11 (20), a vector containing the human apoE gene promoter and its liver enhancer sequence (21), to produce the construct pLIVhC-IV (**Fig. 1**). After digestion with *Sal*I and *Spe*I, the linearized LIVhC-IV fragment was isolated following agarose gel electrophoresis as described (7). Transgenic animals were generated essentially as described previously (22), using FVB/N mice obtained from Charles River Laboratories (Wilmington, MA). The LIVhC-IV DNA was microinjected at 2–3 ng/μl in 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA buffer into the fertilized embryos of superovulated mice. Five independent transgenic founder lines (17% of pups screened) were identified by Southern blot analysis of tail DNA, using an apoC-IV cDNA probe as described (7). Copy numbers of the transgenes were determined by Southern dot blot analysis and quantification using a Fujix Bas 1000 Bio-imaging Analyzer (Fuji Photo Co. Ltd., Japan); human genomic DNA from cultured HepG2 cells was used as a standard.

RNA preparation and RNase protection analysis

Total RNA from mouse tissues was isolated using guanidine thiocyanate (23). RNase protection analysis was performed as described (24), using 5 μg of total cellular RNA per sample. An antisense RNA probe for human apoC-IV mRNA was transcribed as described (7) using [α^{32}P]UTP in the presence of T3 RNA polymerase. A mouse actin mRNA probe (pTRI-actin, Ambion Inc.) was used in this assay to confirm that equivalent levels of RNA were present in each tissue sample.

Isolation of plasma lipoproteins

Total human or mouse plasma lipoproteins (d < 1.21 g/ml) were isolated by density ultracentrifugation in a TLA100.2 rotor using a Beckman tabletop TL100 ultracentrifuge. Plasma samples (1 ml) were centrifuged at d 1.21 g/ml for 4 h at 100,000 rpm and 4°C. Mouse plasma lipoproteins also were isolated by FPLC gel filtration using a Superose 6 HR10/30 column (Pharmacia LKB, Piscataway, NJ). FVB/N mice that were hemizygous for the LIVhC-IV transgene were maintained on normal mouse chow (Purina 5001, 4.5% fat), fasted for

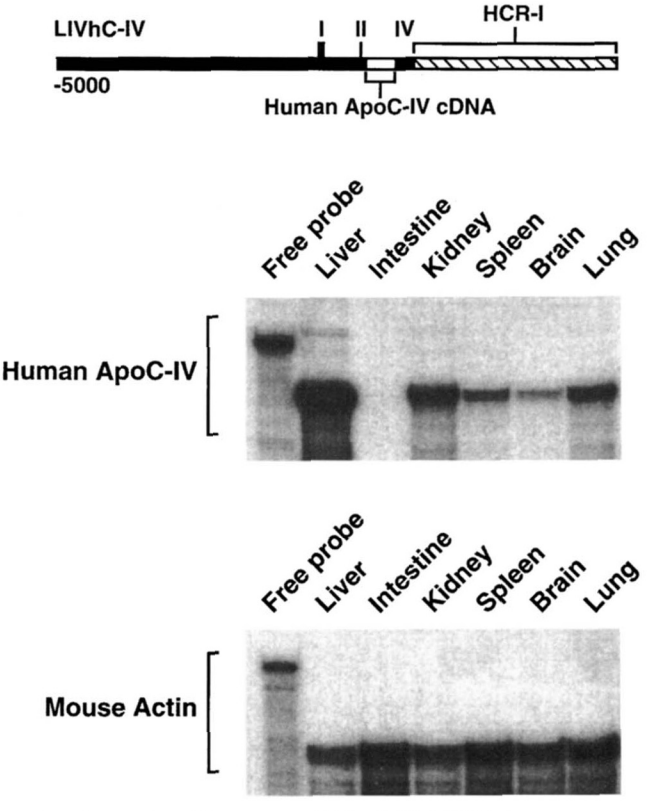

Fig. 1. Expression of human apoC-IV mRNA in LIVhC-IV transgenic mice. Transgenic mice were generated with the construct LIVhC-IV, which contains 5 kb of the 5′-flanking region, the first exon (I), part of the second exon (II), and the polyadenylation sequence of the fourth exon (IV) of the human apoE gene, as well as the human apoC-IV cDNA and sequence containing the liver enhancer, HCR-1 (21), as shown above. Total RNA was isolated from each tissue, obtained from a founder with four copies of the LIVhC-IV construct. Autoradiograms of the dried gel after RNase protection analysis of human apoC-IV mRNA or mouse actin mRNA in mouse liver, intestine, kidney, spleen, brain, and lung are shown. The bands shown here correspond to the protected human apoC-IV or mouse actin mRNA fragments (349 and 250 bp, respectively). The human apoC-IV hybridization probe does not cross-react with mouse apoC-IV mRNA in this assay (data not shown).

4 h, and the plasma was collected from the major tail vein using 1 mg/ml EDTA as an anticoagulant. For each FPLC run, a 250-μl aliquot of six pooled plasma samples was applied to the column, and the eluate was collected in 0.25-ml fractions at a flow rate of 0.5 ml/min. The cholesterol and triglyceride contents of plasma samples and lipoprotein fractions were determined by colorimetric quantitation assays, using reagents obtained from Boehringer Mannheim Diagnostics (product numbers 290319 and 701912, respectively, Indianapolis, IN), as described previously (25).

Production of anti-human apoC-IV antisera

A multiple antigen peptide (MAP) containing eight copies of a peptide corresponding to amino acid residues 30–44 of human apoC-IV was synthesized and used for the production of anti-peptide antibodies (a service of Research Genetics, Inc., Huntsville, AL). Antisera were collected from two 3–9-month-old NZ White rabbits and serum aliquots were stored at -20°C. Antiserum samples collected at week 10 of the immunization schedule were applied to Affi-Gel protein A agarose affinity columns (Pharmacia, Piscataway, NJ) as recommended by the manufacturer. Antibody eluted from Protein A, denoted anti-CIV[30–44] IgG, was dialyzed against phosphate-buffered saline (PBS), then used in the immunoblot analysis described below.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis

Equivalent volumes of plasma or lipoprotein samples were analyzed by SDS-PAGE using 15% or 4–20% gradient polyacrylamide gels as described (25). Proteins separated by SDS-PAGE were transferred onto nitrocellulose sheets by electroblotting (26). The nitrocellulose sheets were incubated for 1 h in 5% (w/v) skim milk, followed by incubation with apolipoprotein-specific antibodies for 1 h at room temperature. Anti-CIV[30–44] IgG was diluted 1 in 1000 in PBS/0.05% (v/v) Tween 20. Antisera recognizing mouse apoA-I, apoB, and apoE were used as described previously (17, 25). Specifically bound antibodies were detected using a horseradish peroxidase-conjugated secondary antibody followed by chemiluminescent detection (ECL kit, Amersham, Arlington Heights, IL).

Enzymatic deglycosylation

Lipoprotein (d < 1.21 g/ml) samples were dialyzed against 0.1 M ammonium acetate, pH 5.0. Samples were lyophilized for glycosidase treatment described below, or desialylated by adding an equal volume of 0.1 M ammonium acetate, pH 5.0, containing 0.5 mg/ml neuraminidase (Sigma, N-5631) for 2 h at 37°C. The addition of neuraminidase was repeated, and after another 2-h incubation at 37°C, the samples were lyophilized and stored at -20°C.

N-glycanase treatment of d < 1.21 g/ml protein, untreated or desialylated, was carried out after denaturation at 95°C for 5 min. Samples were adjusted to 50 μl of reaction volume containing (final concentrations) 25 mM sodium phosphate, pH 7.2, and 0.4 U of N-glycanase (N-glycanase F, Boehringer Mannheim, Germany), and incubated at 37°C for 14 h. O-glycanase digestion was carried out after denaturation of desialylated samples with 0.5% SDS at 95°C for 5 min. Protein solutions were adjusted to 50 μl containing 25 mM sodium phosphate, pH 7.2, 0.5% Nonidet P40, 0.05% SDS, and 20 mU O-glycanase (Boehringer Mannheim, Germany), and incubated at 37°C for 14 h.

RESULTS

Transgenic mice expressing human apoC-IV

To express the human apoC-IV gene in transgenic mice, a construct (LIVhC-IV) containing the human apoC-IV cDNA, under the transcriptional control of the human apoE gene promoter and its endogenous liver enhancer domain, was prepared. This vector was chosen because other heterologous cDNAs previously cloned into pLIV11 were expressed at high levels in transgenic animals, predominantly in the liver (20, 27). We used this vector instead of the intact gene because our previous work indicated that the human apoC-IV gene is poorly expressed in the liver, possibly as a consequence of a TATA-less promoter (7). The inbred FVB/N strain of mice was selected to minimize variations in gene expression between animals. Two independent founder lines were examined for the expression of human apoC-IV mRNA in six different tissues including the liver. By RNase protection analysis, each transgenic line showed the same pattern of apoC-IV transgene expression, with the liver having the highest levels of human apoC-IV mRNA (Fig. 1).

Analysis of human apoC-IV in human and transgenic mouse plasma

To determine whether human apoC-IV was present in human or transgenic mouse plasma, anti-peptide antibodies were raised to amino acid residues 30–44 (EAQEGTLSPPPKLKM) of the predicted human apoC-IV sequence. This region of human apoC-IV was selected to avoid potential antibody cross-reactivity, as it shares less than 30% amino acid homology to the corresponding mouse apoC-IV sequence (7). Furthermore, this region is predicted to contain a β-turn structure, which is known to facilitate apolipoprotein antibody production (28, 29). The isolated plasma lipoproteins (d < 1.21 g/ml) of two independent lines of transgenic mice

and two nontransgenic littermates were analyzed by SDS-PAGE followed by immunoblotting. The anti-CIV[30–44] IgG revealed two major bands (of approximately 18 and 12.5 kDa) in the transgenic lipoproteins, which were not present in normal mouse lipoproteins (**Fig. 2**). Longer exposures of the immunoblots showed the presence of faint additional bands (of about 14.5–15 and 19 kDa) in the transgenic samples (data not shown). Comparison of reduced and non-reduced lipoprotein samples revealed bands of similar electrophoretic mobility (data not shown), indicating that the apoC-IV protein was not disulfide-linked to other plasma proteins via any of its six cysteine residues.

The predicted molecular masses of nascent human apoC-IV and of human apoC-IV without its deduced signal peptide sequence (7) are approximately 14.6 kDa and 11.9 kDa, respectively. Therefore, the presence of 18 kDa and 12.5 kDa isoforms in plasma suggested that apoC-IV had undergone post-translational modification. To determine whether glycosylation could account for these apoC-IV isoforms, plasma lipoproteins from transgenic mice were examined further. Lipoproteins were treated with neuraminidase, which removes sialic acid residues from N- and O-linked sugars, resulting in the identification of four major electrophoretic bands of approximately 12.5, 14.5, 17.5, and 18.5 kDa (Fig. 2). To explain this observation, the original untreated apoC-IV isoforms were separated by gel electrophoresis, isolated, digested with neuraminidase, then examined by immunoblot analysis using anti-CIV[30–44] IgG (data not shown). The 12.5 kDa band was not altered by neuraminidase, suggesting it is not sialylated (Fig. 2). The 18 kDa was sialylated because neuraminidase digestion yielded a 17.5 kDa band. Two additional prominent

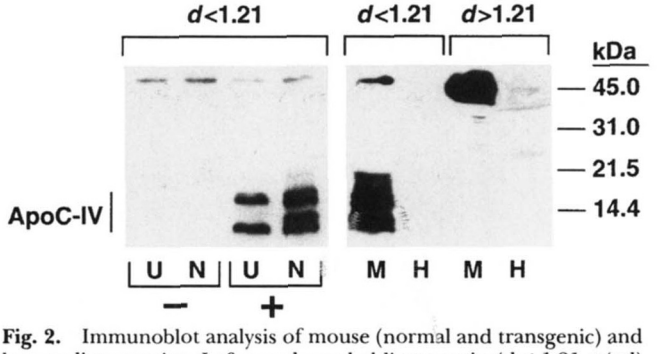

Fig. 2. Immunoblot analysis of mouse (normal and transgenic) and human lipoproteins. Left panel: pooled lipoprotein (d < 1.21 g/ml) fractions of two nontransgenic (-) or two transgenic (+) female littermates were either untreated (U) or treated with neuraminidase (N), and then equal volumes were resolved by denaturing 15% polyacrylamide gel electrophoresis. The proteins were transferred to nitrocellulose and reacted with anti-CIV[30–44] IgG. Right panel: plasma lipoprotein (d < 1.21 g/ml) and d > 1.21 g/ml fractions of neuraminidase-treated transgenic mouse (M) and human (H) samples were resolved by denaturing 15% polyacrylamide gel electrophoresis, and then immunoblotted as described above. The sizes of protein standards are indicated on the right of the panel.

bands, 14.5 kDa and 18.5 kDa, were also detected after neuraminidase treatment. These latter two apoC-IV bands appeared to be derived from sialylated precursors (approximately 15 kDa and 19 kDa, respectively) that were only barely detectable in untreated lipoproteins, as described above. These findings demonstrate that transgenic lipoproteins contain distinct sialylated human apoC-IV isoforms, and that sialylation impairs the ability of the specific anti-peptide antibody to recognize two of these isoforms.

The possible presence of apoC-IV in human plasma was examined by immunoblot analysis. Anti-CIV[30–44] IgG was not able to detect apoC-IV in the d < 1.21 g/ml lipoprotein or d > 1.21 g/ml non-lipoprotein fractions of normal adult human plasma. However, immunoblot analysis of transgenic lipoproteins showed that certain apoC-IV isoforms were readily detected by anti-CIV[30–44] IgG only after desialylation. Neuraminidase treatment of human lipoprotein (d < 1.21 g/ml) or non-lipoprotein (d > 1.21 g/ml) fractions did not reveal the presence of apoC-IV (Fig. 2), under conditions that reduced the molecular mass of sialylated isoforms of human apoC-III as determined by immunoblot analysis (data not shown). Therefore, it is unlikely that apoC-IV is present in normal adult human plasma.

To characterize further the carbohydrate modification of apoC-IV, transgenic lipoproteins were treated with N-glycanase or O-glycanase. N-glycanase treatment of denatured proteins removes N-linked oligosaccharide chains at asparagine, converting it to aspartic acid. Treatment of denatured sialylated or desialylated transgenic lipoproteins with N-glycanase resulted in the disappearance of the higher molecular weight apoC-IV bands (**Fig. 3**). The presence of an asparagine at residue 63 of apoC-IV within a consensus site for N-glycosylation (Asn-X-Thr) is consistent with this finding. These observations suggest that the high molecular weight apoC-IV bands represent isoforms containing a complex N-linked oligosaccharide at asparagine 63 that undergoes variable sialylation. O-glycanase treatment of denatured and desialylated proteins, which removes O-linked oligosaccharide chains at serine or threonine residues, had essentially no effect on the molecular sizes of the apoC-IV isoforms (Fig. 3).

Alterations in plasma lipoproteins in apoC-IV-transgenic mice

To determine whether the expression of human apoC-IV had altered plasma lipid levels, total plasma cholesterol and triglyceride levels were determined for transgenic and nontransgenic littermates. Transgenic mice (fasted for 4 h) had a 2-fold elevation in plasma triglyceride levels compared to controls (**Table 1**), with little difference in cholesterol levels between the two groups. Similar elevations in plasma triglycerides also

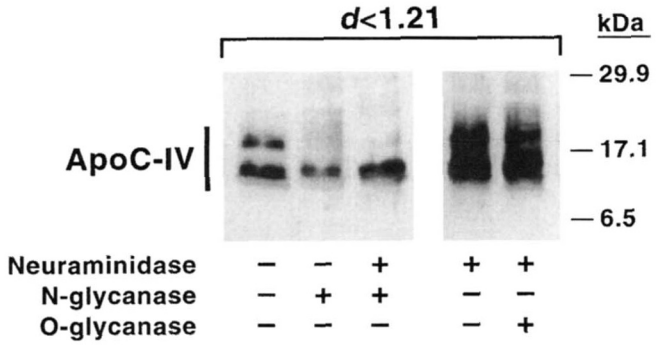

Fig. 3. Immunoblot analysis of glycosidase-treated human apoC-IV transgenic mouse lipoproteins. Pooled plasma lipoproteins (d < 1.21 g/ml) of two female transgenic mice were treated with neuraminidase, N-glycanase, or O-glycanase as described. Equal amounts of untreated or treated samples were resolved by denaturing 15% polyacrylamide gel electrophoresis, then immunoblotted using anti-CIV[30–44] IgG. Both neuraminidase- and neuraminidase/O-glycanase-treated samples were adjusted to the conditions of O-glycanase treatment. The sizes of protein standards are shown on the right.

were observed in male transgenic mice expressing human apoC-IV (data not shown).

To determine whether expression of human apoC-IV had effected the plasma lipoproteins of transgenic mice, plasma fractions were isolated by Superose 6 chromatography, as described in Materials and Methods. Equal volumes of plasma samples from six transgenic female or six nontransgenic female littermates were pooled and applied to the column. The cholesterol and triglyceride levels of the isolated fractions are shown in **Fig. 4.** Previous studies have determined the distribution of the various mouse plasma lipoproteins in fractions collected from Superose 6 chromatography (25). Compared to nontransgenic littermates, mice expressing human apoC-IV had elevated triglyceride and cholesterol levels associated with the region of the chromatographic profile corresponding to very low density lipoprotein (VLDL) (fractions 22–26). In addition, transgenic mice showed an altered cholesterol profile in the LDL/large high density lipoprotein (HDL) region (fractions 38–48), relative to control animals. Transgenic mice had larger cholesterol-containing particles in the LDL/large HDL region (fractions 38–42), and the region with intermediate HDL species (fractions 44–48) appeared to contain slightly lower levels of cholesterol (Fig. 4). The small HDL region (fractions 50–56) contained similar levels of cholesterol in both transgenic and nontransgenic samples.

The presence of human apoC-IV in the lipoprotein fractions was determined by SDS-PAGE followed by immunoblot analysis using anti-CIV[30–44] IgG. In the lipoprotein fractions of transgenic animals, human apoC-IV was found mainly in the VLDL region (fractions 22–25), as shown in **Fig. 5.** Low levels of human apoC-IV also were detected in the fractions containing intermediate density lipoproteins (IDL), LDL, and HDL. As expected, the apoC-IV that was detected in these untreated (i.e., not digested with neuraminidase) lipoprotein fractions corresponded to the two major apoC-IV bands detected in the untreated total plasma lipoproteins (Fig. 2). The band in the nontransgenic lane of fractions 49–56, in the region of apoC-IV, is due to cross-reactivity of anti-CIV[30–44] IgG with an underfined mouse plasma protein. It had a slightly greater size than the lower apoC-IV band and was found in the plasma of only one of the six normal littermates (data not shown). The presence of human apoC-IV in the VLDL of transgenic mice was also confirmed by silver staining of VLDL proteins (fractions 22–25) separated by SDS-PAGE (**Fig. 6**). Four major protein bands were observed in transgenic VLDL, that were not present in nontransgenic VLDL. The lowest and second highest molecular size bands corresponded to the two major apoC-IV forms detected in lipoproteins (not treated with neuraminidase) by immunoblot analysis (Fig. 2 and Fig. 5). The other two apoC-IV species represent the isoforms that were not readily detected by anti-CIV[30–44] IgG (Fig. 2). Human apoC-IV levels in transgenic mouse plasma were estimated by densiometric analysis, and found to be similar to the apoC-III levels, approximately 5–10 mg/dl.

Examination of the apolipoprotein content of pooled Superose 6 chromatography fractions by SDS-PAGE and immunoblot analysis showed that the distribution of mouse apoB and apoE were similar in transgenic and nontransgenic fractions (Fig. 5). However, human apoC-IV transgenic mice had increased levels of apoB-100 and apoB-48 in the VLDL and large remnant lipoprotein-containing fractions, compared to normal controls (fractions 22–29, Fig. 5). In addition, densiometric scanning of these gels showed that the VLDL (fractions 22–25) of transgenic mice had a 2-fold increase in the apoB-100:apoE ratio, and a 40% increase in the apoB-48:apoE ratio, relative to the apoB:apoE ratios of corresponding nontransgenic fractions. Large remnant lipoproteins (fractions 26–29) of transgenic mice showed a 3-fold increase in the apoB-48:apoE ratio, relative to the ratio of normal mice. Thus, the apoB-containing triglyceride-rich lipoproteins of human apoC-IV transgenic mice had a relative reduction in apoE content when compared to

TABLE 1. Plasma lipid levels of nontransgenic and human apoC-IV transgenic female mice

	Nontransgenic	Transgenic
	mg/dl	
Cholesterol	102 ± 18	116 ± 13
Triglyceride	156 ± 46	324 ± 21

Data are presented as mean ± standard deviation, with n = 6.

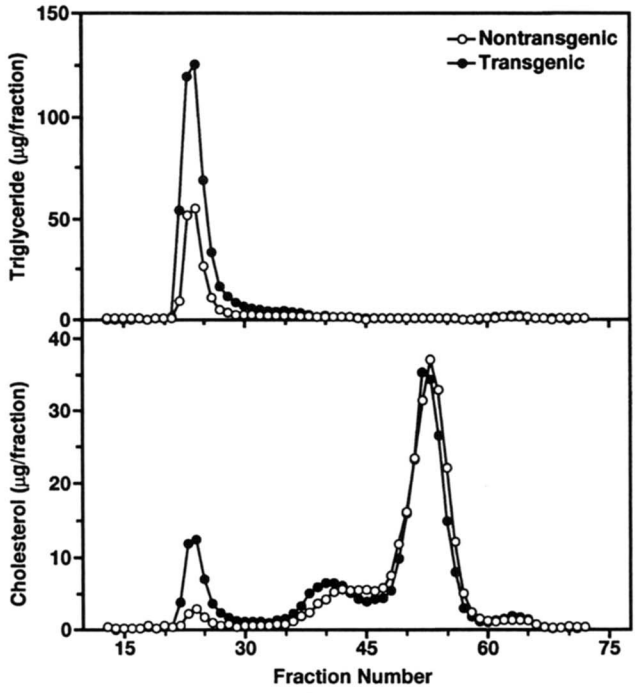

Fig. 4. Superose 6 chromatography of transgenic and nontransgenic mouse plasma. A 250-µl aliquot of six pooled transgenic or nontransgenic (normal littermates) plasma samples were injected into a Superose 6 column, and 0.25-ml fractions were collected. Triglyceride (top panel) and cholesterol (bottom panel) levels were determined for each fraction; (○-○) nontransgenic plasma; (●-●) transgenic plasma.

the corresponding nontransgenic lipoproteins. Immunoblot analysis of fractions 38–47, which are known to contain LDL and large HDL (25), showed no change in the levels of apoB-100 and apoB-48 in both transgenic and nontransgenic lipoproteins. However, the transgenic lipoproteins in fractions 38–47 contained higher levels of mouse apoA-I relative to nontransgenic controls (Fig. 5). These observations may indicate that the altered cholesterol profile of this region is in part due to the appearance of larger apoA-I-containing HDL particles.

DISCUSSION

We have expressed a newly described human apolipoprotein, apoC-IV, in the plasma of transgenic mice. Analysis of this animal model supports the proposal that the human apoC-IV gene is a member of the apolipoprotein gene family (7). In addition, expression of human apoC-IV in transgenic mice resulted in an interesting model of hypertriglyceridemia. We observed changes in the plasma triglyceride levels that resembled the hypertriglyceridemic phenotypes found in transgenic mice expressing apoC-I, apoC-II, and apoC-III (16–19).

The human apoC-IV in transgenic mice was found associated with plasma lipoproteins, mostly in the VLDL fraction. This finding is consistent with the predicted lipid-binding structure of the apoC-IV molecule, which indicated two amphipathic α-helical regions that are typical of the lipid-binding domains of the other apolipoproteins in this gene family. Plasma apoC-IV was not found to be disulfide-linked to any other plasma protein, and it was present in four distinct isoforms, of 12.5–19 kDa as determined by SDS-PAGE. The high molecular

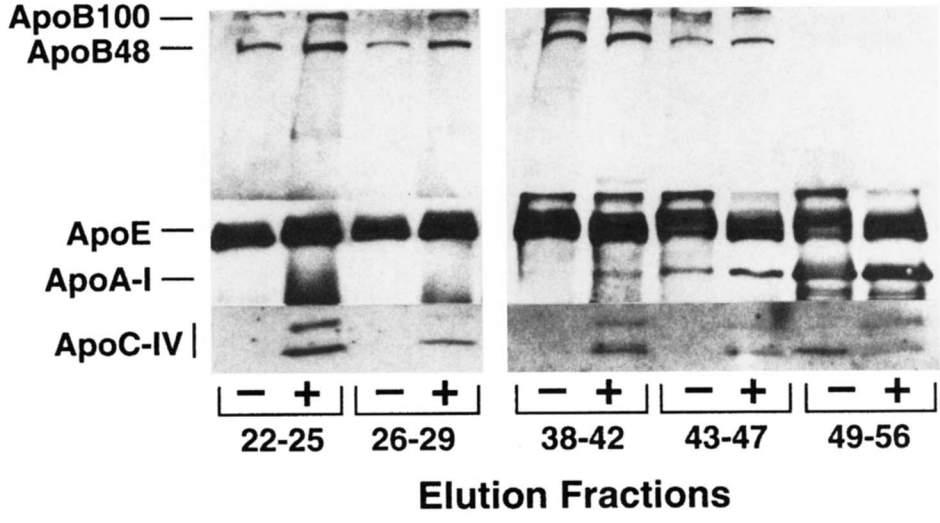

Fig. 5. Immunoblot analysis of lipoproteins separated by Superose 6 chromatography. Gel filtration fractions of nontransgenic (-) or transgenic (+) plasma samples were pooled, and equal volumes were resolved by denaturing 4–20% gradient polyacrylamide gel electrophoresis. The two panels (indicating separate gels) show the distributions of mouse apoB-100, apoB-48, apoE, and apoA-I, as well as human apoC-IV, determined by immunoblot analysis using specific antibodies. The migration positions of the specific apolipoproteins are indicated on the left.

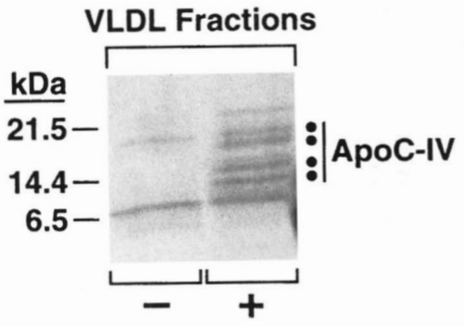

Fig. 6. Detection of human apoC-IV in VLDL of transgenic mice by silver staining. Equal volumes of nontransgenic (-) or transgenic (+) VLDL fractions obtained after Superose 6 chromatography were resolved by denaturing 4–20% gradient polyacrylamide gel electrophoresis and visualized by silver staining (37). The bands corresponding to the apoC-IV isoforms are indicated (●).

mass isoforms of apoC-IV were determined to be due to complex N-linked oligosaccharides having variable sialylation, thereby contributing to the observed heterogeneity. Other human apolipoproteins, such as apoC-III and apoE, also are found in the plasma in different sialylated isoforms (30, 31). However, the physiological significance of the variable sialic acid modifications of these apolipoproteins remains unknown.

The expression of human apoC-IV in mice resulted in elevated levels of plasma triglycerides, due to an accumulation of VLDL. Transgenic mice expressing the three known human C apolipoproteins, apoC-I (19), apoC-II (18), and apoC-III (16, 17) are known to be hypertriglyceridemic. The present observation of hypertriglyceridemia in human apoC-IV transgenic mice suggests that the human C apolipoproteins all share a common property that enables each of them to modulate triglyceride metabolism via a similar mechanism.

Recent studies in transgenic mice showed that overexpression of human apoC-II or apoC-III inhibited apoE-mediated uptake of triglyceride-rich VLDL (16–18). In vitro studies have shown that apoC-I and apoC-II both inhibit the binding of β-VLDL to the LRP-receptor (14, 32), predominantly by the displacement of apoE from β-VLDL. Similar studies have shown that the addition of apoC-I, apoC-II, or apoC-III can inhibit the apoE-dependent interaction of VLDL and IDL with the LDL receptor (13). Thus, an excess of C apolipoprotein impairs the apoE-mediated interaction of triglyceride-rich lipoproteins with receptors. Considering these findings, the elevated plasma triglycerides and increased apoB:apoE ratio of triglyceride-rich lipoproteins in human apoC-IV transgenic mice, relative to the corresponding values of nontransgenic controls, are consistent with an accumulation of VLDL. It is noteworthy that the expression of human apoC-IV in mice had no direct effect on the lipolytic activities of plasma. The plasma total lipase and hepatic lipase activities of human apoC-IV mice were comparable to the levels of nontransgenic littermates (H. Dichek and C. Allan, unpublished observations).

We were unable to detect human apoC-IV in adult human plasma using the anti-apoC-IV antibody. This finding supports our previous observation of only low levels of apoC-IV mRNA in human liver, relative to that of apoC-II mRNA (7). The low expression of the human apoC-IV gene may be due to the lack of a TATA-box motif in the promoter, a unique characteristic of this gene locus. Taken together, these observations suggest that human apoC-IV may not be active in normal adults. A comparison of the known apoC-IV sequences of different species (mouse, rat, and monkey) reveals a high degree of conservation in the coding regions of the apoC-IV gene (7), implying that it may have a useful physiological function.[2] For example, the apoC-IV gene might be expressed at higher levels during an earlier developmental stage, or during inflammation or disease. Other clusters of evolutionarily related genes, such as the human albumin or globin multigene loci, contain genes that are only expressed in a developmental stage-specific manner (33, 34). In addition, lipoproteins contain proteins that are only expressed at high levels during specific responses, such as the increased levels of apoJ and serum amyloid protein found associated with lipoproteins during acute immune responses (35, 36). Whether or not human apoC-IV shares any of these characteristics remains to be determined.

In summary, we have shown that the human apoC-IV gene encodes a lipid-binding protein, a finding that supports its inclusion into the apolipoprotein multigene family. In addition, apoC-IV overexpression in transgenic mice affected plasma triglyceride metabolism, resulting in hypertriglyceridemia. This particular lipid phenotype is found in transgenic mice that express each of the other previously characterized human C apolipoproteins. Overall, our results unequivocally demonstrate that human apoC-IV is a member of the apolipoprotein family. However, as it appears to be absent in human plasma, the normal physiological role of human apoC-IV remains unclear. Whether this human protein has an undefined specific role in lipid metabolism or functions in a process unrelated to lipoprotein metabolism remains to be determined.

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[2]After this manuscript was submitted, a report by Zhang, L-H., L. Kotite, and R. J. Havel (1996. *J. Biol. Chem.* **271:** 1776–1783) identified an apoC-IV homolog in rabbit plasma lipoproteins.

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